Vijay Singh Sharanagat
Dharmesh Chandra Saxena
Kshitiz Kumar
Yogesh Kumar *Editors*

Starch: Advances in Modifications, Technologies and Applications

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Editors

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Editors
Vijay Singh Sharanagat
Department of Food Engineering
National Institute of Food Technology,
Entrepreneurship and Management Sonipat
Haryana, India

Dharmesh Chandra Saxena
Department of Food Engineering
and Technology
Sant Longowal Institute of Engineering
and Technology
Punjab, India

Kshitiz Kumar
Department of Food Processing Technology
AD Patel Institute of Technology
Gujarat, India

Yogesh Kumar
Department of Food Engineering
and Technology
Sant Longowal Institute of Engineering
and Technology
Longowal, Punjab, India

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Preface

Starch is the most used natural polymer at the industrial level and forms more than 85% of the hydrocolloids used in food system. Native or unmodified starch has some inherent shortcomings such as formation of highly viscous and rigid gel upon cooking, undergo syneresis and retrograde quickly. Hence, starch is modified to alter its structure that either hydrolyze the starch to short molecules or/and add chemically substituted group to it. Modification improves the functional properties of starch such as increased gel-forming ability, cold water dispersibility, stability of dispersion and shear and thermal stability, which open up a vast spectrum of application in food industry. Modified starches are increasingly used in bakery, snack, meat product, sauces, beverages, dairy confectionary and nutritional food as thickening, emulsifying binding agents and fat replacer. With the help of modification, it is possible to create tailor-made ingredients from starch which can be used in specific formulation of food. The demand for modified starch is going to increase in coming years due to increased intake of processed and ready-to-eat food products. The global market for modified starch was estimated as USD 13.7 billion in 2022 and expected to grow at compound annual growth rate of 4.9% from 2022 to 2030.

Starch modification is carried out by physical, chemical, enzymatic and genetic methods. Unlike chemically modified starch, physically modified starch is not considered as food additives and can be used to develop "clean label" product. Over the last two decades, international research has progressed a great deal in the modification methods of starch obtained from various plant sources with a focus on the development of novel methods for modification. The use of dual and multi-modification techniques, wherein two chemical or physical modification techniques are used simultaneously or successively or in combination with enzymatic modification, has helped in improving the properties of native starch and increased the versatility of modified starch utilization. In recent years, the use of non-thermal methods such as ultrasound, high pressure, microwave, pulsed electric field has been explored for the modification of starch with great effect.

This book focuses on recent work done on the starch modification and its effect on structural, thermo-physical and functional properties of starch. The application of the modified starch by different techniques has been included in each chapter.

Chapters 1 and 2 cover the overview of starch production and its modification techniques. The need for modification and different types of modification mentioning the recent work has been presented in these chapters. Chapters 3, 4, 5, and 6 cover the detailed aspect of chemical modification of starch through oxidation, hydrolysis, crosslinking and esterification using octenyl succinic anhydride. The mechanism along with the method of modification and its effect on chemical, functional, pasting, thermal, morphological structure and digestibility of starch are discussed. Chapters 7, 8 and 9 cover the physical modification of starch through dry heat treatment, heat moisture treatment and annealing. The effect of these modifications on solubility, swelling power, paste clarity, pasting property, digestibility and glycemic index along with their applications are mentioned. Chapters 10, 11, 12, 13, 14, 15, and 16 present the use of novel methods for the modification of starch covering technologies such as high hydrostatic pressure, ultrasonication, pulsed electric field, cold plasma, microwave and gamma irradiation. Additionally, some case studies along with current research gap and future scope are discussed in these chapters. Chapters 17, 18, and 19 describe some environment-friendly methods such as enzymatic modification and fermentation as well as germination as method of starch modification. The use of modern equipment such as scanning electron microscope (SEM), X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), differential scanning calorimeter (DSC) and stress/strain-controlled dynamic rheometers for characterization of structure, thermal and rheological properties has enabled the researchers a better insight into the changes taking places during modification. The book covers in each chapter the analysis of starch molecules with these equipment.

Several book chapters and reviews are available on starch modification indicating the richness of ongoing scientific work in this field. However a dedicated book covering each modification techniques in details along with recent advances and application was not available. This book is an effort to fill that void and bring at one place all the work related to starch modification in a single volume. This would be very useful for the food technologists, researchers, academicians, scientists as well as industries.

The editors would like to express their deep gratitude to all the authors and co-authors for accepting our invitation and contributing in completion of the book. We also extend our thanks in advance for any suggestion for the improvement of the book.

Haryana, India	Vijay Singh Sharanagat
Punjab, India	Dharmesh Chandra Saxena
Gujarat, India	Kshitiz Kumar
Longowal, Punjab, India	Yogesh Kumar

Contents

Chapter 1
Starch: Current Production and Consumption Trends

Yogesh Kumar, Deep Shikha, Fabiola Araceli Guzmán-Ortiz, Vijay Singh Sharanagat, Kshitiz Kumar, and Dharmesh Chandra Saxena

1.1 Introduction

Starch is an important component of many diets worldwide and provides a slow, steady release of energy to the body. It is also used in various industrial and commercial applications, such as pharmaceuticals, textiles, paper, glue, packaging, and printing materials. The properties of starch can vary depending on its source, and it can be modified through cooking and processing to change its texture, taste, and other characteristics. Starch exists in the form of granules consisting of glucose units joined together by glycosidic linkages, which are mostly comprised of two components amylose (15–30%) and amylopectin (70–85%). Amylose is a linear polymer with a molecular weight of less than around 500,000 Da linked together by α-1,4 glycosidic bonds, depending on its physiological perspective. Amylopectin molecules, in contrast, have a molecular weight between ranges between 10^7 and 10^9 Da linked by α-1,4 and α-1,6 glycosidic bonds and are substantially larger and heavily branched (Krithika & Ratnamala, 2019; Park & Kim, 2021). For commercial applications, starch is mainly classified based on its amylose content: low (<20% amylose), medium (21–25%), and high (>26%) (Juliano, 1992).

Y. Kumar (✉) · D. Shikha · D. C. Saxena
Department of Food Engineering and Technology, Sant Longowal Institute of Engineering and Technology, Punjab, India

F. A. Guzmán-Ortiz
CONACYT- Universidad Autónoma del Estado de Hidalgo, Carretera Pachuca, Hidalgo, Mexico

V. S. Sharanagat
Department of Food Engineering, National Institute of Food Technology, Entrepreneurship and Management, Sonepat, Haryana, India

K. Kumar
Department of Food Processing Technology, AD Patel Institute of Technology, Gujarat, India

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The size of starch granules is microscopical; their diameter ranges from 0.1 to 100 μm, and their morphology is mostly oval, ellipsoidal, spherical, smooth, angular, and lenticular, depending on the botanical source. The size distribution of these granules can be uni, bi, or polymodal, and they are present individually or collectively in amyloplasts (Jane et al., 1994). Two types of granules exist in common cereals such as wheat, barley, and rye: type A, large and with a lenticular shape; and type B, spherical and smaller in size (Tester et al., 2006; Vamadevan & Bertoft, 2015). The morphology of starch granules is characterized mainly by the presence of a Maltese cross or birefringence, which can be observed under polarized light, indicating a high order in the amylose and amylopectin layer structure. When a molecular disorder occurs, the starch loses birefringence and gelatinization increases. The starch is dispersed in molecular components and swells; amylose leaching modifies properties such as viscosity and clarity. These are key parameters for analysis and implementation in the industry.

Starch can contain lipid molecules such as phospholipids and free fatty acids, which are commonly associated with the amylose fraction (Tester et al., 2004). The presence of lipid complexes in starch granules is observed as a hydrophobic nucleus within the helix formed by amylose chains in starches, mostly cereals. These complexes represent between 0.15% and 0.55% of the amylose fraction. The lipid structures vary according to the botanical source; for instance, corn starch contains free fatty acids and triglycerides, while wheat, rice, barley, and rye starches usually contain a significant amount of phospholipids. In contrast, phosphate monoesters are widely distributed in potato starch and other native starches from roots and tubers (Srichuwong & Jane, 2007). These lipid molecules can significantly reduce the swelling capacity of starch despite representing a very small fraction (Morrison & Azudin, 1987). On the other hand, starch can contain up to 0.6% of the protein associated with the molecule. This protein and lipid proportion is usually located on the surface of the starch granule, affecting and/or modifying its functionality. Additionally, they contain a relatively small amount (<0.04%) of minerals, such as calcium, magnesium, phosphorus, potassium, and sodium (Pérez & Bertoft, 2010). Phosphorus is another component in starch, found as phosphates and phospholipids. Phosphates are generally linked to the amylopectin fraction by covalent bonds, as phospholipids tend to create complexes with amylose (Singh et al., 2003).

Besides these starch components, other factors, including the presence of proteins and lipids and environmental factors, also affect the functionality of various grain starches. Therefore, various components as additives, such as salts, sugars, and sugar syrups, have also been used to improve or modify the characteristics as well as the functionality of starch (Shikha et al., 2019). In addition to physical modifications (hydrothermal and pre-gelatinization), chemical methods, including esterification, grafting, cross-linking, and etherification etc., as well as enzymatic modifications such as treatment with hydrolases and transferases of starch, have also been performed to enhance its applications (Chen et al., 2015; Ashogbon & Akintayo, 2014; Hj Latip et al., 2021; Zhong et al., 2022). However, to overcome the problems associated with native starch, researchers continuously explore different starch sources with desirable physicochemical properties such as syneresis, turbidity, and freeze-thaw stability to improve its industrial applications (Wani et al., 2013). It is important to characterize different starches based on their product-specific end-use as the properties of native starch are not optimal enough for many applications and require further modification using additives or ingredients to improve the functional properties (Raina et al., 2006).

The modification of starch not only enhances the paste clarity and sheen, film formation, texture of paste and gel, and adhesion but also reduces the retrogradation, pastes gelling tendency, and syneresis of gel (BeMiller, 1997). Therefore, to overcome such limitations, starch modifications are widely practiced in food processing industries. The modification of starch results in the stabilization of starch granules slows the rate of retrogradation, enhances solubility, and improves the pasting properties and gel texture during processing. Several starch-based food items like bakery goods, vermicelli, syrups, jellies, and edible coating have starch as a prime component (Abegunde et al., 2013). Despite this, native starch imparts various detrimental effects, like reduced solubility, deprived paste clarity, etc. (Sun et al., 2017). Also, starch retrogradation and recrystallization are two major aspects that are considered unfavorable under certain conditions of using native starch. Hence, the unpredictable processing attributes of native starches bound the appropriate utilization of starch (Krstonošić et al., 2011). So, to conquer such adverse effects of starches, several methods were designed with great security, *viz.* physical, chemical, and enzymatic treatment methods (Chiu et al., 1998). These techniques allow for modifying the starch polymer, altering its physicochemical and structural properties, and increasing its value in the food and non-food industries.

1.2 Overview of Starch Sources and Production

Starch, as a major carbohydrate source, is a primary component of various plant-based food materials, including grains sources such as corn, high amylose corn, waxy starch, wheat, rice, millet sources, tubers, and roots including potato, cassava, yam, and sweet potato, legumes (soybean, green pea, navy bean, lentil bean, cowpea, and groundnut) (Waterschoot et al. 2015; Pérez & Bertoft, 2010; Zhong et al., 2022). In the United States, maize (corn) is the primary source of starch for industrial applications, accounting for about 80% of total starch production. Corn is an abundant and versatile crop well-suited to produce high-quality starch, making it a popular choice for manufacturers. The USA is one of the largest producers and exporters of corn in the world, and the demand for corn-based starch continues to grow due to its widespread use in various industries, including food and beverage, paper and packaging, and chemical and pharmaceutical industries. Additionally, demand for corn starch is driven by the growing use of gluten-free ingredients in the food and beverage industry due to the rising incidence of gluten intolerance and celiac disease.

Cassava is a major source of tuber starch and is primarily produced in Southeast Asia and Brazil. Cassava is a drought-tolerant crop that can be grown in various soil types, making it an important food crop for subsistence farmers in many parts of the world. Cassava is also a valuable source of starch for industrial applications, and the demand for cassava-based starch continues to grow in regions such as Southeast Asia, Africa, and South America. In addition to cassava, other tuber crops such as sweet potato and yam are also important starch sources in some regions, particularly in Africa and South America. Most of the starch production in Europe comes from cereal grains, with maize (corn) being the largest source, followed by wheat. According to the European Starch Industry, maize is the largest source of starch in Europe, accounting for 47% of the total production, followed by wheat (39%) and potato (14%) (Schwartz et al., 2009; Mitchell, 2009). Potato is also an important starch source in Europe, although to a lesser extent. The European Union uses around 8.6 million tons of starch and its derivatives, mostly in food (51%) and non-food uses such as paper making (47%).

The global starch market was estimated at 119.6 million metric tons in 2020 and is projected to reach 160.3 million metric tons by 2026, with a compound annual growth rate of 5%. Additionally, the global starch market was valued at $97.85 billion in 2020 and is projected to grow at a compound annual growth rate of 7% from 2020 to 2028. This indicates a positive outlook for the industry and suggests that demand for industrial starch is expected to increase in the coming years. The growth of the food and beverage industry, as well as the increasing use of industrial starch in various industries, are likely to be key drivers of this growth. The U.S. starch market is estimated at 33.4 million metric tons in 2021, accounting for 27% of the global market.

Starch production is a significant industry worldwide, with millions of tons produced yearly. The technology and methods used for starch production have greatly advanced in recent years, making the process more efficient and cost-effective. Overall, the starch market is expected to grow in the coming years due to the increasing demand for food, biofuels, and industrial applications. The increasing demand for natural and organic ingredients, the growing demand for biofuels, and the growing demand for convenience foods will likely drive the market's growth.

1.3 Need for Starch Modification

Native starch mainly consists of two components, amylose, and amylopectin, which contribute to its structural and functional properties and make it useful as a thickening agent, additive, and gelling agent. However, native starch has certain limitations, such as poor solubility and swelling properties and the tendency of amylose to retrograde and form solid and tough gels, while amylopectin forms weak, soft gels. Moreover, native starches have also been identified as lacking applications during processing under different pH, temperature, and shear conditions. Starches are modified by physical, chemical, and enzymatic methods to overcome these limitations. Physical modifications involve the application of temperature, pressure, and shear conditions to alter the particle size and enhance properties such as solubility and swelling. Chemical modifications involve using chemicals, involvement of certain functional groups, etherification, and esterification to obtain new derivatives and desired qualities such as digestibility and nutritional value. This method is an efficient and inexpensive way to develop starch products with desirable properties (Ashogbon & Akintayo, 2014; Zhong et al., 2022). In addition, enzymatic modifications involve using enzymes to overcome the limitations of native starches. While all three methods can be effective, physical modifications are preferred for consumer health and safety. Unmodified starches have also been identified as lacking in their applications during processing under different pH, temperature, and shear conditions, as well as being resistant to enzymatic hydrolysis. Therefore, starches must be modified to increase their functionality using physical, chemical, and enzymatic methods or combinations thereof. Studies of starch structure and composition have shown that modifications can improve starch properties for various applications (Khunae et al., 2007; Watcharatewinkul et al., 2010; Vieira & Sarmento, 2008).

Modified starches are excellent flavor carriers, meaning they can enhance the taste and aroma of food products. This makes them a popular ingredient in ready-to-drink mixes, where they can help improve the beverage's overall flavor profile. In addition, modified starches can add a slight amount of viscosity to a drink, which can help to create a smooth and silky texture. In the baking industry, modified starches can help improve the texture and mouthfeel and enhance the stability and shelf life of products such as pasta, soup, and mayonnaise to improve their quality. In recent decades, modified starches have also been beneficial for emulsifying end-use products, particularly those containing flavored oils. They can be used as the particle in Pickering emulsion to stabilize the oil droplets, making them a popular ingredient in the food, personal care, and pharmaceutical industries. Additionally, the properties of modified starch, such as water binding, flocculation, adhesiveness, pH stability, acidic stability, thickening, shear stability, and film-forming abilities, make it popular in various applications.

Among the different modified starch, the most popular are pregelatinized, cross-linked, and resistant starch. Pregelatinized starch is widely used in instant and convenience foods due to its quick solubility and dispersibility. Crosslinked starch is commonly used in frozen and refrigerated foods due to its heat, acid, and shear resistance. Resistant starch is gaining popularity as a dietary fiber ingredient due to its potential health benefits, such as improved digestion, glycemic control, and the gut microbiome. Therefore, due to the increased demand for processed foods, the demand for modified starch is expected to grow, especially in regions like the US, Canada, Japan, China, and Europe. The global market for modified starch is estimated to be worth \$12.1 billion in 2023 and is expected to grow to \$19.8 billion by 2033, with an average annual growth rate of 5%. The modified starch market is currently dominated by North America, with a share of 42%. However, China and India are the fastest-growing markets due to the increasing demand for convenience foods and the expanding middle-class population. Conclusively, the increasing demand for convenience and processed foods, the growing awareness about the functional benefits of modified starch, the rising population and urbanization, and the expanding application scope of modified starch in various industries will drive the global modified starch market in the next decades.

1.4 Industrial Utilization and Future Trends

Starch is a versatile and widely used ingredient in various industries, including food and non-food. Its properties as a natural polymer make it an excellent additive, stabilizing agent, and thickener in the food industry. Starch is also used in non-food industries such as paper, textile, pharmaceuticals, and cosmetics, where its properties as a binder, adhesive, and filler are useful in product development applications. Most commercially available starch is chemically modified or altered using cross-linking or oxidation processes to enhance its functional properties, improving its utilization in food and non-food industries (Abbas et al., 2010; Sharma et al., 2015; Verma & Srivastav, 2022). Starch from various sources such as wheat, corn, potato, and tapioca are gluten-free starches, which increases their value for people with gluten allergies, as shown in Table 1.1 (Horstmann et al., 2016).

Table 1.1 Industrial utilization of starch from major starch-source

Starch-source	Utilization/function	Reference
Corn	Corn syrup production, thickeners in baby products, increase fiber content in cake, gravies, puddings, sauces, soups, etc.	Guadarrama-Lezama et al. (2016); Li et al. (2015); Sitohang et al. (2015)
Potato	Film-making properties, pasting, gelling applications,	da Rosa Zavareze et al. (2012); Pongjaruvat et al. (2014)
Rice	Freeze-thaw stability and retrogradation application in frozen foods, Flavor encapsulation: volatile aromatic compounds such as ethyl hexanoate, limonene and β-ionones Bakery foods, paper industry, emulsion stabilizer, textile industry	Sun et al. (2017); Shrivastava et al. (2018); Márquez-Gómez et al. (2018); Mun & Shin (2018); Villanueva et al. (2019); Yang et al. (2017)
Wheat	Cookies production, gelling properties etc.	Majzoobi et al. (2016)
Tapioca	Thickeners in soup production, gluten-free products	Li et al. (2013); Wongsagonsup et al. (2014); Agudelo et al. (2014)
Sweet potato, chestnut, quinoa, and underutilized starches	Emulsion stabilizer, bread production, starch industry, gel stabilizer, corn-starch substitute, and many other applications.	Cruz et al. (2013); Tsatsaragkou et al. (2014); Spada et al. (2017)

In the USA, the food industry consumed 57% of total starch consumption in 2020, followed by paper (26%), textile (7%), and pharmaceutical industry (4%). The consumption pattern of starch in various industries is similar in many parts of the world, although the exact distribution may vary by region and country. In addition, the food industry (sweeteners, starches and flours, and other ingredients such as hydrocolloids and emulsifiers) in the European Union accounts for around 60% of the total starch consumption, followed by the paper and board industry (25%) and industrial applications (15%) such as textiles, adhesives, and bioplastics.

The future trends of starch consumption and utilization are likely to be influenced by various factors such as technological advancements, changing consumer preferences, and sustainability concerns. As consumers become more health-conscious and demand transparency in food labeling, there may be a shift towards natural and minimally processed starches in food products. Additionally, as the trend toward plant-based diets continues to grow, there may be an increased demand for starches derived from alternative sources such as pulses, legumes, and tubers. Ongoing research into the starch modification and functionalization may also enable starch application in new areas of various industries. However, the most interesting development in the near future could be the use of starch-based biodegradable materials and packaging due to growing concerns over environmental sustainability and the need to reduce waste.

1.5 Conclusions

Starch is an essential component in our daily lives and is widely used in various industries. However, native starch has certain limitations, modifying an essential step in starch processing. The global production and consumption of starch have continued to increase in recent years due to the growth of the population and the rising demand for processed foods and industrial applications. The increasing demand for convenience foods and the expanding middle-class population in developing countries like China and India will allow the starch market to grow and innovate. Therefore, research and development become crucial to meet the industrial expectations of starch and to develop new and improved products that meet the changing demands of consumers and industries. The upcoming chapters will provide great knowledge about the current developments in starch modification.

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Chapter 2
Modification of Starch

Babatunde Olawoye, Olusola Samuel Jolayemi, Temitope Yemisi Akinyemi, Morgan Nwaogu, Timilehin David Oluwajuyitan, Oyekemi Olabisi Popoola-Akinola, Oladapo Fisoye Fagbohun, and Charles Taiwo Akanbi

Abbreviation

ANN	Annealing
CHPTAC	Cationic monomers 3-chloro-2-hydroxypropyltrimethyl ammonium chloride
DS	Degree of substitution
ECH	Epichlorohydrin
EO	Ethylene oxide
EPTAC	3-epoxypropyltrimethylammonium chloride
GRAS	Generally Regarded as Safe
GTAC	Glycidyltrimethylammonium chloride
H_2O_2	Hydrogen peroxide
H_2O_2	Inorganic peroxides
HES	Hydroxyethyl starch
HMT	Heat moisture treatment
HP	High pressure
HPS	Hydroxypropyl starch
NaCIO	Sodium hypochlorite
NaClO	Sodium hypochlorite

B. Olawoye (✉) · O. O. Popoola-Akinola
Department of Food Science and Technology, First Technical University Ibadan,
Ibadan, Nigeria

O. S. Jolayemi · T. Y. Akinyemi · M. Nwaogu · T. D. Oluwajuyitan
Department of Food Science and Technology, Federal University of Technology,
Akure, Nigeria

O. F. Fagbohun
Department of Biology, Wilmington College, Wilmington, Ohio, USA

C. T. Akanbi
Department of Food Science and Technology, Obafemi Awolowo University Ile-Ife,
Ile Ife, Nigeria

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$NaIO_4$	Periodate
$NaIO_4$	Sodium periodate
OSA	Octenyl succinyl anhydride
RE	Reaction efficiency
RS	Resistant starch
SDS	Slowly digestible starch
STMP	Sodium trimetaphosphate
STPP	Sodium tripolyphosphate
SV	Setback viscosity
XRD	X-ray diffraction

2.1 Introduction

Starch is one of the most abundant naturally occurring biopolymers obtained through different botanical sources, including cassava (tapioca), potato, wheat, rice, maize, etc. (Masina et al., 2017). It is a storage polysaccharide composed of two α-glucan polymers encrypted in its structure – amylose and amylopectin that jointly accounts for 98–99% of its net weight (Haroon et al., 2016). Structurally, amylose is majorly linear, with one end of its monomeric units linked together by a comparatively more hydrolysable α-1-4 glycosidic linkage as compared to a more rigid, compact α-1-6 glycosidic bond in amylopectin (Fig. 2.1). Amylose makes up to 20–25% of starch molecules and amylopectin is about 70–80% in most natural plant-based starches (Pratiwi et al., 2018).

Starch global popularity as a functional food and food ingredient is due to several competitive advantages over other biopolymers. These include cheap and renewable sources, low cost of production, biodegradability, eco-friendliness, malleable chemical composition, and suitability in various food and industrial applications (Wang & Copeland, 2015; Maniglia et al., 2020). The relevance of starch in food systems is both nutritional and technological, which depend on the nature and chemical compositions of the starch. Nutritionally, starch accounts for most carbohydrate components of foods, serving as the indispensable energy content of human diets (Wang et al., 2021). Technologically, the capacity of starch to form aqueous dispersions of diverse rheological properties makes it suitable as a thickener, emulsifier, stabilizer, binder, surfactant, adsorbent, adhesive, and biodegradable plastic (Lee & Chang, 2019; Otache et al., 2021; Kim & Jung, 2022).

As an essential food ingredient, starch is commonly utilized in food industries as a bulking, thickening, gelling, and water retention agent. However, starch in its native form has restricted industrial and domestic applications due to its undesirable tendency to undergo retrogradation quickly, poor solubility at low temperatures, high resistance to hydrolytic enzymes, and low thermal stability (Haroon et al., 2016). Native starch also presents other technical problems such as low mechanical resistance and high-water vapor permeability when used as biofilms (Martins & Martins, 2021; Fonseca et al., 2015). Numerous disadvantages of native starch limiting its use necessitate adopting safe and sustainable modification techniques.

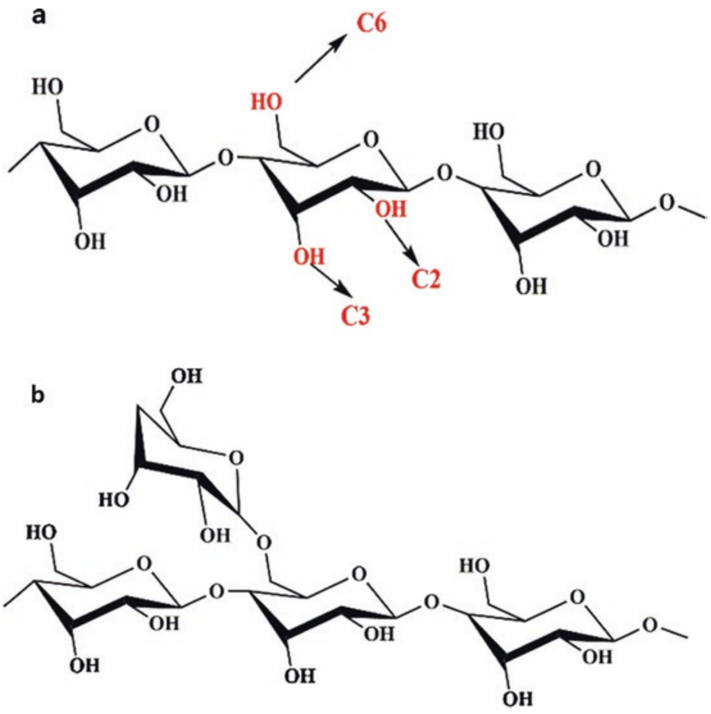

Fig. 2.1 Amylose (**a**) and amylopectin (**b**) molecules. (Adapted from Wang et al., 2020a)

Structural alterations of starch molecules through different chemical, physical, enzymatic, or genetic modulations as a stand-alone method or in conjunction with each other have been proposed with remarkable outcomes. Studies have shown that modifying starch improves its functional properties, thus making it suitable for a wide range of industrial applications. Moreover, due to the availability of modified starch, producers of starch-based products in the food and non-food sectors can meet the market demand for new products with specific functionalities (Banura et al., 2017; Punia, 2019). Hence, this chapter focuses on advances in modification, technologies and applications of starch.

2.2 Starch Modification

2.2.1 Chemical Modification of Starch

Within the last decade, literature has been inundated with studies on various forms of chemical modification of starch, such as oxidation, hydrolysis, esterification, etherification, cationization, phosphorylation (cross-linking) and modification with octenyl succinyl anhydride (OSA) all made possible by the number of reactive hydroxyl functional groups on starch structural backbone, making it amenable to many oxidoreduction process (Chen et al., 2018). This section will attempt to summarize these processes with emphasis on their recent advances.

2.2.2 Oxidation of Starch

There are many well-known methods of starch oxidation which include, wet, semi-dry, and dry oxidation based on the amount of effluent generated and overall environmental impacts of the procedure (Tolvanen et al., 2009). All these procedures rely mainly on the number of primary hydroxyl groups embedded within the anhydroglucose repeating units of the starch molecule which are available for oxidative transformation into carboxyl and carbonyl derivatives under well-controlled reaction conditions (Zhang et al., 2012b; Chen et al., 2015a). This transformation eliminates the inherent defects of native starch and confers significant improvement on the techno-functional properties on the oxidized starch. For instance, lower solubility, pasting temperature, gelatinization, and retrogradation enthalpies were observed in ozone and sodium hypochlorite hydrolyzed oxidized sago, potato and waxy corn starches (Sumardiono et al., 2021; Zhou et al., 2016; Sandhu et al., 2008).

However, during oxidation, the conversion of hydroxyl groups present at C-2, C-3 and C-6 into their corresponding carboxyl or aldehydes and carbonyl derivatives may initiate depolymerization thus compromising structural integrity of the starch (Tolvanen et al., 2009). Therefore, the effectiveness and or the degree of starch oxidation procedure are measured by the resultant carboxyl, carbonyl derivative formation as well as extent of structural degradation (Chen et al. 2015b). Some of the rate determining factors influencing the efficiency of starch oxidation includes the botanical origin of the starch, the intended properties of the starch, potency of the oxidizing agents and presence or absence of catalyst (Sangseethong et al., 2010; Zhang et al. 2012a). Inorganic peroxides (H_2O_2), sodium hypochlorite and periodate (NaClO, $NaIO_4$), ozone, oxygen/air, nitrogenous compounds (nitric oxide HNO_3, dinitrogen oxide N_2O_4), metallic oxides are among the commonly used oxidizing agents in starch oxidation (Lewicka et al., 2015). Other forms of oxidations with the use of non-conventional oxygen sources such as photo-oxidation, electrolytic oxidation, and combustion reaction, have been found promising (Castanha et al., 2017;El-Sheikh et al., 2010). Despite the environmental concerns due to potential toxic effluents produced during the application of some of these oxidants, hydrogen peroxide, sodium hypochlorite and sodium periodate constitute the most applied reactants in starch oxidation.

2.2.2.1 Hydrogen Peroxide (H_2O_2) Oxidation of Starch

Hydrogen peroxide is the most widely applied oxidizing agents among the non-toxic waste generating. Other benefits of hydrogen peroxide in starch oxidation include low cost, high content of active oxygen and significantly high oxidation potential (Zhang et al., 2012b; Lewicka et al., 2015). The interaction of hydrogen peroxide with other functional groups of electrophilic behavior, is relatively low as it loses it oxidizing potentials. This can be eliminated using metallic catalyst (Karić et al., 2020). However, these metal-mediated oxidized starch is not suitable for foods, cosmetic and pharmaceutical products due to heavy metals contamination. Ability of the oxidized starch to form a complex with these heavy metals have made the process less desirable for personal care products. During hydroperoxide oxidation, starch depolymerization is not uncommon. In a semi batch starch oxidation procedure conducted by Tolvanen et al. (2009) using a constantly fed H_2O_2 with iron complex (iron tetrasulfophthalocyanine, FePcS) as a catalyst, there was a rapid decomposition of H_2O_2 at pH 10 and over 30% solid starch was loss due to depolymerization. Similarly, due to depletion of hydrogen bonds, the resultant starch lost its crystallinity and birefringence while enhancing it thermoplastic and hydrophobic properties (Prachayawarakorn & Kansanthia, 2022; Zhang et al., 2012b).

2.2.2.2 Sodium Hypochlorite (NaCIO) and Periodate ($NaIO_4$) Oxidation of Starch

Similar to hydrogen peroxide oxidation of starch, this process increases the carboxyl (COOH) and carbonyl (CO) groups by oxidation of primary hydroxy group at a rate proportional to concentration of active chlorine (Kuakpetoon & Wang, 2008). However, in the study of Fonseca et al. (2015) the difference in potato starch treated with 1 and 1.5 g/100 g active chlorine was not statistically significant. In the same vein, hypochlorite modified starch exhibited improved properties such as starch granule structure, pasting characteristics, swelling power, solubility, and thermal properties in comparison to the native starch. Spier et al. (2013) and Kuakpetoon and Wang (2008) reported appearance of pores and holes on the surface of oxidized starch. These morphological differences in oxidized starch granules varied among different native starch. Cassava, potato, and jicama starch granules were not affected by oxidation (Jawad et al., 2013). The reduced viscosity, retrogradation, swelling and high pasting temperature of hypochlorite-oxidized starch has been attributed to depolymerization of starch polysaccharides especially amylose (Sandhu et al., 2008). Hence, oxidized starch is characterized by higher thermal stability, resistance to mechanical and enzymatic breakdown. This oxidant also decreased starch digestibility as slowly digestible (SDS) and resistant starch (RS) contents increased during oxidation (Zhou et al., 2016).

In regards to sodium periodate, the treatment resulted into formation of aldehyde group and cleavage of C2 and C3 carbons bond to form dialdehyde starch (Fig. 2.2)

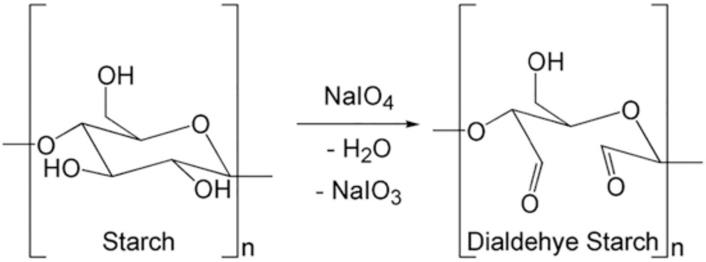

Fig. 2.2 Sodium periodation of starch by $NaIO_4$. (Adapted from Kariuki et al., 2022)

which has been found useful in food and paper industries, as well as a basic ingredient in the manufacture of biodegradable plastics and films (Ziegler-Borowska et al., 2018). Dialdehyde starch is converted to dicarboxylic starch when it is oxidized. After gelatinization, the products are oxidized to get more desirable qualities (reduced viscosity and better water solubility) (Zhang et al., 2010). Both intramolecular and intermolecular hydrogen bonds are severed during gelatinization, making oxidation easier. The influence of sodium periodate concentration on physicochemical parameters of di-aldehyde cassava starch regarding its aldehyde and carboxyl contents, solubility, swelling power, thermal properties, pasting properties, relative crystallinity, and molecular weight distribution were studied by Wongsagon et al. (2005). They reported that the aldehyde and carboxyl content increased linearly with periodate concentration, relative crystallinity reduced and X-ray diffraction remained intact. It was also observed that, like hypochlorite, sodium periodate raised the starch pasting and gelatinization temperatures, peak viscosity as well as the breakdown viscosity.

2.2.2.3 Ozonation of Starch

Ozone is a potent and eco-friendly industrial disinfectant with a GRAS (Generally Regarded as Safe) status. It is a powerful and environmentally harmless oxidant when compared to other oxidizers (Maniglia et al., 2020). Its application as starch and hydrocolloids oxidizing agent have gained prominence in recent years. One of the byproducts of ozone breakdown is hydroxyl radical. This radical is capable of absorbing hydrogen, transfer electrons or reacts with oxygen radical (Satmalawati et al., 2020). Many researchers have indicated that ozone as an oxidant is a greener option to various chemical oxidants for starch (Hui et al., 2009; Klein et al., 2014; Sandhu et al., 2012).The production of hydrogen ions, electron transfer, and interaction with ozone radicals are all part of the starch ozonation pathway. The carbonyl, carboxyl, reducing sugar levels, pH, apparent amylose content, and molecular size, increased as the ozonation period increased, indicating a molecular level transformation of starch (Castanha et al., 2017). Reyes et al. (2021) reported substantial changes in crystallinity and irregularities in granule shape after starch ozonation. The ozonized starch had similar pasting qualities to sodium hypochlorite treated starch and no hazardous residues were observed (Chan et al., 2011). Other desirable attributes demonstrating the efficacy of ozonation on starch includes enhanced pasting qualities, gel texture and paste clarity (Lima et al., 2020; Castanha et al., 2017). Industrially, ozonation of starch produced gel of better printability at a much wider gelatinization temperature range (Maniglia et al., 2019).

2.2.3 Hydrolysis of Starch

Starch co-exists with other macromolecules in its natural matrices and the degree of purity of starch vary based on the isolation technology. In the food industry, two general chemical methods of starch isolation are acid and alkaline hydrolysis (Fig. 2.3) (Egharevba, 2019). Effect of acid or alkaline hydrolysis on starch structural alterations and how these changes influence its behavior during processing and application have been elucidated in several studies (Oladele et al., 2020; Falade & Ayetigbo, 2021). Acid hydrolysis of starch involves treatment of starch granules with one or more dilute alcoholic or aqueous solution of mineral acids such as hydrochloric, sulphuric, or nitric acids at a temperature below which loss of crystallinity of its granules occurs (Wang & Copeland, 2015). Most starches have a two-stage hydrolysis pattern, with a fast initial rate and a slower rate after that. The quick initial rate is assumed to be due to the hydrolysis of the amorphous portion of starch granules, whereas the slow rate corresponds to a simultaneous hydrolysis of the amorphous and crystalline portion. The loose packing of starch chains in amorphous parts of starch granules is hypothesized to make them more vulnerable to acid hydrolysis than the crystalline regions (Wang, Blazek, et al., 2012a).

Alkaline treatment of starch can be achieved using hot aqueous alkaline solutions of sodium, potassium or calcium hydroxides to produce starch of higher reducing value (Egharevba, 2020). Dextrin or maltodextrin, maltose, and glucose are all results of starch hydrolysis. Reaction conditions (temperature, moisture, pH, and duration), amount/strength of hydrolyzing reagents, presence or absence of catalyst, and degree of polymerization determine the final composition of the resulting hydrolyzed starch. For instance, a single hydrolysis phase was found to be sufficient for waxy maize starch chains, while high amylose maize and sago starch required double phase hydrolysis (Li & Hu, 2021). Hydrolytic techniques have been employed to create starch and starch derivatives with improved functional properties

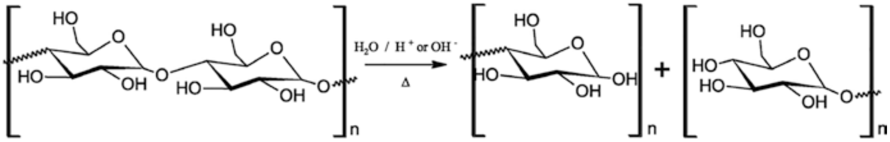

Fig. 2.3 Hydrolysis of starch molecule with acid. (Adapted from Egharevba, 2019)

and specific applications. In the work of Martins et al. (2018) on acid hydrolytic modification of rice and potato starches, swelling power, solubility, and amylose content of starches were all reduced because of the modifications. Biodegradable films produced from acid hydrolyzed rice starch were preferred to the unmodified ones with regards to tensile strength, solubility, and crystallinity (Martins & Martins, 2021). Rice and potato starches exhibited reduced retrogradation, lower structural crystallinity and higher gelatinization temperature after hydrolysis. Similarly, hydrochloric acid treated cassava starch showed a better emulsion-stabilizing properties than its native form (Fonseca-Florido et al., 2018). Over the years, thermo-alkali treatment known as nixtamalization has been utilized to stimulate distinct techno-functional properties on starch. According to Palacios-Fonseca et al. (2013), amylose/amylopectin, lipid, and protein content, as well as the granule shape and size of the final product, were all affected by the acid and alkali hydrolysis that is very similar to nixtamalization. Combined effects of hydrolysis and other methods of modification have produced novel starch with broader synergistic enhanced physicochemical properties (Sumardiono et al., 2017).

2.2.4 *Esterification of Starch of Starch*

Esterification reaction is the substitution of a nucleophilic acyl molecule with a compound that has an acid chloride, acid anhydride, or carboxylic acid structure under the influence of an acid catalyst (Fig. 2.4) (Kim & Jung, 2022). The hydrogen bonding capacity of amylose or amylopectin is decreased as the hydroxyl group of glucose is esterified by this chemical process. This structural manipulation in starch can reduce its moisture reactivity, thus increasing its stability (Tian et al., 2018). Furthermore, by esterifying the hydroxyl group of the reactive glucose to produce fatty acid starch esters, the native morphology of starch is modulated for increased hydrophobicity and thermoplasticity. In addition to that, features like flexibility, elasticity, solubility, unique rheological properties, impact, chemical resistance, and capacity to prevent crosslinking at high temperatures (Adak & Banerjee, 2016;

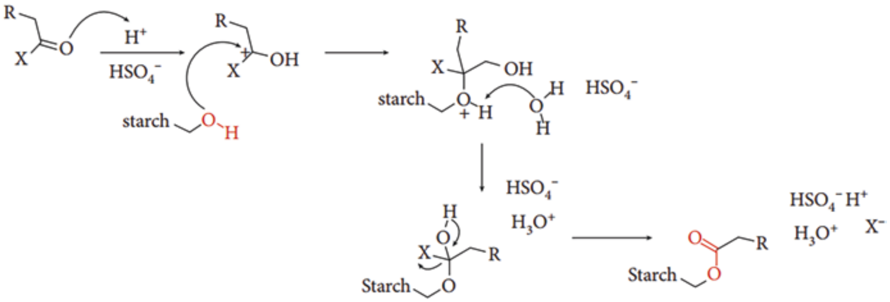

Fig. 2.4 Fischer's esterification of starch. (Adapted from Kim & Jung, 2022)

Konieczny & Loos, 2018; Sumardiono et al., 2019) also gets enhanced. Industrial utilization of the esterified starch includes it being used as coatings, binders, adhesives and medication delivery systems (Adak & Banerjee, 2016).

Research have suggested that fatty acid starch esters can be made by reacting 80 percent amylose-containing starch with some vinyl carbonates derivatives such as vinyl laurate or vinyl stearate (Winkler et al., 2013, 2014). Effective starch esterification has been done with the use of folic acid to produce starch-folic acid derivatives with a potential utilization as a colloidal delivery system (Borah et al., 2019).

Extruded double esterified sorghum starch using phosphorylation and acetylation provided a superior microcapsulation for bioactive compounds than other wall materials during storage (García-Gurrola et al., 2019). Citrate-esterified thermostable debranched starch with higher resistance to digestive enzymes have also been reported (Liu et al., 2020c). Other recent advancement in starch esterification includes the use of phosphates (Rożnowski et al., 2021; Zhang et al., 2020b), maleic anhydride (Roy Goswami et al., 2020), octenyl succinic anhydride (Lopez-Silva et al., 2021; Hao et al., 2019), acetylation and propionation (Konieczny & Loos, 2018).

Like other chemical methods of starch modification, the degree of substitution (DS) is a significant factor that influences the end use of the starch. There are three levels of substitutions, high, medium, and low (Otache et al., 2021). Low and minimally substituted starch is an essential ingredient in food sector as a thickening or stabilizer. However, when the degree of substitution is high, the formulated starch is more desirable for thermoplastic and biodegradable materials (Zhang et al., 2019a). Regarding DS, esterification can take place on the entire starch chain to generate traditional starch esters, or it can take place on the outer surface of the starch molecule, leaving the underlying crystalline structure intact, thus resulting in starch of novel physicochemical properties (Otache et al., 2021).

2.2.5 Etherification of Starch of Starch

Etherification of starch is accomplished by replacing the hydroxyl groups on dehydroglucose monomeric units of the starch structure with ether linkages (St-OR) via nucleophilic substitution reaction between an alkyl halide and alkoxide according to Alexander Williamson in 1850 (Fig. 2.5). Non-ionic or alkyl ethers, anionic, cationic, and amphoteric, starch etherification processes are the four main categories of etherification reactions (Akinterinwa et al., 2020; Haroon et al., 2016). Sodium hydroxide (NaOH) is widely employed as an alkaline initiator (Fig. 2.6) to activate the chemical substitutions by producing nucleophilic (St-O-) sites at C2, C3 and C6 O-H bonds through deprotonation (H^+ abstraction) (Masina et al., 2017; Tian et al., 2018). The starch etherification reaction includes hydroxypropylation, carboxymethylation, hydroxyethylation, and certain substitution reactions using monomeric reagents like acrylic acid and acrylamide (Hebeish et al., 2013).

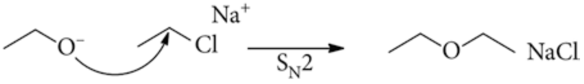

Fig. 2.5 Alexander Williamson's Etherification mechanism. (Adapted from Kim & Jung, 2022)

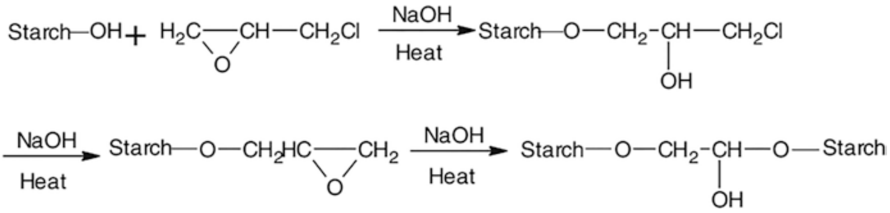

Fig. 2.6 Alexander Williamson's Etherification mechanism. (Adapted from Zhang et al., 2012a, b)

Generally, oxygen-containing ethers are more polar than alkyl types because oxygen is more electronegative than carbon. Hence, etherification of starch by converting its hydroxyl group into ether structure enhances starch-water interaction thereby increasing its solubility and overall applicability. Solvents capable of generating hydrogen bonds, such as water or ethanol, as well as polar solvents like dimethylformamide or dimethyl sulfoxide have been utilized as reaction solvents in this process (Sharma et al., 2021). Utilization of starch ethers in a variety of foods and industrial products as edible coatings, flocculants, medicine delivery, additives, papermaking, and water–oil emulsions, are not uncommon (Akinterinwa et al., 2020).

Carboxymethylation of starch is another straightforward, quick, and stepwise process whereby primary and secondary alcohol groups of the polysaccharide is etherified with carboxymethyl groups (Chakka & Zhou, 2020) based on the Williamson's reaction. The initial alkoxide groups are formed from the deprotonation of starch hydroxyl groups by sodium hydroxide and subsequent nucleophilic substitution reaction between the alkyl halide (monochloroacetic acid) and alkoxide to form carboxymethyl-starch. Duan et al. (2019) and Yang et al. (2011) studied carboxymethylation of different polysaccharides using isopropanol as the organic reaction medium with appropriate degree of carboxymethylation. CMS (carboxymethyl starches) are utilized in a variety of applications. Their use as organic adsorbent is predicated on increased swelling capacity and the introduction of carbonyl and carboxylate groups with potential capacity to chelate heavy metal. Wang, Song, et al. (2012b) formulated carboxymethyl modified corn starch with a significant chelating affinity for heavy metal ions such as Ni^{2+}, Cd^{2+}, Cu^{2+},Pb^{2+}, and Zn^{2+} at appropriate pH and degree of substitution. In addition to heavy metal scavenging capacity, CMS exhibited increased in swelling power and water solubility (Bakouri & Guemra, 2019). The degree of substitution (DS) increased with increase in ratio of monochloroacetic acid to anhydroglucose unit, while DS of carboxymethylated phytoglycogen was higher than that of carboxymethylated waxy starch under the same modification conditions. Based on the DS, carboxymethyl modification resulted in a substantial increase in resistance and slowly digestible starch contents of the resultant starch (Liu, Lu, et al., 2020b). Other innovative application of CMS is in controlled drug delivery system (Pooresmaeil & Namazi, 2021).

In the case of hydroxypropylation and hydroxyethylation of starch molecules, the starch hydroxyl groups are substituted by hydroxyethyl or hydroxypropyl groups, respectively. During hydroxypropyl (HPS) and hydroxyethyl (HES) starches production, ethylene, and propyleneoxides with NaOH (initiator) are commonly utilized, but other methods have also been effective. In comparison to CMS, HPS and HES have increased swellability and solubility (Masina et al., 2017; Fu et al., 2019). Hydroxypropylated starches found application widely in food industries because they lessen the tendency of starch to retrograde while retaining the starch's capacity to offer body, bulk, and other functions to food product. The degree of retrogradation of gel cake produced from hydroxypropylated starch reduced substantially and hydroxypropylated waxy rice starch was more successful in retarding retrogradation than hydroxypropylated waxy corn starch (Han et al., 2020). During hardness ($CaCO_3$) removal from water, hydroxypropyl sago starch performed similarly with other modified starches (Racho & Namseethan, 2017). However, hydroxyethylated starch is a non-ionic starch ether produced from substitution reaction of starch with ethylene oxide (EO) in a non-homogenous alkaline medium at mild temperature (30–40 °C). HES-EO has a superior rheological and film properties thus making it a suitable thickening agent in textile industries. HES produced from ethylene carbonate (EC) exhibited higher degree of substitution and a well uniformly distributed hydroxyethyl groups throughout the starch chain compared to HES-EO (Zhong et al., 2020). Generally, HES is of less application in food industry due to health concerns (Hepworth-Warren, 2021).

2.2.6 Cationization of Starch

As earlier mentioned in etherification process, cationic starches are produced by the chemical interaction between starch and reagents containing amino, imino, ammonium, sulphonium, or phosphonium groups (Pal et al., 2005). In this process, the cationic monomers 3-chloro-2-hydroxypropyltrimethyl ammonium chloride (CHPTAC) or 3-epoxypropyltrimethylammonium chloride (EPTAC) are transformed into 2-epoxypropyltrimethylammonium chloride (EPTAC) which is the true reactive species, in an alkaline medium (Fig. 2.7). To generate cationized-starch, the reagent's (EPTAC) chlorine atom performs an intramolecular nucleophilic substitution with the starch free hydroxyl groups under wet, dry, or intermediate process conditions (Ayoub et al., 2004). Under a dry condition, the reagent is sprayed over the dry starch and the mixture is extruded to produce dry-cationized starch while in the semi-dry approach, reagent is sprayed onto the starch and the

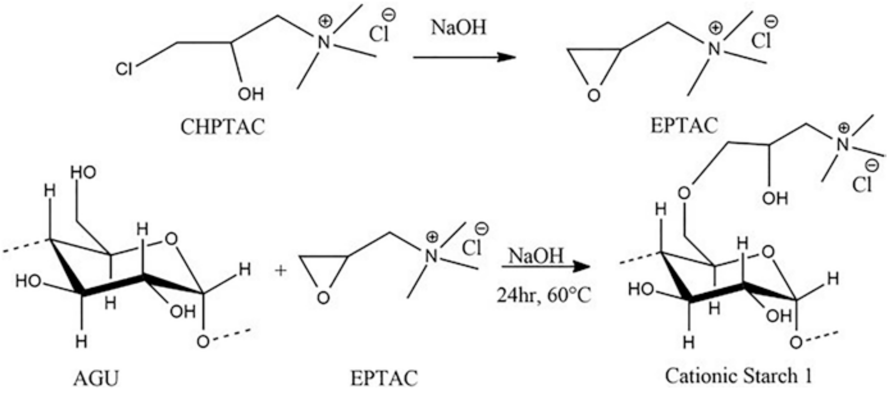

Fig. 2.7 Starch cationization mechanism. (Adapted from Sharma et al., 2020)

mixes are thermally treated. Both strategies reduced the amount of effluent generated and thus prevent environmental pollution.

Pi-xin et al. (2009) using corn starch, cationic reagent (EPTAC), and dioxane–THF–water (dioxane–THF–water) medium found that the modified corn starch granules shape and crystallinity were damaged, and these were attributed to cationic penetration of the starch granules and the alkaline reaction medium. Lappalainen et al. (2015) achieved high DS (0.30–0.35) values of potato peel waste when cationized with glycidyltrimethylammonium chloride (GTAC). A substitution value as high as 1.14 was achieved from EPTAC cationized enzymatically-debranched corn starch, in an alkaline medium and due to its morphological integrity, the cationized starch exhibited high nanoparticles encapsulation efficiency (Liu et al., 2019). In a mild condition employed by Nasir et al. (2020), an optimized cationized sago starch of high solubility and degree of substitution (1.195) was produced. As stated by Pi-xin et al. (2009), there is an irrecoverable loss of starch crystallinity during cationization which has been attributed to adverse effect of solvent on starch structural integrity. Recently, a more eco-friendly solvent-free method developed using betaine hydrochloride (BHC) derived from green sources, as the cationic reagent resulted into a modified starch of comparable DS, higher solubility, swelling capacity and decreased viscosity (Sharma et al., 2021). Other applications of cationized starch involve development of nano-biocomposites of optimum tensile strength, water vapor permeability and rheological properties (Wang et al., 2018). Efficient biomass flocculants, and selective adsorbent of high turbidity-reducing capacity were developed from cationized starch after modulating its DS (El-Naggar et al., 2018; Kumar et al., 2019). Those that are suitable for paper production must essentially be of low DS as described in these reviews (Chemelli et al., 2020; Sharma et al., 2020).

2.2.7 Cross-Linking of Starch

A cross-link is a bond or a short string of bonds that connects two polymer chains. These linkages might be covalent bonds or ionic bonds, and the polymers can be synthetic or natural (such as starch). In starch, cross-linking agents such as sodium trimetaphosphate (STMP), sodium tripolyphosphate (STPP), epichlorohydrin (ECH), and phosphorus oxychloride chloride have been widely used to modify native starch (Haq et al., 2019). Figure 2.8 depicts the reaction mechanisms of these cross-linking reagents. Adipic–acetic mixed anhydride and a combination of STMP and STPP are two other cross-linking agents in starch system. Application of ECH in food system has been so far discouraged in developed countries due to the health implication of 3-monochloropropane-1,2-diol (α-chlorohydrins) which has been found carcinogenic (Cho et al., 2008). There are many parameters that influence the efficiency of starch cross-linking process which include botanical origin of the starch, cross-linking reagent concentration and composition, level of substitution, pH, reaction time, and temperature (Ashogbon & Akintayo, 2014). Cross-linking reagents dictate the morphological characteristics, pasting and functional properties of cross-linked starch. For instance, in the work of Kim et al. (2017), STMP and STPP treated amorphous granular potato exhibited varying physicochemical properties. Therefore, based on these reagents, cross-linked starch is classified into three categories, the first is a mono-starch phosphate, which is formed by esterification of starch with ortho-phosphoric acid, sodium or potassium ortho-phosphate, or STPP. The second is di-starch phosphate which is formed when a native starch is treated with STMP or $POCl_3$. The final form of cross-linked starch is phosphated di-starch phosphate, which is the outcome of combining mono- and di-starch phosphate treatments (Gunaratne & Corke, 2007; Jyothi et al., 2006).

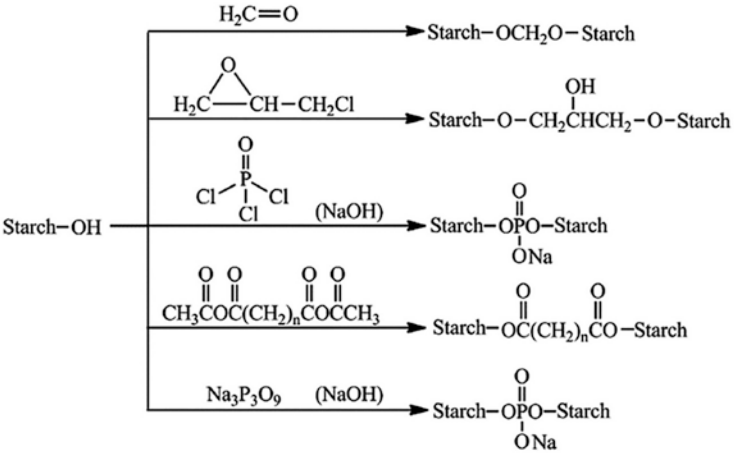

Fig. 2.8 Starch cross-linking reactions with some reagents. (Adapted from Chen et al., 2015a)

In the past decades, there are many studies that characterized the functional, pasting and physical properties differences between cross-linked starch and their corresponding native form using one or more of these categories. In the work of Koo et al. (2010) cross-linking corn starch with STMP and STPP at ratio 99:1 (w/w) substantially decreased the swelling factor, solubility, and paste clarity of corn starch. When comparing cross-linked starch of varying origin to their native counterpart, reduced water solubility, poorer paste clarity and lower swelling power were observed (Gunaratne & Corke, 2007; Mirmoghtadaie et al., 2009). Cross-linking generally improves the binding between starch chains, resulting in a product that is less prone to swelling. Within the microstructure of the starch, Koo et al. (2010) observed no difference in crystallinity between the cross-linked and the native corn starch. This confirmed the widely held belief that cross-linking occurs mostly in the amorphous portions of starch granules and has no effect on starch crystalline patterns.

In the recent work of Feng and Wen (2017) cross-linked starch-xynthate produced from co-polymerized acrylamide and sodium acrylate, in the presence of initiators (potassium persulfate and sodium hydrogen sulphite) and a cross-linker (N-N methyl bisacrylamide), showed no change in crystallinity. This further buttressed the claim that starch cross-linking occurred at the amorphous area and has no effect on crystallinity. The cross-linked starch increased the proportion of resistant starch (RS) and should be used as an alternative nutritious fiber in the food sector (Siroha & Sandhu, 2018). Biduski et al. (2018) established the significance of processing conditions such as gelatinization and retrogradation method on the properties of starch hydrogels produced from both STMP cross-linked and native rice starch. Heat induced gelatinized hydrogels produced from STMP cross-linked rice starch showed low final viscosity and breakdown rapidly in water, while alkaline-induced gelatinization resulted in a well-set hydrogel. A starch-based nanocomposite film/coating formulated from adipic acid cross-linked cassava starch, was found effective in extending the shelf life of pears (Dai et al., 2019). The films presented comparatively better mechanical and water vapor barrier properties than those from native starch. Optimized edible film of low coefficients of oxygen and water permeability and high resistance to microbial growth and lipid oxidation was formulated from cross-linked maize starch and grape juice by Yildirim-Yalcin et al. (2021). A similar observation was recorded by Dhull et al. (2021) in his comparative assessment of active starch edible films produced from STMP cross-linked and native pearl millet starch. Cross-linked starch films had higher thickness, opacity, thermal and tensile strength, with smooth surface, while maintaining lower moisture, solubility, and vapor permeability.

2.2.8 Modification with Octenyl Succinic Anhydride

Octenyl succinylated starch (OS-starch) is a hydrocolloid with amphiphilic qualities that has been esterified with octenyl succinic anhydride (OSA) to introduce a partly hydrophobic character (Altuna et al., 2018; Bhosale & Singhal, 2006). The reaction of a polysaccharide (such as starch, dextrins and cellulose) with a substituted cyclic dicarboxylic acid anhydride as shown in Fig. 2.9 produced modified starch with emulsifying capacity via three methods. The aqueous method, which consists of a reaction in an aqueous slurry under a mild alkaline condition (pH range 7–11), the dry method, which consists of a reaction between the acid and the previously alkali-treated starch without the use of water, and the organic suspension method, which uses an organic liquid instead of water as the dispersion medium, were all described (Altuna et al., 2018). The most common method for synthesizing OS-starch now involves floating the starch in its granular form in distilled water, adding OSA dropwise while stirring and keeping the pH around 8 with NaOH. The reaction is normally carried out at a mild temperature (25–35 °C) and it is continued until the slurry's pH stabilizes, or it can be stopped by adding HCl to lower the pH. This starch esterification route using OSA has been thoroughly explored, and reaction conditions have been adjusted to improve reaction efficiency (RE) and degree of substitutions (DS) which are otherwise usually low (Bello-Flores et al., 2014; Sharma et al., 2016; Simsek et al., 2015; Wang et al., 2016; Zhang et al., 2017; Zheng et al., 2017), compared to the desired functional, physicochemical properties and end application of the modified starch.

Hence, much effort has been expended in recent years to improve the traditional method of synthesis of OS-starch, with the primary goals of increasing DS and RE values, reducing reaction time, changing the distribution of ester groups, and/or producing OS-starch with enhanced or specific properties (Wang et al., 2016; Agama-Acevedo & Bello-Perez, 2017). There are divided opinions on distribution of OSA within the starch granules and within amylopectin and amylose molecules. In waxy corn starch, where the amorphous portions reside in the branching sites of the amylopectin, OSA groups were reported to be evenly distributed in the interior, amorphous regions of amylopectin and on granule surface (Shogren et al., 2000), while another study claimed the opposite (Bai et al., 2014). A better distribution of

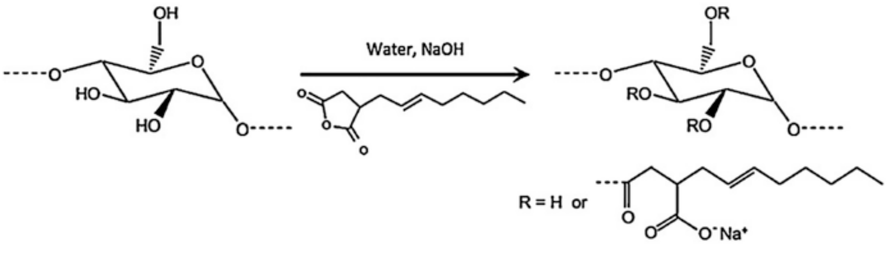

Fig. 2.9 Mechanism of OSA modified starch. (Adapted from Sweedman et al., 2013)

the OS groups within the granules is thought to give superior emulsifying capabilities, hence some contributors have concentrated their goals on reaching the inner regions of the starch granules with the substitution (Chen et al., 2014), while some have suggested mechanical, ultrasonic, hydrothermal, chemical, and enzymatic pretreatments for better outputs (Zhang et al., 2019b; Jiranuntakul et al., 2014; Huang et al., 2010). Recently, atmospheric pressure plasma jet pretreatment, exhibited positive influence of OSA modification of cassava starch, by inducing more OS interaction with the starch internal granules, reducing reaction time, improving DS and contributing more hydrophilic groups to the starch structure (Ji et al., 2022).

Only starch modified with octenyl succinic anhydride is approved for food uses among all dicarboxylic acid anhydride modifications, with 3% maximum allowable level of OSA (Xu et al., 2012). The food and industrial applications OSA-modified starch are predicated on the hydrophobic and hydrophilic groups in its starch alkenyl ester linkages (Hui et al., 2009). The octenyl succinate is connected to the starch via an ester bond, causing OSA-modified starch to form strong films at the oil-water interface, resulting in coalescence-resistant "Pickering" emulsions (Bhosale & Singhal, 2006). Complete separation of oil and water phases is prevented because the glucose moiety or residual of starch binds to water while the lipophilic, octenyl part binds to oil, which has contributed to the widespread use of OSA-modified starch in food additives, clouding agents, salad dressings, creams, fragrances, emulsion paints, lattice coatings, and additives. OSA modification raises the resistant starch fraction of the starch which could be harnessed as dietary fiber in functional food formulation (Xu et al., 2012). OS-starch is used extensively in the food sector as an emulsifier, encapsulating agent, and fat substitute.

OSA modified starches often display reduced gelatinization temperature and enthalpy, poorer digestibility, higher swelling power, and increased paste viscosity and paste clarity as a result of the additional hydrophobic groups introduced and accompanying changes in the granule structure (Altuna et al., 2018). A recent study shows OSA-starch could serve as an excellent protective carrier to active substances and controlled release of this active substance was the fastest among other matrices such as gum Arabic, water soluble soy polysaccharides and gelatin (Łupina et al., 2020). Prior to substance encapsulation both the wall materials and the bioactive (nucleus) must be dispersed in water and then spray dried. In the case of OSA-starch as an encapsulant, when the nucleus materials is lipophilic in nature, encapsulation is easier due to hydrophobic interaction (Romero-Hernandez et al., 2021).

2.3 Physical Modification of Starch

Physical modifications of starches include those treatments that use non-chemical methods such as moisture, heat, shear or radiation to alter starch properties. It has gained wider acceptance due to the absence of chemical deposits in the modified starch (Zia-ud-Din et al., 2015; Banura et al., 2017). Therefore, starches subjected to physical modification treatments are considered safe and eco-friendly (Punia, 2019). Physically modified starches are produced using diverse combinations of temperature and moisture, irradiation, shear pressure, and mechanical attrition. Generally, physical modification techniques induce changes in the molecular arrangements of the starch polysaccharide within granules, and such structural changes yield notable alterations in the properties and functionalities of the starch (Punia, 2019). Physical methods for starch modification can broadly grouped into two categories (Punia, 2019):

1. Thermal modification: Example- pre-gelatinization, annealing and heat-moisture treatment
2. Non-thermal modification: Example- high-pressure processing, pulsed electric field, ultrasonication, micronization etc.

2.3.1 Heat Treatment

Pre-gelatinization also referred to as heat treatment, is a process that involves heating starch in the presence of water until it forms a gel, followed by drying. Different methods used in this process are extrusion, spray drying and drum drying, (Noman et al., 2016; Punia, 2019). The starch is heated and dried under conditions allowing either a slight or no re-association of its molecules. The characteristics of pre-gelatinized starch include; completely degraded granular structure and absence of optical birefringence (Punia, 2019). Upon pre-gelatinization, starch is imparted with other core functionalities such as higher swelling ability, improved solubility and cold water dispersion (Noman et al., 2016). The cooking conditions, starch source, and drying influence the degree of these alterations.

Regarding drum drying, starches are either pre-gelatinized in one or two-step process. For the one-step process, the cooking of starch until it gels and dehydration of its paste is carried out simultaneously by filling the drums with starch slurry. In contrast, the slurry is first cooked using a heat exchanger and then dehydrated by drum drying in the two-step process. Currently, spray drying is considered the most utilized method for the microencapsulation of ingredients. Notably, it has good drying and emulsifying properties, high solubility, non-hygroscopic behaviour, and is affordable (Punia, 2019). The extrusion cooking process results in the physical and chemical transformation of starch. A combination of elevated temperature and pressure causes the starch to gelatinize, disrupting intermolecular bonds, resulting in rupture of the grains and increased water absorption. The degree of change in starch depends on properly selected process parameters and the residence time of raw material in the extruder. This modification helps to create desired functionalities in the starches, including the extent of gel formation and viscosity of the gel (Moscicki et al., 2013).

The improved pasting ability and solubility of pre-gelatinized starches in cold water make it suitable for use as thickeners in products, such as soups, baby food, and desserts (Noman et al., 2016). Extruded starch is also an important ingredient in the production of ready-to-eat products, as a gelling agent, structure stabilizer and water- or fat-absorbent fillers (Moscicki et al., 2013).

2.3.2 Hydrothermal Treatment

Heat moisture treatment (HMT) is one of the methods used for physical modification of native starch and it has been reported to improve various functional properties of starch and widen their industrial application. Hydrothermal treatment modifies the physico-chemical properties of starch without destroying their granular structure. Hydrothermal modification occurs due to the transition in starch polymers from the amorphous to the semicrystalline region. HMT involves treatment of starch granules at moisture levels lower than 35% (w/w) for 15 min to 16 h and at a temperature range that is below gelatinization temperature but higher than the glass transition temperature, usually in the range of 84–120 °C (Pepe et al., 2015; Punia, 2019; Goel et al., 2020).

Hydrothermal modification can only take place when the starch polymers in the amorphous phase are in the mobile rubbery state. A few minute is sufficient to bring about detectable changes in the physicochemical properties of the starch. As hydrothermal treatment occur below the gelatinization temperature of starch granules, the granular structure of starch is preserved and starch remains in mobile rubbery state during modification (Noman et al., 2016; Punia, 2019).

HMT causes the rearrangement of amylose and amylopectin chains in the starch, and therefore may modify its X-ray pattern, crystallinity, swelling power, amylose leaching, pasting, and gelatinization properties, as well as its susceptibility to enzymatic or acidic hydrolysis, which also affect the rheological properties starch (Pepe et al., 2015).

Pepe et al. (2015) investigated the effect of heat-moisture treatment on structural, physicochemical, and rheological characteristics of arrowroot starch. The starches were conditioned to a moisture content of 28% and heated at 100 °C for 2, 4, 8 and 16 hrs respectively. Their work revealed that modified starches had higher pasting temperature and lower peak viscosity while breakdown viscosity practically disappeared, independently of the treatment time. Gelatinization temperature and crystallinity were also found to increase, while enthalpy, swelling power and solubility decreased with the treatment. Gels from modified starches, independently of the stress conditions, were found to have more stable apparent viscosities than gels from native starch. Furthermore, heat-moisture treatment caused a reorganization of starch chains that increased molecular interactions. This increase resulted in higher paste stability and strengthened gels that showed higher resistance to shearing and heat, even after acid or sterilization conditions. It is therefore noted that treatment time of 4 h was enough to deeply change the physicochemical properties of starch.

2.3.3 Annealing

Annealing (ANN) which is another hydrothermal process, consists of holding starch granules in an excess of water (generally >39% w/w) at a temperature that is above the starch's glass transition temperature and below its gelatinization temperature (BeMiller, 2018). ANN increases the crystallinity, improves mechanical properties and heat stability whereas weakens the structural relaxation. Annealing is done to increase mobility of starch molecules and to prevent the beginning of gelatinization process by approaching glass transition temperature (Tg) in the presence of water or glycerol like solvents (Punia, 2019).

Literatures have reported that annealing is restricted unless the moisture content exceeded 60% by weight of the mixture. Annealing decreases swelling power and solubility of starch, delays gelatinization, increases susceptibility to amylase, and changes pasting curves (Siswoyo & Morita, 2010). Siswoyo and Morita (2010), subjected breadfruit starch to annealing at 45–60 °C for 24 h and found out that annealing retarded the retrogradation of breadfruit starch and also increased the resistance of the starch to enzymatic hydrolysis.

2.3.4 High Pressure Treatment

High pressure (HP) is a non-thermal processing technology for food preservation that inactivates microorganisms related to foodborne diseases with minimal effects on food organoleptic and nutritional properties. Since HP is a green and environmental-friendly technology and can alter non-covalent chemical linkages with minimal effects on covalent linkages, it can be used to modify starch to produce desired properties (Castro et al., 2020).

Punia (2019), reported that when heating a starch suspension at pressure higher than 200 MPa, gelatinization takes place at a lower temperature range than when heating at ambient pressure. Furthermore, if the pressure is high enough, gelatinization can occur even at room temperature. This treatment destroys the noncovalent bonds which resulted into serious structural damages such as protein naturalization and starch gelatinization.

It is reported that high pressure of 400 and 600 MPa, increased the resistant starch content of lentils (Ahmed et al., 2016). On increasing the pressure, both swelling, and solubility of lotus seed starch granules increased in the range of 55–75 °C when compared to the native samples. This increment of swelling and solubility at lower temperatures at higher pressures may be due to amylose aggregation under pressure, which interferes with the lipid-starch bounds. While at higher temperatures and pressure, swelling and solubility of starch decreases and this can be attributed to amylose molecular rearrangement. Swelling is mainly caused by amylopectin. Because starch granules are often intact or partially destroyed after HP processing, amylose solubilization is limited. This may be due to the stabilization of the amylopectin by the remaining amylose, which prevents some crystalline structures from melting. It is noted that this swelling and solubility reduction at a higher temperature and higher pressure can be due to granular compression and strengthening of the starch molecular bounds (Castro et al., 2020).

Furthermore, high pressure processing reduces the texture, colour and moisture content of treated starches but had no significant effect on the protein, and fat contents of treated samples (Castro et al., 2020).

2.3.5 Ultrasonic Treatment

Ultrasound treatment (the application of sound waves at or above a frequency range of 15–20 kHz) is a effective non-thermal physical starch modification method. In contrast to chemical methods, ultra-sonic treatment is considered to be environmentally friendly as it reduces usage of chemicals, waste production and energy consumption (Sujka, 2017; Punia, 2019).

Ultrasound treatment influences starch pores, which can also affect the texture of the food material. Sujka (2017), subjected rice, corn, wheat, and potato starch granules suspended in water or ethanol to ultrasound treatment at frequency of 20 kHz and power 170 W for 30 min. Measurement of porosity in terms of specific surface area revealed that ultrasound treatment, increased the porosity of starches. This study showed that ultrasound treatment formed new pores in potato starch and this has been demonstrated to improve frying quality of potato as the oil absorbed decreased in ultrasonic pretreated potato compared to untreated samples (Zhang et al., 2021). Rahaman et al. (2021), used High power ultrasound (at 40 kHz voltage), to modify corn and cassava starch. Their research showed a slight loss in starch crystallinity, a hollow-like indentation on the granules' structure, and no apparent change in granule size, all of which suggest that starch can be modified to have a variety of beneficial effects in the food industry.

2.3.6 Deep Freezing and Thawing

Freezing technology, as a means of storage, is one of the most extensive methods, which can effectively retard the development of deterioration caused by chemical, microbial and enzymatic reactions in food. However, the frozen products might undergo a long period of frozen time from semi-finished goods, production, storage and transportation, distribution, selling to final consumption, which will expose them to series of temperature fluctuations, going through the repeated freeze-thaw process (Liu et al., 2020a).

Freeze-thaw treatment has the capacity to change the granule morphology of starch, increasing the porosity, and damaging starch granules. The porosity of potato starch granules is reported to increase more than 4 times after 10 freeze-thaw cycles (Wang et al., 2020a).

As a major component of frozen products, the changes in structures of starch play an important role in products' quality. Literature reveals that the phase change of the water squeezed the internal structure of the starch granules after freeze-thaw cycle, which increased the starch channel, the roughness of starch surface and weakening of the crystal structure. It has also been reported that the deep-freezing process (in liquid nitrogen) resulted in an increased crystallinity of granules, but it had a minor effect on the granule aqueous solubility and characteristics of gelation. Oven-dried potato starch was quickly blended with water (water/starch 1.5:1 v/v) and frozen in liquid nitrogen, the samples were allowed to equilibrate for about 30 mins to room temperature after nitrogen evaporation with no more than five freeze-thaw treatment. The surface showed more roughness and oblong scratches after the freezing/thawing cycle (Szymońska et al., 2003). Studies have also revealed that the structural and functional properties of non-gelatinized waxy rice starch after 1, 3, 7, and 10 freezing/thawing cycles in −20 °C resulted in an increased damaged appearance in the starch granules and amylopectin leaching of waxy rice starches (Tao et al., 2015). Recent studies have also revealed that the structural and physicochemical properties changed with the number of pores on the surface of starch granules increased under −20 °C freezing and 25 °C thawing within 20 cycles (Liu et al., 2020a).

Liu et al. (2020a, c), studied the effect of repeated freeze-thaw cycles treatment on the structural and physicochemical properties of maize starch. The study was carried out under repeated freeze-thaw treatment with 0, 3, 6, 12, 18 and 24 times at −20 °C, −40 °C and −80 °C, respectively. The result of the study showed the peak, trough, final and setback viscosities, pasting temperature, water holding capacity, transparency and hydrolysis rate of samples significantly ($P < .05$) increased, while the breakdown viscosity decreased with the increase of freeze-thaw numbers. They concluded that increasing freezing temperatures from −80 to −20 °C, had no significant effect on the structure and the integrity of the maize starch. So, it can be conclusively said that repeated freezing and thawing could be used to modify starch functionalities to allow for increased applicability in food systems.

2.4 Radiation Treatment

Irradiation as a method of treating food products is a physical treatment that involves exposing them directly to electromagnetic rays to enhance their quality, safety and extend their shelf-life. It does not result in a noticeable increase in temperature, requires no laborious sample preparation and is time saving (Punia et al., 2020; Sunder et al., 2022). The utilization of the irradiation technique for starch modification has recently begun to draw much attention due to its safety, cost-effectiveness and environmentally friendly attribute. It has also proven to be more advantageous than other methods of treating starch regarding its ability to retain and enhance nutrients and the quality of the products after application (Sudheesh et al., 2019). Previous concerns have been about the possible deposition of radioactive particles on irradiated products. However, several researchers have rebutted this misconception; irradiated foods pose no threat to human health at different doses (Farkas & Mohácsi-Farkas, 2011; Sudheesh et al., 2019). Although several irradiation methods are employed in the food industry and other sectors, there is a booming interest in applying microwave and gamma irradiation to alter the starch structure to obtain the desired functionality.

2.4.1 Microwave Irradiation

Microwave irradiation involves exposing foods to an electromagnetic wave of frequencies ranging between 300 MHz and 300 GHz, equivalent to the wavelength ranging between 1 mm and 1 m. It has been considered a sustainable method for modifying starch due to its numerous and unique advantages. It is cheap, starts up within a short time, heats up quickly and is highly efficient (Brasoveanu & Nemtanu, 2013; Zhang et al., 2020a). The impact of microwave treatment on starch is dependent on both the processing conditions of the microwave and starch attributes. These factors include the type of starch, moisture content, power, and exposure time (Wu, 2018). Concerning the mechanism of action of microwave radiation, Brasoveanu and Nemtanu (2013) identified four stages: dielectric relaxation of water molecules, water vaporization, high internal pressure generation, expansion of granules and granules degradation. Thus, it appears that the moisture content of the native starch is the most influential factor with regard to starch modification using microwave radiation. More so, microwave treatment only produces a low concentration of free radicals that rapidly becomes negligible in hydrated foods. Therefore, it is regarded as a safe method of starch modification.

Generally, microwave irradiation results in the repositioning of starch molecules leading to alteration in starch properties. Some of these properties which are of utmost importance to food producers are solubility and swelling ability, pasting properties, gelatinization, and starch granule structure (Brasoveanu & Nemtanu, 2013; Wu, 2018; Ma et al., 2020a; Miernik & Jakubowski, 2021). Regarding the structure of the granule starch, microwave can either influence the structure of part or the whole of the granules. Whether it induces a complete or partial structural change depends on the starch type and processing conditions. As a result of temperature increase and dehydration, the treatment leads to reordering the crystalline structure and chains. Brasoveanu and Nemtanu (2013) reported that while there was a notable decrease in the crystalline portion of wheat and normal starches, changes in waxy corn starch were negligible. It has been proven that while the swelling ability of irradiated starch is negatively correlated with the temperature, the solubility increases with increase in temperature. For example, a study by Ma et al. (2020a, b) on microwave irradiated starches from hull-less barley showed that the swelling ability decreases with the power and exposure time, but solubility was improved. Further, it revealed that the attributes of amylose and amylopectin after microwave treatment of starch are the main factors influencing the swelling capacity and solubility. These characteristics include amylose-amylopectin ratio, extent and length of branching, molecular conformation, and molecular weights of the fractions after degradation. The alteration in the swelling ability and solubility of starch may be attributed to the internal reordering of the crystalline region of the starch and reduction in the hydrogen bonds within the chains (Ma et al., 2020a, b; Zailani et al., 2021). It appears that the reduced interchain hydrogen bonds increased the hydrophilicity of the starch.

Microwave irradiation causes a noticeable alteration in the pasting properties of starch by lowering its viscosity. The viscosities are often influenced by the starch composition (amylose and moisture content), type, and irradiation dosage during irradiation. A comparative study on maize starches by Yang et al. (2017) revealed that microwave-irradiated waxy maize starches had lower viscosities than the native starches. Notably, the peak viscosity and breakdown viscosity lowered with increasing time. However, at such an extended period of irradiation, the pasting temperatures rose continuously. The report suggested that the starches may resist swelling to a great extent due to the low viscosities. The results were further supported by the low values obtained for the setback viscosity of the starch. The low setback viscosity (SV) implies that the waxy maize starch had a little resistance to shearing and thus reduced swelling ability. Supposedly, this SV-lowering effect of microwave irradiation indicates that it could be beneficial for curtailing temporary retrogradation. Therefore, it enhances the starch quality to become more desirable for making that baked products. Another study on microwave irradiated barley starches by Ma et al. (2020a, b) reported a similar trend. The starch viscosities dropped continuously as the irradiation time and power rose. The study attributed the low values of viscosities to the weak intermolecular forces of the starch. It indicated that the extent of the change induced on the starch viscosities depends on the degree of disruption of the starch granules. Shah et al. (2016) also reported similar findings for starch from Indian horse chestnuts.

For the effect of microwave treatment on the gelatinization temperature of the starches in most cases, the temperature increases after irradiation (Wu, 2018). However, the alterations vary over a wide range of initial moisture content of the starch. This variation is due to the strong positive relationship between gelatinization and the initial moisture content of the starch. Further, Wu (2018) reported that gelatinization only occurs at a moisture content equal to or greater than 30%. This report is supported by Ma et al. (2020a, b), who believes that the increase in gelatinization temperature after microwave treatment reflects the disruption of intra and intermolecular hydrogen bonding caused by high moisture content.

2.4.2 Gamma Irradiation

Several findings have shown gamma irradiation to be a highly beneficial, most preferred physical and non-thermal method for altering starch in the food industry (Dikkala & Shirisha, 2018; Bhat et al., 2022; Sunder et al., 2022). The technique involves the exposure of starchy foods to an in-depth penetration of high-energy gamma rays from a radioactive isotope. According to FDA, only cobalt-60 or cesium-137 are acceptable isotopes for gamma treatment of foods. Regarding the radiation dose, the purpose for which the modification is carried out is a determining factor. A low (less than 2 kGy), medium (2–10 kGy), higher (greater than 10 kGy) dose of gamma radiation are used to enhance the quality of food products, extend the shelf life and sterilize the foods respectively (Sunder et al., 2022). It is reported that gamma irradiation induces structural changes in starchy foods. These alterations affect several functional properties that are crucial to harnessing the potential of starchy foods for food and non-food applications (Han et al., 2020). As revealed by recent studies, starch type, moisture content, and irradiation dose are significant factors influencing the structural alterations of starch induced by gamma irradiation. For instance, Punia et al. (2020) used the scanning electron microscope to examine the morphological changes in gamma-irradiated starches from lotus seeds. The report showed that the impact of the treatment on the structure of starch granules was insignificant at a low dose (5–10 kGy) but became more pronounced as the dose rose. Gani et al. (2013) reported similar varying and a more noticeable structural change at elevated dose for gamma-irradiated starches from lotus stem. Additionally, molecular degradation has been higher in wet starches than in dried form (Zhu, 2016). Concerning crystallinity of starch granules, there are three forms; A-type, B-type, and C-type, which has recently been reviewed by Sunder et al. (2022). It was reported that starch granules of B-type showed more resistance to gamma irradiation than those of A-type and C-type, which are more sensitive (Chung & Liu, 2010; Sunder et al., 2022). Starch treatment with gamma irradiation results in hydrolytic degradation of its polymeric chain. This degradative effect of gamma treatment reduces the molecular sizes of amylose and amylopectin. However, the former seems to be less susceptible than the latter. The difference in susceptibilities may result from the smaller size of the amylose molecules (Singh et al., 2011; Zhu, 2016).

The structural changes discussed above are the leading causes of the alterations in physico-chemical properties induced by gamma irradiation. Based on most reports, under this treatment, the capacity of starch granules to retain water or swell negatively correlates with the irradiation dose. This relationship may be attributed to the differing level of fragmentation of amylopectin at low and high irradiation doses. Punia et al. (2020) observed a significant decrease in the swelling ability of irradiated locus seed starch at a high dosage. This decrease yields improved starch texture after cooking (Gani et al., 2013). When starch is irradiated with gamma rays, the number of hydrogen bonds between chains decreases, increasing polarity, and simple sugars with higher affinity for water are formed. These occurrences are responsible for irradiation-induced increasing solubility of starch. The report of Dar et al. (2018) which mentions a significant increase in the solubility of gamma-irradiated buckwheat as the dosage rises agrees with this statement. Pasting properties in terms of viscosity of starch has been observed to be dose-dependent. Its relationship with dose is similar to that of swelling capacity discussed earlier. The continuous reduction of pasting properties with rising dosage could be due to the degradative effect of gamma irradiation on amylose and amylopectin in starch (Bashir & Aggarwal, 2017). At elevated irradiation doses, the breakdown viscosity and the setback viscosity reduce due to starch paste's instability and their possibility to retrograde. The irradiation treatment brings about a significant reduction in pasting and gelatinization temperatures at higher doses. Punia et al. (2020) observed a decrease in gelatinization temperature of starch from lotus seeds from 143.21 °C to 140 °C upon irradiation. It was revealed that the reduction could be consequential to the breakage of inter and intramolecular bonds in the starch. All reports examined concerning syneresis of gelatinized irradiated starch showed that syneresis values decrease as dosage increases. The lower values for syneresis could be due to the generation of fractions with a molecular weight with a higher affinity for water.

2.5 Other Modification Techniques

2.5.1 Enzymatic Modification of Starch

Enzymatic modification of starch is a biological means of transforming native starch into a more physically, chemically, and biologically stable starch through enzyme-mediated redox process by lipases, amylases, proteases, peroxidases, or laccases. Enzymatic method has gained more popularity owing to their safety, mild conditions, and substrate specificity, all of which comply with "clean label" requirements. Depending on the intended end results, starch can be modified using enzymes alone as the modifier in the case of enzymatic hydrolysis or as a catalyst in acylation/esterification, and copolymerization (Karaki et al., 2016b).

2.5.1.1 Enzymatic Hydrolysis of Starch

Porous starches are enzymatic modified starches that are gaining popularity due to their high adsorption capacity. These starches have a lot of pores from the surface to the center of the granules, thus present large surface area and are good natural absorbents (Li et al., 2021). One of the incentives of using enzymatic hydrolysis in the production of porous starch instead of the usual physical and chemical methods, is the zero toxic effluent production. In an abundant-water system, porous starch is often made by using α-amylase at a sub-gelatinization temperature (Xu et al., 2018). In the work of Benavent-Gil and Rosell (2017) porous starch was produced from cassava using α-amylase from *Bacillus amyloliquefacie* and there was no significant difference in the starch granule pore sizes. When aided with a small amount of glucoamylase/amyloglucosidase, hydrolytic effect of α-amylase on porous starch was more substantial (Zhang et al., 2012a). Similarly, in a recent work of Zhai et al. (2022) using maltogenic α-amylase (MA) to hydrolyze wheat starch granule, at sub-gelatinization temperature, MA acted equally on both amorphous and crystalline regions of the starch. There was a linear correlation between the rate of retrogradation and degree of hydrolysis of the starch. Thus, MA-modified starch could be used to formulate starchy foods that are less prone to staling.

2.5.1.2 Enzymatic Acylation/Esterification of Starch

Acylation is an enzyme-mediated reactions between short chain fatty acids and starch molecules that leads to increase in hydrophobicity of the starch through conversion of its primary hydroxyl group into acyl groups (Karaki et al., 2016a). Cassava and maize starch were acylated using coconut-oil derived fatty acids in the presence of lipase from *Thermomyces lanuginosus*, fungal or bacterial (Rajan & Abraham, 2006; Rajan et al., 2007). The resultant modified starch exhibited improved physicochemical properties and was found suitable in the production of biodegradable plastic due to its hydrophobicity. In the pharmaceutical industry, hydrophobic starch is used for microencapsulation and controlled drug delivery systems. Enzyme-aided esterification of cassava starch with biologically (*Candida antartica* Lipase B) synthesized rosin acid, further improved starch hydrophobicity, and conferred new characteristics such as improved viscosity and emulsifying properties on the polymer (Lin et al., 2015). At the same time, the rosin acid treated starch appeared cloudy and less thermally stable than the native starch. Using an ionic liquid as a solvent, *C. rugosa* lipase was employed to synthesize long-chain fatty acid starch-esters (starch-laurate) (Song et al., 2013). During the procedure, native starch's morphological and crystalline features were largely disrupted. Starch-laurate was not as thermally stable as the native starch. In a solvent-free method, a lipase was previously employed to acylate corn starch with palmitic acid. Palmitic acid was added to starch to increase its hydrophobicity and possibly its emulsifying capacity (Xin et al., 2012).

Enzymes have long been used to change native starches and create products with different solubility, viscosity, and gelation qualities that have a wide range of applications in the food, paper, textile, and other sectors. Li et al. (2009) investigated the effects of protease N and alcalase on rice starches. Rice starches made with protease N showed higher pasting viscosities, larger elastic moduli and yield stresses than that of alcalase. However, there were no substantial variation between granule morphology, crystalline pattern, thermal properties, and average molecular weights of both enzyme-modified starches. Recently, a new approach to produce alkaline phosphatase modified starch was proposed (Wu et al., 2022) where phosphate groups were grafted onto the starch structure. This enzymatic phosphorylation produced starch containing ester and cross-linked linkages. Morphologically, the starch surface appeared rough and both crystallinity and enthalpy reduced significantly because of structural disruption.

2.5.1.3 Enzymatic Co-polymerization of Starch

Starch can be polymerized by enzymatically grafting it with another polymer to produce a co-polymer of specific functional properties. In the work of Shogren et al. (2009) horseradish peroxidase (HRP) enzyme was employed in co-polymerization of starch with acrylamide in the presence of acetate buffer and an aqueous medium containing H_2O_2 and pentanedione (Fig. 2.10). Pentanedione was oxidized to a free radical in this reaction, which subsequently took a proton from starch to produce carbonyl radicals, which act as initiators for the copolymerization of acrylic monomers. The polyacrylamide chain was covalently linked to the starch backbone, via the abstraction of glycosidic hydrogen atoms (Hollmann & Arends, 2012; Shogren et al., 2009). The resultant polyacrylamide-starch exhibited high water absorption capacity and could be employed in textile and papermaking industries.

Preparation of grafted starch-polyphenol co-polymer using *Trametes versicolor* laccase as an oxidoreductase enzyme to produce starch-polyphenolic polymer has also been reported (Shogren & Biswas, 2013). Laccase accelerated the graft co-polymerization of waxy maize starch and sodium lignosulfonate – a sulfate-containing water-soluble polyphenolic polymer that can be used to make anionic starch sulfates. Starch-sodium lignosulfonate graft co-polymer displayed good

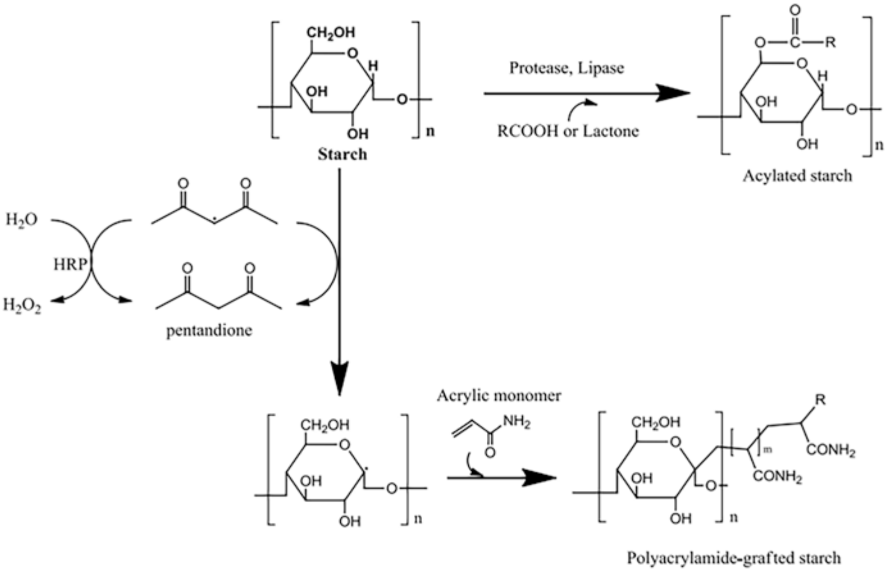

Fig. 2.10 Enzyme-catalyzed co-polymerization of starch

antioxidant activity due to the phenol moiety. The graft co-polymer could be employed in food additives, cosmetics, and packaging.

2.5.2 Fermentation of Starch

There is a growing interest in the application of flexible, adaptable, and eco-friendly, methods of starch modification in which the products are completely devoid of any residual chemical reagents and other byproducts contamination. Starch fermentation is specifically a biochemical process involving the activities of amylolytic-enzymes-producing microorganisms on starch molecules under a reaction with well-controlled conditions (Petrova et al., 2013). Literature abounds with examples of fermentation-modified starches with altered physicochemical, nutritional, and technical properties (Díaz et al., 2018; Jorge et al., 2018; Ye et al., 2019; Zhao et al., 2019). Fermented rice flour had lower breakdown and setback pasting properties than non-fermented rice flour (Yuan et al., 2008). Gels prepared from fermented rice flour had lower hardness and brittleness and higher cohesiveness than non-fermented rice gels (Lu et al., 2007), and the fermented rice gel was retrograded more slowly than the non-fermented rice gel. Yuan et al. (2008) discovered that starch isolated from spontaneously fermented maize had dramatically different pasting characteristics and significantly higher gel strength. Reyes et al. (2016) found that fermentation of corn starch with *Saccharomyces cerevisiae* caused structural changes in the starch granules, resulting in improved crystallinity, reduced hydrolysis susceptibility, and improved thermal properties. It was also found that by regulating the quantity of waxy rice fermentation, the digestibility may be reduced (Zhang et al., 2016).

Fermentation could be spontaneous in which amylolytic enzymes such as α-amylase, β-amylase, α-glucosidase, amyloglucosidase, amylopullulanase are produced from the lactic acid bacteria (LABs), which are the dominating microorganisms in the reaction medium, causing starch fermentation and the generation of lactic acids (Fig. 2.11). Putri et al. (2012) and Yuan et al. (2008) found that spontaneous lactic acid fermentation improved the textural qualities of maize starch noodles. According to the author, fermentation can alter the amorphous area of the starch granule and its chemical components, modifying the physical qualities of rice flour and the texture of rice noodles. Similarly, in a recent study, spontaneously fermented sweet potato starch was found to indicate no variations in granular morphology, granule size distribution, crystalline structure, or gel adhesiveness as compared to the native (Ye et al., 2019). The authors observed that spontaneous fermentation reduced the amylose content, the average molecular weight of the starch, amylopectin chain length, peak viscosity, trough viscosity, and final viscosity of the starch. Cassava starch was reported to be modified by fermentation coupled with sun-drying, resulting in a sour starch with a reduced viscosity peak, higher paste clarity, and solubility (Demiate & Kotovicz, 2011).

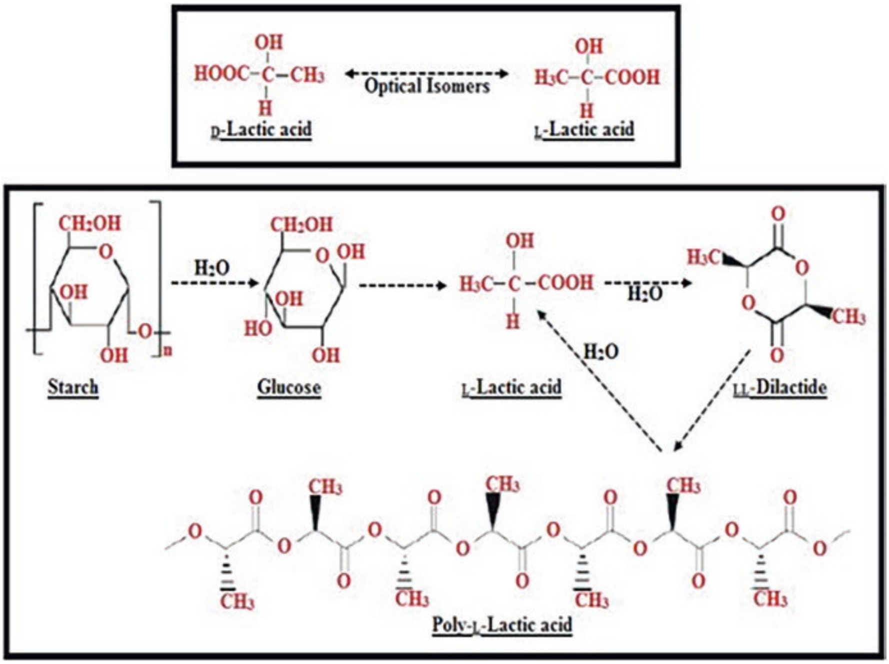

Fig. 2.11 Lactic acids production during spontaneous fermentation of starch. (Adapted from Ghaffar et al., 2014)

Back-slopping fermentation is a strategy for speeding up the fermentation process by employing a starter from an earlier fermentation with unknown microbial populations while lowering the danger of fermentation failure (Leroy & De Vuyst, 2004). In a comparative study on waxy maize starch (Teixeira et al., 2019), spontaneous fermentation produced starch of more and larger pores than back-slopping fermentation which was attributed to surface degradation. However, the thermal properties of the starch remained unaffected. A similar observation was reported on naturally fermented wheat starch by Zhao et al. (2019). According to their study, fermentation enhanced the crystalline layer and decreased the amorphous portion of the starch granules. Natural fermentation had more influences on the starch than back-slopping as it decreased peak viscosity, final viscosity, and setback values while increasing peak time and paste temperature values.

Short-term co-fermentation (yeast and lactobacillus strains) on starch digestibility and multi-scale structures were studied on rice starch (Tu et al., 2021). The starch granular surface eroded during fermentation due to degradation, resulting in decreased structural compactness, relative crystallinity, and molecular weight. The degraded starch molecular fractions increased as the number of starter cultures rose, thus facilitate starch reassembly and subsequent increase in slowly digestible starch content. Therefore, by varying the number of starter cultures used in the fermentation, digestibility, and morphology of starch could be reasonably controlled.

2.5.3 Germination of Starch

Germination is another biochemical process that occurs after uncooked seed kernel, root and tuber horticultural produce absorb water at a required interval of respiration, to activate and release endogenous enzymes for physical and chemical changes (Ma et al., 2020b). These enzymes aids in the escape of the kernel capsules and the exposure of the kernel roots from the kernel embryos. Biopolymers such as carbohydrates, proteins, lipids etc. are partially or completely hydrolyzed and degraded into their monomeric units, nutrients such as vitamins and minerals are released and antinutritional factors are reduced or eliminated during seed germination (Lee et al., 2018; Sangsukiam & Duangmal, 2017).

Unlike fermentation that can take place spontaneously or by microbial inoculation (back-slopping) on a pure isolated starch, preprocessed or whole starchy plant materials, only raw unprocessed grains or roots/tuber plant can germinate or sprout. Thus, modification of starch using germination implies the physicochemical, thermal, and functional properties differences between starches obtained from germinated and native nongerminated plant sources. The effects of germination depend on starch botanical origin and germination duration (AL-Ansi et al., 2021). In recent years, nutritional and functional benefits of germination on the starch fraction of cereals, legumes, roots, and tubers plant have been adequately explored. In the work of (Chinma et al., 2021)**,** increasing germination period up to 72 h on an underutilized legume plant (Fig. 2.12) increased the physicochemical, functional, thermal, and overall nutritional qualities of the beans while reducing its antinutritional compounds. After 6 days of germination, starch content of oats dropped by 40% (Tian et al., 2010). During germination (0–5 days) of three brown rice varieties, starch, amylose, and amylopectin levels all declined significantly (Wu et al., 2013).

Previous research has shown that germination can alter the structural and physicochemical properties of starch isolated from a variety of seeds, including brown rice, mung bean, black bean, oat, sorghum, and millet (Liu et al., 2020b; Wunthunyarat et al., 2020). Starch obtained from brown rice exhibited reduced foaming capacity and molecular weight after germination, which is a desirable attribute in bread making (Wunthunyarat et al., 2020). AL-Ansi et al. (2021) assessed the impact of germination on the molecular, physicochemical, and morphological features of highland barley starch. Similar to the observation of Chinma et al. (2021), germination for 72 hours considerably decreased total starch content while enhancing amylose concentration and gelatinization temperatures of the starch. Pasting temperatures, particle size, and swelling capacity, all reduced after germination. Furthermore, after 72 h germination, average molecular weight, relative crystallinity, gelatinization temperature ranges, and gelatinization enthalpy declined compared to others. Ma et al. (2020b) also demonstrated similar observation on germinated maize from varying cultivars. The total starch, amylose, and amylopectin concentrations, and average molecular weight all decreased significantly with germination. All the samples had A-type starch X-ray diffraction (XRD) patterns,

Fig. 2.12 Images of raw and germinated Bambara groundnut. (Adapted from Chinma et al., 2021)

and the relative crystallinity of the starch decreased over time. According to Liu et al. (2020c), the crystallinity of mung bean starch increased during the first 12 hours of germination and subsequently decreased as germination progressed up to 72 hours, which may be explained by hydrolysis of the amorphous sections of the starch granules.

Wu et al. (2013) observed substantial changes in the pasting and rheological properties of brown rice flour, whereas only minor changes were observed in isolated starch. Scanning electron micrographs of the flour revealed that the continuous matrix structure of flour was severely damaged after germination. However, there was evidence of pits and holes on some granule surfaces in isolated starch after 72 h germination. Similar granules surface modification occurred in germinated quinoa starch (Xing et al., 2021). Average molecular weight of the starch remained unaffected after germination, but the polydispersity value of germinated brown rice was higher than that of non-germinated.

Nutritionally, in conjunction with maltogenic amylase, germinated brown rice flour produced starch of high slowly digested starch (SDS) content that have been found suitable in the management of type 2 diabetic (Le Nguyen et al., 2021). Germination lowers starch digestibility, increased resistance starch (RS), slowly digested starch (SDS) content and reduces rapidly digested starch (RDS) fraction of maize starch (Ma et al., 2020b).

2.6 Conclusions

Modification of starch enhance its utilization potentials over native starch due to better retrogradation tendency, solubility, mechanical resistance, thermal stability, among other functional and rheological properties. Furthermore, modification of starch reduces limitations of native starches such as low and high-water vapor permeability when used as biofilms. Application of advance modification and technologies of starch on industrial scale of food production should be encouraged.

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Chapter 3
Oxidation of Starch

Smita Dimri, Aditi, Yograj Bist, and Sukhchran Singh

3.1 Introduction

Starch is a naturally occurring polysaccharide found in plants. Due to its compact structure, renewability, and low cost, it is used in various industries such as food products, textiles, adhesives, paper making, and glass fiber sizing. Modified starches are used in tabletting, cosmetic formulations, and biodegradable plastics. Non-digestible starch is also being explored as a new application in nutraceuticals. However, native starches have limited functionality due to retrogradation and syneresis, as well as poor processability, solubility, and thermal stability. These properties can hinder their practical use. The characteristics of starch are predominantly impacted by the proportion of amylose to amylopectin, the average chain length of these two molecules, and how amylopectin is arranged within the semi-crystalline regions of starch granules. Since natural starches do not possess optimal features for industrial use, the molecular makeup of amylose and amylopectin can be altered via physical, chemical, enzymatic, or a combination of these methods. These modifications enhance the positive attributes, reduce the defects, and add new traits to native starch, thereby increasing its functional versatility.

Oxidation is one of the most common methods of chemical modification. The process involves the oxidation of primary or secondary hydroxyl groups of the glucose units. Hydroxyl groups undergo conversion first into carbonyl groups, and subsequently into carboxyl groups. The extent of oxidative substitution is indicated by the ratio of carboxyl and carbonyl groups present in the oxidized starch.

S. Dimri · Aditi · Y. Bist (✉) · S. Singh (✉)
Department of Food Engineering and Technology, Sant Longowal Institute of Engineering and Technology, Punjab, India
e-mail: Yograjbisht16@gmail.com

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The degree of oxidative substitution is determined by the quantity of carbonyl and carboxyl groups in the oxidized starch. The location of the hydroxyl group and glycosidic bond in starch plays a significant role in determining its properties during chemical modification (Lewicka et al., 2015). These groups occur primarily at the hydroxyl groups of the C2, C3, and C6 positions that make up the starch molecule's polymeric chain (Wurzburg, 1986). When the glucose ring part of –CHOH–CHOH– is located in place of carbon C-2 and C-3 atoms, the starch contains three hydroxyl groups (triol). The glycosidic bonds present at carbon C-2 and C-3 atoms form a hemiacetal. Due to the presence of three hydroxyl groups in glucose, starch is prone to substitution reactions, increasing the number of potential modifications that can be made to it (Tomasik, 1999).

Oxidation is a process that can enhance the functionality and reactivity of native starch by introducing additional functional groups, such as carbonyl and carboxyl groups, into the modified starch. These groups are typically absent in the inert native starch, and their presence can increase the overall functionality and usefulness of the modified starch. The effectiveness of oxidation depends on the botanical origin of the starch, the type of oxidant used, and the process conditions. In addition, the oxidation reaction may loosen intermolecular bonds and partially depolymerize the polymer chains (Fortuna et al., 2002). Oxidation modification reduces starches' relative crystallinity, viscosity, swelling power, retrogradation tendency, and pasting temperature (EI-Halal et al., 2015b). The oxidation of starch is carried out in the presence of oxidizing agents like air and oxygen and the presence of catalysts such as transition metal ions. These components work together to facilitate the oxidation process. (Lewicka et al., 2015). The preferred oxidants are sodium hypochlorite, hydrogen peroxide, and sodium periodate (Tomasik & Schilling, 2004). Other oxidants include per-acetic acid (CH_3CO_3H), potassium permanganate ($KMnO_4$), chromic acid (H_2CrO_4), and nitrogen dioxide (NO_2).

The physicochemical and structural properties of oxidized starch depend on several factors, including the botanical source, molecular structure, size of amorphous lamellae, packing of crystalline lamellae, reaction conditions, and type of oxidizing agent used (Sangseethong et al., 2010). Oxidized starch is also used in the food industry as a thickening and gelling agent and stabilizer in emulsions and suspensions. Oxidized starches improve the texture and quality of baked goods, such as bread and cakes, by enhancing their volume, crumb structure, and moisture retention. In addition, it is used in the production of noodles, instant foods, and confectionery products, such as jellies and candies. The use of oxidized starch in the food industry is regulated by the food safety authorities, and it must meet specific purity and quality standards to ensure its safety for human consumption. Overall, the application of oxidized starch in various industries is expected to continue to grow, as its unique properties make it a versatile and valuable ingredient in many different products.

3.2 Modification and Mechanism

3.2.1 Oxidation with Hydrogen Peroxide

Hydrogen peroxide (H_2O_2) has the potential to act as an oxidant of organic compounds. It is environment-friendly and produces nontoxic waste products, such as water. H_2O_2 has a high content of active oxygen (47%) and a high oxidation potential (1.77 V), making it an effective and low-cost oxidant (Arts et al., 1997). However, H_2O_2 has limited reactivity towards most organic functional groups because it has an electrophilic character and behaves like a nucleophile, which lacks oxidizing properties. To eliminate these unfavorable nucleophilic characteristics, H_2O_2 is activated by the presence of metal ion catalysts such as Cu (II), Ti (III), Fe (II), Co (II), W (VI), and Fe (III). This activation allows for enhanced oxidizing properties of H_2O_2. It is important to note that H_2O_2 with metal catalysts cannot be used for starch modification for food, cosmetics, and pharmaceuticals due to the heavy metal ions present in the resulting products, which can be strongly complexed by the oxidized starch (Arts et al., 1997; Kesselmans et al., 2004).

The oxidation of starch with H_2O_2 in the presence of a metal catalyst is a very complex phenomenon, and it is hypothesized to follow either a radical or ionic mechanism. In the presence of a metal catalyst, hydrogen peroxide (H_2O_2) rapidly decomposes, resulting in the production of hydroxyl radicals (-OH). These highly reactive radicals quickly react with carbohydrates, specifically extracting hydrogen atoms from C-H groups on sugar rings, which results in the formation of new radicals (R-CHOH). During the H_2O_2 process, carbonyl functional groups are primarily formed, with some carboxyl groups also produced (Sangseethong et al., 2010). Generally, the degree of oxidation in H_2O_2 is below 2.78% (w/w). To enhance the number of functional groups in modified starches, metal ions are introduced during the H_2O_2 oxidation process. Copper, iron, and tungstate are the most effective metal catalysts (Vanier et al., 2017) (Fig. 3.1).

In a study by Zhang et al. (2012), the oxidation of corn starch was investigated using varying concentrations of a metal catalyst, $CuSO_4$. The results indicated that a concentration of 0.5% $CuSO_4$ was effective in reducing the reaction time from 72 h to only 1 h. However, using a higher catalyst concentration resulted in insufficient oxidation and undesirable color changes in the starch. Potato starch was oxidized using H_2O_2 with tetrasulfophthalocyanine (FePcS) as the catalyst. Tolvanen et al. (2009) found that pH 8.4 and pH 10 were suitable for forming carbonyl and carboxyl groups. Different concentrations of H_2O_2 were used to oxidize corn starch, resulting in a degree of oxidation ranging from 0.096 to 0.554. The oxidized corn starch had a higher content of aldehyde groups than carboxyl groups. As the degree of oxidation increased, the relative crystallinity, intrinsic viscosity, and thermal stability decreased. The water absorption intensity of oxidized starch decreased at lower levels of oxidation but improved at higher levels (Zhang et al., 2009). Cassava starch was oxidized using hydrogen peroxide (H_2O_2) and sodium hypochlorite (NaOCl) at pH 10, with reaction times of 30, 60, 120, and 300 min (Sangseethong

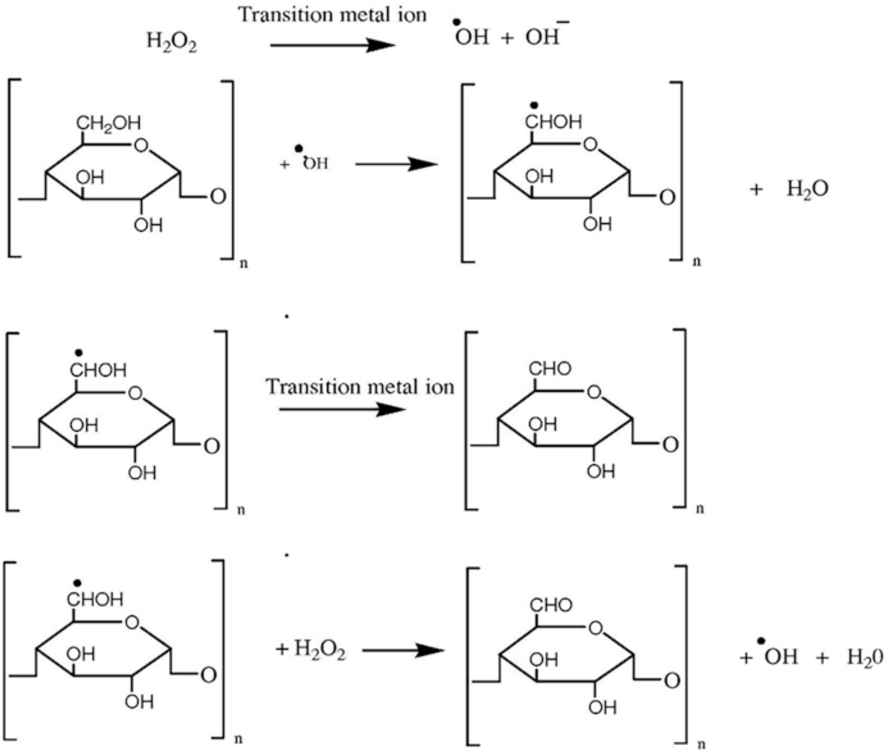

Fig. 3.1 Hydrogen peroxide oxidation of starch. (Reprinted from (Vanier et al., 2017), with permission from Elsevier)

et al., 2010). Hydrogen peroxide favored the formation of carbonyl groups, while sodium hypochlorite favored the formation of carboxyl groups. Both oxidized starches showed similar molecular distribution and viscosities, but hydrogen peroxide achieved these properties in a shorter amount of time. El-Sheikh et al. (2010) used 2.7% H_2O_2 to oxidize rice starch, with UV irradiation as the photoinitiator. They found that a starch ratio of 1:8 and temperatures of 60 and 70 °C resulted in higher carbonyl and carboxyl content.

3.2.2 Oxidation with Sodium Hypochlorite (NaOCl)

Sodium hypochlorite is widely used in the industrial production of modified oxidized starch due to its availability and ability to modify starch properties. This oxidation process takes place in a solution of gelatinized starch. Sodium hypochlorite selectively oxidizes hydroxyl groups of starch into either aldehyde or carboxyl groups. The extent of this oxidation process depends on various factors, such as reaction temperature, reaction time, pH, sodium hypochlorite concentration, molecular structure, and starch origin (Vanier et al., 2017). The oxidation process only modifies a small proportion of the glucose units and partially breaks down the polysaccharide chains ((Lewicka et al., 2015). The percentage of carbonyl and carboxyl groups present in starch affects its physicochemical properties. Starch that has undergone oxidation with sodium hypochlorite is more resistant to amylase activity, more stable at higher temperatures, and can form complexes with calcium ions while exhibiting polyelectrolyte properties.

Sodium hypochlorite-oxidized starch is utilized as a food additive under the code name E 1404. It functions primarily as a thickener for creams, puddings, sauces, and jellies, and as a stabilizer in cake fillings and ketchup production. It is also utilized as a gelation agent. Additionally, the paper industry uses oxidized starch to enhance the tear resistance of paper (Lewandowicz & Mączyński, 1990; Pietrzyk et al., 2006) (Fig. 3.2).

The oxidation of starch with sodium hypochlorite in the industry is commonly conducted at mild to moderate alkaline conditions. This is because the pH level strongly affects the reaction rate between starch and hypochlorite, with a faster reaction rate at a pH close to 7 and a slower rate at pH 10. By maintaining a mild to moderately alkaline pH, more carboxyl groups can be formed, which can inhibit starch retrogradation and stabilize the viscosity of the starch dispersion (Rutenberg & Solarek, 1984; Sangseethong et al., 2009). Additionally, Wurzburg (1986) noted that mild to moderate alkali conditions can enhance the stability of the starch dispersion. Halal et al. (2015b) discovered that when active chlorine (1–2%) was used to oxidize barley starch at pH 9.5, a greater quantity of carboxyl groups was produced than carbonyl groups. Meanwhile, Sánchez-Rivera et al. (2009) employed sodium hypochlorite to oxidize banana starch at pH 7.5 and 11.5 for durations of 1–4 h. They discovered that carboxyl group formation was influenced by the pH, with pH 11.5 producing a greater quantity of carboxyl groups. In the case of cassava starch oxidation with sodium hypochlorite by Sangseethong et al. (2009), the effects

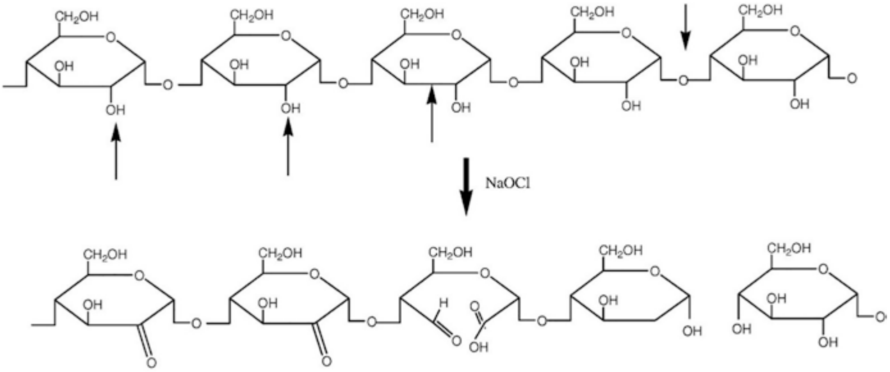

Fig. 3.2 Sodium hypochlorite oxidation of starch. (Reprinted from (Vanier et al., 2017), with permission from Elsevier)

of sodium hypochlorite were investigated at pH values of 7, 8, 10, and 11. It was discovered that pH 8 and 9 resulted in the formation of dicarboxyl groups at C2 and C3, which weakened the bonds at the C1 position. As a result, the depolymerization of starch via beta-elimination was facilitated.

3.2.3 Oxidation with Sodium Periodate ($NaIO_4$)

The oxidation of starch with sodium periodate cleaves the C2 and C3 carbon bonds, forming an aldehyde group, as shown in Fig. 3.3. This oxidation process is carried out after gelatinization, as intra- and inter-molecular hydrogen bonds are cleaved during gelatinization, making the chemical reactions more accessible to the starch, and commonly called oxidative diol-scission reaction (Veelaert et al., 1994; Zhang et al., 2009). Periodate starch exhibits unique characteristics as it produces highly reactive dialdehyde groups. It is used in various applications such as paper, textiles, drugs, proteins, and biodegradable plastics packaging (Tomasik, 2000; Zhang et al., 2007). Furthermore, periodate starch reduces viscosity and provides better water solubility. However, the industrial use of periodate is limited due to its expensive nature.

3.2.4 Oxidation with Ozone

Hydrogen peroxide and sodium hypochlorite are commonly used as chemical oxidizing agents. However, these agents generate wastewater and leave unwanted residues in food products (An & King, 2009). In contrast, ozone is a cleaner and more powerful oxidant that requires limited downstream purification processes. The end products of ozone oxidation are carbon dioxide, inorganic ions, water, and less toxic by-products (Chan et al., 2011).

Hydrogen peroxide and sodium hypochlorite are frequently utilized as oxidizing agents, but they produce wastewater and undesired residues in food products (An & King, 2009). On the other hand, ozone is a superior and more environmentally friendly oxidant that necessitates minimal post-treatment processes. Ozone oxidation results in carbon dioxide, inorganic ions, water, and fewer hazardous by-products (Chan et al., 2011).

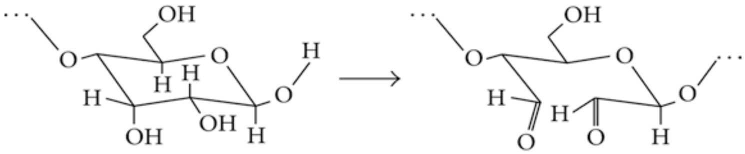

Fig. 3.3 Oxidation with Sodium periodate (Lewicka et al., 2015)

According to An and King (2009), ozone can be applied to starch either in a gaseous phase or an aqueous solution. During the ozone oxidation process, ozone (O_3) naturally changes into oxygen (O_2) quickly and does not leave any residue in the final product. The oxidation of starch with ozone can occur through two methods: direct and indirect. The direct method involves the reaction between the substrate and molecular ozone in an acidic medium. However, this method is slower and less commonly used. On the other hand, the indirect method involves carrying out the reaction in an alkaline medium, where ozone is first decomposed before reacting with the starch substrates. Hydroxyl radicals are one of the products of ozone decomposition, and they can react through three mechanisms: removal of hydrogen, electron transfer, and addition of O_3 radicals, as shown in Fig. 3.4 (De Moraes et al., 2000).

Several studies have investigated the effects of ozone-oxidized starches on different sources like rice (An & King, 2009), corn, sago, and tapioca (Chan et al., 2009), yam (Oladebeye et al., 2013), wheat (Sandhu et al., 2012), and cassava (Klein et al., 2014). The oxidation of starch using ozone depends on the starch source, the methods used for ozonization, the phase of ozone (gaseous or aqueous), reaction time, pH medium (alkaline or acidic), and ozone concentration. Ozone is a strong oxidant that affects the swelling, granule morphology, solubility, thermal

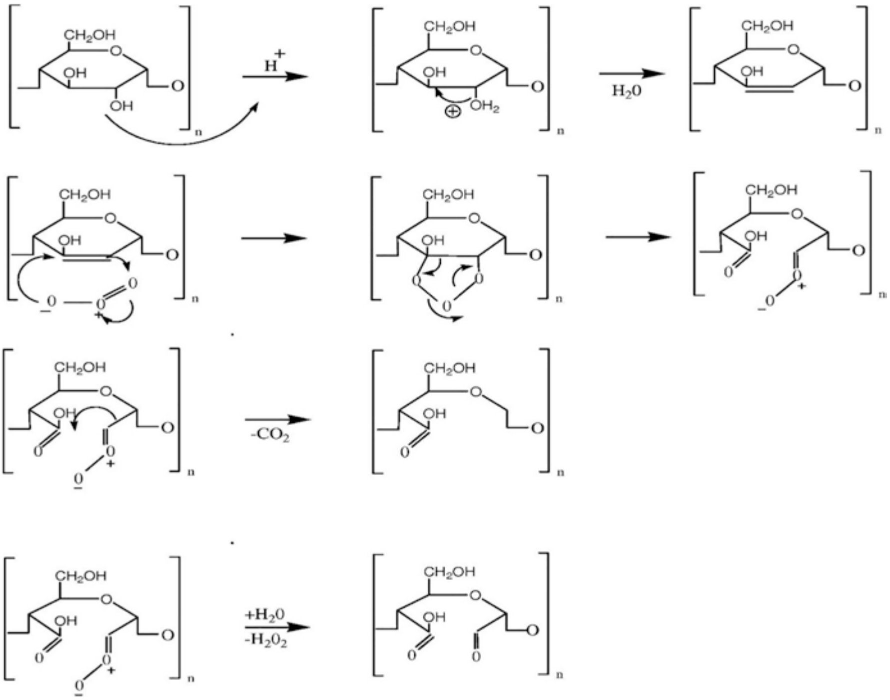

Fig. 3.4 Ozone oxidation of starch. (Reprinted from (Vanier et al., 2017), with permission from Elsevier)

profile, and functional groups of native starch (Chan et al., 2009). The use of ozone as a modification method could be considered safer for both the environment and consumers.

3.3 Effect of Oxidized Starch on Different Properties

The effect of oxidizing agents on the functional, pasting, structural, morphological, and thermal properties of oxidized starches are shown in Table 3.1.

3.3.1 Physicochemical Properties

The interaction between amorphous and crystalline regions of starch granules is indicated by the swelling power, which is typically reduced by oxidation. This is due to the disintegration of granule structures caused by oxidation (Vanier et al., 2017). Wang and Wang (2003) observed that the swelling power of both waxy and normal corn starches was reduced by sodium hypochlorite treatment. This reduction in swelling power was attributed to the hydrolysis of amylopectin chains at higher temperatures, as well as the sponge-like structure of the starch granules, which absorbed water during heating. However, during centrifugation, this sponge-like structure could not retain the water, resulting in separation. Additionally, the oxidation process could increase the interaction between amylose and amylopectin, leading to stronger intramolecular bonds and changes in the arrangement of the crystalline region of the starch. A reduction in the swelling power of oxidized starch was reported for carioca bean starch (Vanier et al., 2012), potato starch (Zhou et al., 2016), and normal and waxy corn (Sandhu et al., 2008). Zhou et al. (2016) reported an increase in resistant and slowly digestible starches, coupled with a decrease in rapidly digestible starches, was associated with a decrease in the swelling power of potato starch. However, Sangseethong et al. (2009) reported an increase in the swelling of cassava starch granules oxidized using 3% sodium hypochlorite. A similar study was reported by (Wojeicchowski et al., 2018; Olayinka et al., 2013; Sukhija et al., 2016) for carioca beans, white sorghum, and elephant foot yam. The increase in swelling power was due to hydrogen bonds within the broken starch granules, which are responsible for the formation of crystal double helices during excessive water heating, leading to granular swelling.

The leaching of amylose and small amounts of amylopectin during the swelling process determines the solubility. The increase in solubility provides a less rigid starch granules structure. Olayinka et al. (2013) reported an increase in solubility due to granules swelling which weakens the intragranular bonds and amylose in starch granules leached into the water phase. Many other authors reported a similar finding for oxidized starches like Banana starch (Sanchez-Rivera et al., 2005), corn (Chong et al., 2013), carioca bean (Vanier et al., 2012), finger millet (Afolabi et al.,

Table 3.1 Effect of different oxidizing agents on functional, pasting, structural, morphological, and thermal properties of oxidized starches

Oxidizing agent	Starch source	Process parameters	Degree of oxidation	Functional properties	Pasting properties	Structural and morphological properties	Thermal properties	Uses	Reference
Sodium hypochlorite	Banana	Oxidizer conc. (0.25–2.0%), Reaction time and temp 50 min, 35 °C, pH 9.5, catalyst active chlorine	Carbonyl (%) 0.0015–0.004 Carboxyl (%) 0.013–0.076	Water solubility (↑), Swelling power (↓)	Peak and breakdown viscosity (↑), Final viscosity (↓)	_	Gelatinization initial temp increases (↑), Gelatinization enthalpy (ΔH) (↓)	_	Sanchez-Rivera et al. (2005)
Sodium hypochlorite	Digitaria exilis	Oxidizer conc. 0.0025–0. 15, temp 30 °C, pH 10–11, catalysts bromide, cobalt, iron, manganese, and copper salts	_	Water, oil absorption emulsion, and foaming capacity increases (↑)	_	_	_	Gum arabic replacer, yarn sizing, in glass industry for coat glass fibres	Isah (2018)
Sodium hypochlorite	Corn	Active chlorine 0–2 g, temp, and time 35 °C, 15–30 min pH 9–10, ultrasound 200 W, amplitude100%	Carbonyl (%) 0.1745 Carboxyl (%) 0.3969	Swelling power (↓), Solubility (↑)	Peak and breakdown viscosity (↑), final viscosity (↓)	_	_	_	Chong et al. (2013)

(continued)

Table 3.1 (continued)

Oxidizing agent	Starch source	Process parameters	Degree of oxidation	Functional properties	Pasting properties	Structural and morphological properties	Thermal properties	Uses	Reference
Sodium hypochlorite	Carioca bean	Oxidiser conc. 0.5–1.5% (w/w), temp 35 °C, pH 9.5, time 50 min	Carbonyl (%) 0.056 Carboxyl (%) 0.057	Color improvement, solubility increased, gel hardness and swelling decreased	Decrease (↓) in pasting temp, peak viscosity, breakdown, final and setback viscosities.	C- type pattern decrease (↓) in relative crystallinity. Surface imperfections observed.	Gelatinization initial temp (T_0) decreases (↓) increase (↑) in enthalpy of gelatinization	–	Vanier et al. (2012)
Sodium hypochlorite	Finger millet	Oxidizer conc. 10%, reaction time 240 min pH 9.0–9.5	–	Swelling power and solubility increased (↑)	Decrease (↓) in pasting temperature, increase (↑) in peak, hot, breakdown, and final viscosities	Increase (↑) in crystallinity,	Increase (↑) in initial, peak, and conclusion temperature, decrease (↓) in enthalpy of gelatinization.	In powder compacts and tablet, fillers in capsule formulation	Afolabi et al. (2012)
Sodium hypochlorite	Banana	Oxidizer conc. 1%, reaction time 30–240 min, pH 7.5, 11.5 Reaction time 35 °C	Carboxyl (%) pH 7.5, (0.014–0.084), pH 11.5, (0.175–0.327)	–	Decrease (↓) in pasting viscosity,	Increase (↑) in crystallinity	Increase (↑) in initial, peak and conclusion temperature, decrease (↓) in enthalpy of gelatinization	–	Sánchez-Rivera et al. (2009)

Sodium hypochlorite	Cassava	Oxidizer conc. 3%, reaction time 30–300 min, pH 8–11 Reaction time 30 °C	% carboxyl and carbonyl was higher at pH (8–9) and lower at pH (10–11)	–	Viscosity decreases (↓) for fresh and stored paste with time and pH	–	Decrease (↓) in initial, peak temperature, and increase (↑) in conclusion temperature, also decrease (↓) in enthalpy of gelatinization	–	Sangseethong et al. (2009)
Sodium hypochlorite	Normal corn, waxy corn	Oxidizer conc. 1%, reaction time 50 min, pH 9.5, reaction time 35 °C	Normal corn (Girja), (waxy corn) Carbonyl (%) 0.032, 0.021 Carboxyl (%) 0.036,0.019	Swelling and solubility decreases (↓) in normal and waxy corn after oxidizing	Decrease (↓) in pasting temperature, peak, breakdown, and final viscosities	Size-small to large, Shape- oval to polyhedral	Decrease (↓) in initial, peak, and conclusion temperature for normal and waxy corn. Also decrease (↓) in enthalpy of gelatinization	–	Sandhu et al. (2008)

(continued)

Table 3.1 (continued)

Oxidizing agent	Starch source	Process parameters	Degree of oxidation	Functional properties	Pasting properties	Structural and morphological properties	Thermal properties	Uses	Reference
Sodium hypochlorite	Cassava	Oxidizer conc. 0.16–1.84%, reaction time 50 min, pH 2.5–7.9, reaction time 20–45 °C	Carbonyl (%) Oven and sun-dried cassava 0.044, 0.071 Carboxyl (%) Oven and sun-dried cassava 0.037, 0.065	–	Decrease (↓) in, peak, hold, and final viscosities of sun-dried fermented cassava, Gel hardness Increase (↑)	–	–	Biscuit making	Dias et al. (2011)
Sodium hypochlorite	Sword bean	Oxidizer conc. 10%, reaction time 240 min, pH 9.0–9.5	Degree of oxidation 0.08%	Water binding capacity, solubility, and swelling increases (↑) with temp for both native and oxidised starch	Peak viscosity decreases (↓) trough, final viscosity increases (↑) for oxidised starch	Native starch Type B pattern with smooth ellipsoids and indentation in Centre, C pattern with cavity at Centre	Decrease (↓) in initial, and conclusion temperature for oxidized starch. Also increases (↑) in enthalpy of gelatinization	–	Adebowale et al. (2006)

Hydrogen Peroxide	Potato	Oxidizer conc. 2%, temp 40 °C, Time 15 min, catalyst Cu II ions (0.3%)	Carbonyl (%) 1.309 Carboxyl (%) 0.221	Water binding capacity, intrinsic viscosity, decreases (↓) and solubility increases (↑)	Pasting temperature, peak, hot, paste, and cold paste viscosity reduced (↓)	–	Initial and peak temperature increases, while conclusion temperature and enthalpy of gelatinization decreases (↓)	–	Pietrzyk et al. (2012)
Hydrogen Peroxide	Corn, pea, sweet potato	10% starch conc. 80 °C, time 60 min, catalyst CuSO4 (0.5%)	40.5%,	Reduction in intrinsic viscosity (↓), transmittance increases (↑) with temperature	–	Type A pattern	No glass transition temperature was observed up to 200 °C.	Food, paper, textile, and plastics	Zhang et al. (2012)
Hydrogen Peroxide	Cassava	Oxidizer conc. 3%, temp 40 °C, time 30–300 min, pH 10, time 30 catalyst $CuSO_4$	–	Reduction in hot paste viscosity (↓)	–	Native starch round shape Oxidized starch Rough surface after 300 min	Initial, peak, conclusion temperature increase (↑) with time and enthalpy of gelatinization decreases (↓) with time	–	Sangseethong et al. (2009)

(continued)

Table 3.1 (continued)

Oxidizing agent	Starch source	Process parameters	Degree of oxidation	Functional properties	Pasting properties	Structural and morphological properties	Thermal properties	Uses	Reference
Hydrogen Peroxide	Corn	Oxidiser conc. 0.7 to 2%, Time 1440 min, pH 7, temp 25 °C	Carboxyl content 10.2–44.6%	Intrinsic viscosity Decrease (↓)	–	Crystallinity decreases (↓) and SEM images with irregular surfaces	Thermal stability decreases (↓)	–	Zhang et al. (2009)
Hydrogen Peroxide	Rice	Oxidiser conc. (0–0.4%) Time 240 min, temp 60 °C	Carboxyl content (5.76–23.0) Carbonyl content (12.46–15.05)	–	Apparent viscosity decreases (↓)	–	–	Sizing of cotton yarns	El-Sheikh et al. (2010)
Hydrogen Peroxide	Potato	Oxidiser conc. 5.3, time 60–420 min, pH 2.0–10.0 temp 40–90 °C Catalyst FePcS, WO_4^{2-}	Maximum degree of substitution of carboxyl groups at pH (10), and carbonyls at pH 8.4	–	–	Oxidized starch loses smoothness	–	–	Tolvanen et al. (2009)

Hydrogen Peroxide	Maize	pH- 8, time 15–180 min, catalyst Ferrous sulfate (0.07%)	Carboxyl content 0.039%,	Solubility Increases (↑) and Retrogradation decreases (↓)		A type pattern	Initial, peak, conclusion temperature increase (↑) and enthalpy of gelatinization decreases (↓)		Liu et al. (2014)
Hydrogen Peroxide	Potato	Oxidiser conc. 2.0, time 60 min, temp 313 °C Catalyst Cu (II)	L*decreases (↓), a* b* increases (↑)	–	–	–	–	–	Labonowska et al. (2011)
Ozone	Potato	Ozone conc. 47 mg L^{-1}, gas flow 0.5 L min^{-1}, time 15–60 min	Carboxyl and carbonyl content increases with time (↑)	Apparent amylose content decreases (↓), and paste clarity increases (↑) with time	Peak, trough, and final viscosity decreases (↓) And pasting temperature increases (↑)	–	Irregular shape with rough surface	–	Castanha et al. (2017)
Ozone	Potato	Ozone conc. 47 mg L^{-1}, gas flow 0.5 L min^{-1}, time 15–60 min, pH 5.5,	Carboxyl and carbonyl content increases with time (↑)	Water holding capacity increases (↑) gel firmness increases (↑), High apparent viscosity (↑)	–	–	–	Viscous pastes or higher water retention products	Castanha et al. (2019)

(continued)

Table 3.1 (continued)

Oxidizing agent	Starch source	Process parameters	Degree of oxidation	Functional properties	Pasting properties	Structural and morphological properties	Thermal properties	Uses	Reference
Ozone	Cassava	Aqueous phase pH (3.5–9.5), 25 °C, time 60 min Gas conc. 13 mg L^{-1}	Carbonyl (0.002–0.011), and carboxyl (0.000–0.028)	–	Decrease in peak, trough, setback, breakdown, and final viscosity	Decrease in relative crystallinity	–	–	Klein et al. (2014)
Ozone	White Cocoyam	Gaseous phase, reaction time 5–15 min, vessel speed 150 rpm, Reacted ozone 1.83 (m Mol)	Carbonyl and carboxyl content 0.711, 0.311 (%)	Swelling power, solubility, and amylose content increases, (↑), gel strength decreased (↓)	Peak, hot paste, cold paste, setback viscosity, and peak time decreases (↓)	C_A-type pattern	Initial, peak, conclusion temperature increase (↑) and enthalpy of gelatinization decreases (↓)	–	Oladebeye et al. (2013)
Ozone	Rice	Aqueous phase Reaction time 15–30 min, oxidiser flow rate 170 mL/min, catalyst Lysine (6%)	–	Swelling strength and cooking stability increases (↑) Retrogradation tendency decreases (↓)	Peak and final viscosity reduces (↓)	–	–	Thickening agent	An and King (2009)

Ozone	Wheat	Gaseous phase reaction time 30 min, oxidiser flow rate 2.5 L/min, Ozone conc. 1.5 mg/kg	Carboxylic content increases (↑) with time	Swelling power increases (↑)	Breakdown viscosity increases (↑) and no significant change was observed in peak and final viscosity	A type of pattern and intact granules with no damage to surface	No change in transition temperatures and enthalpies	–	Sandhu et al. (2012)
Ozone	Wheat	Aqueous phase reaction time 15–60 min, temp 5 °C Ozone conc. 0.00042 g	–	–	Pasting temperature, peak, trough, breakdown, final viscosity decreases, (↓)	–	–	–	Çatal and Ibanoğlu (2014)
Ozone	Corn, sago, tapioca	Gaseous phase, time 1–10 min, Vessel speed 150 rpm	–	Corn and sago starch gel firmness increases (↑) with time	–	–	No significant changes were observed in gelatinization temperature	–	Chan et al. (2011)

(continued)

Table 3.1 (continued)

Oxidizing agent	Starch source	Process parameters	Degree of oxidation	Functional properties	Pasting properties	Structural and morphological properties	Thermal properties	Uses	Reference
Ozone	Corn, Sago	Gaseous phase, Gas flow 8 ml/s, Time 1–10 min, vessel speed 150 rpm	Carbonyl content Corn (0.025–0.061%), sago (0.066–0.085%) and carboxyl content corn (0.011–0.063%), sago (0.002–0.048)	Intrinsic viscosity decreases (↓) And varying solubility and swelling power with time	Variation in pasting temperature, peak, hot paste, and breakdown viscosity with time	–	–	–	Chan et al. (2009)
Sodium Periodate	Potato, corn	Oxidizer conc. 0.7 M, 5–10 mL, magnetically stirred at 40 °C for 3 h	Aldehyde groups content corn (25–45%), and potato (21–33%)	–	–	Compact solid mass with many pores and cavities	–	Films	Ziegler-Borowska et al. (2018)

Sodium Periodate	Corn	Oxidizer conc. 0.6 Mol/L temp 35 °C, time 120 min	Aldehyde group contents 74.5%	–	Pasting viscosity decreases (↓)	A-type pattern destroyed, and SEM image showed circular middle depression	Initial, peak temp increases (↑), conclusion temp, enthalpy of gelatinization decreases (↓)	–	Zuo et al. (2017)
Sodium Periodate	Waxy maize	Oxidizer conc. 0.125, 0.25 g, pH 3, temp 30 °C, Time 2 h.	–	–	–	A-type pattern, SNC (platelet shape), SNP (irregular shape with multiple edges and corner)	–	Oil in water Pickering emulsion	Chen et al. (2019)
Sodium Periodate	Pea	Oxidizer conc. 20–95%	Aldehyde group contents 84.3–81.1%	–	Intrinsic viscosity decreases (↓)	A-type pattern up to 30% concentration	–	Thermoplastic starch	Zhang et al. (2009)
Sodium Metaperiodate	Tapioca	Oxidizer conc. 0.05–0.3 N, pH 3, temp 32 °C, time 1 h.	Aldehyde and carboxyl contents increase (↑) with conc.	–	Swelling and solubility higher (↑) at 60 and 70 °C, but lower (↓) at 80 and 90 °C	A-type pattern	Initial, peak temp increases (↑), enthalpy of gelatinization decreases (↓)	–	Wongsagon et al. (2005)
Sodium Periodate	Potato	Oxidizer conc. 20 g, pH 5, temp 30 °C	Dialdehyde content 1.5–25%	–	–	B-type pattern, degree of crystallinity decreases (↓)	–	–	Fiedorowicz and Para (2006)

2012), sword bean (Adebowale et al., 2006), maize (Liu et al., 2014), white cocoyam (Oladebeye et al., 2013 and elephant foot yam (Sukhija et al., 2016).

The intrinsic viscosity of starch is strongly correlated with its molecular weight, and it is observed that oxidation leads to a reduction in intrinsic viscosity. Furthermore, the intrinsic viscosity of modified starch is influenced by the oxidizing agent's type and concentration. The decline in intrinsic viscosity of corn and sago starches was reported by Chan et al. (2009). This reduction is attributed to the partial depolymerization of starch polymers, which subsequently leads to a decrease in molecular weight as oxidation levels increase. Similar findings were reported in other studies on corn by Pietrzyk et al. (2012) and Zhang et al. (2009), and on corn, pea, and sweet potato by Zhang et al. (2012).

The light transmittance of the starch paste is determined by the number of swollen starch granules present, which refract the light passing through. This relationship is reciprocal, meaning that higher light transmittance leads to a more transparent paste. According to Sangseethong et al. (2009), oxidized cassava starch paste demonstrated higher transmittance than native starch. Similarly, Zhang et al. (2012) reported that oxidized corn, pea, and sweet potato starches also had higher light transmittance compared to their native counterparts.

3.3.2 Pasting Properties

The impact of oxidation on the gelatinization and starch retrogradation properties is commonly evaluated using the Brabender viscoamylograph or rapid visco analyser (Vanier et al., 2017). Studies have indicated a significant decrease in the pasting temperature (Ptemp) of oxidized starch from various sources, including potato, corn, rice, and cocoyam. The reduction in P_{temp} has been attributed to the carboxyl group generation and the acetyl group introduction, which weakens the hydrogen bond within starch molecules (Ma et al., 2021; Halal et al., 2015b; Kuakpetoon & Wang, 2006). However, the changes in pasting temperature do not hold for all starches, and some differences may be observed within the varieties, as observed in lima bean (Okekunle et al., 2020). The increase in pasting temperature observed in potato starch may also be due to the formation of hemiacetal cross-linked aldehyde groups that lower amorphous region mobility and improve the stability of the swollen starch (Zhang et al., 2022).

The peak and final viscosity of oxidized lima bean and corn starch have been more significant than that of native starch. This is due to starch stabilization by forming hemiketal or hemiacetal cross-links, which easily overcomes the destabilization caused by depolymerization (Okekunle et al., 2020; Kuakpetoon & Wang, 2006). However, on extensive oxidation, the values drop due to depolymerization. The depolymerization partially cleavages the glycosidic bonds between the starch molecules observed in some starches like potato, barley, and lima bean starch. The extent of oxidation greatly influences the viscosity of modified starch (Zhang et al., 2022; Okekunle et al., 2020; Halal et al., 2015b).

The breakdown viscosity of starch was used to analyze its stability under shear and heat treatment. The oxidized starches derived from lima beans were found to have a lower breakdown viscosity, which enhances their ability to withstand higher levels of heating and shear stress (Okekunle et al., 2020). This is possible due to the amylose degradation and largening of space in the amylose chain, indicating lesser chances of retrogradation (Zhang et al., 2022). Similar results were reported for oxidized potato, and barley starches, which exhibit improved stability against mechanical stirring and heating as evidenced by their lower breakdown viscosity compared to native starch (Pietrzyk et al., 2012: Halal et al., 2015b).

Setback viscosity reflected the retrogradation tendency of potato, barley, and corn starch paste and decreased with an increase in oxidation (Zhang et al., 2022; Halal et al., 2015b; Kuakpetoon & Wang, 2006). Oxidations favor the leaching of amylose molecules and degrade the starch molecules, lowering the retrogradation rate. Furthermore, the introduction of carbonyl and carboxyl groups through oxidation inhibits the re-association of starch chains, thereby reducing the retrogradation tendency (Halal et al., 2015b).

3.3.3 Thermal Properties

Differential scanning calorimeters are utilized for investigating the thermal characteristics of starches (Okekunle et al., 2020). The transition temperatures include gelatinization onset temperature (T_o), the peak of gelatinization (T_p), and the conclusion temperature (T_c). Oxidation does not cause significant changes to the onset gelatinization temperature (T_o) of barley starch. However, gelatinization temperature (T_o) decreases with oxidation for corn starch; it may depend on the botanical source (Halal et al., 2015b). Oxidation of lima bean starches causes reduced thermal stability, which indicates reduced onset temperature (T_o) and enthalpy of gelatinization. Oxidation often causes the addition of a carboxyl group (CHO-) to the starch molecule, which improves the water adsorption and swelling of starch, reducing the gelatinization temperature. However, it may also be observed that these results vary widely based on the botanical origin and oxidizing agent (Okekunle et al., 2020).

The loss of molecular order and crystallinity in starch can be determined by its enthalpy, which represents the thermal energy involved in gelatinization (Sandhu et al., 2008). The drop in gelatinization enthalpy of oxidized barley, cassava, and corn starch was higher at lower active chlorine concentrations. This may be attributed to the partial degradation of crystalline lamellae starch and the weakening of starch granules which further lowers the energy of gelatinization (Halal et al., 2015b). However, waxy corn starch exhibits a higher enthalpy linked with a strong association of the double helix bond of amylopectin chains (Sandhu et al., 2008).

3.3.4 Retrogradations and Syneresis

In the retrogradation process, the starch gel initially rapidly crystalized the amylose molecules and, subsequently, the amylopectin. Retrograded starch and refrigerated storage have significant industrial importance because they affect the sensory, structural, and mechanical properties of starch-based products. Liu et al. (2014) reported the retrogradation properties of oxidized maize starch decreased after 24H. The decrease in retrogradation rate may be due to an increase in oxidization content which decreases the amylose content. The other possible reason may be that the introduction of negatively charged carboxyl groups hinders the chain rearrangement, which lowers the retrogradation rate. The rice starch with ozone and the addition of lysine provide the minimum retrogradation tendency and superior cooking stability (An & King, 2009). The charged amino acid, with the presence of ozone, enhances ionic interactions, which could form more carboxyl groups.

Syneresis is a crucial physical characteristic of starch gels that measures the amount of water expelled from a gel during standing or after undergoing freezing and thawing cycles over a specified period. High syneresis values indicate significant retrogradation of starch gels due to weak or insufficient interactions between starch granules. The extent of syneresis is influenced by several factors, such as the amylose content, degree of polymerization of amylose and amylopectin, degree of starch chain association, and length of amylopectin side chains (Hoover & Sosulski, 1991). Nep et al. (2016) reported that the oxidation of Borassus aethiopum starch with sodium hypochlorite resulted in a reduced syneresis value. This inhibition of syneresis can be attributed to the presence of acetyl groups, which enhance the water retention capacity of the starch.

3.3.5 Structural and Morphological Properties

The structural properties of oxidized starch depended upon starch source, type of oxidizer, and concentration. The difference in the degree of crystallinity depends upon crystal size, amylopectin content, amylopectin chain length, arrangement of amylopectin within the crystalline domain, and interaction between double helices (Hoover & Ratnayake, 2002). The oxidized starch generally does not change the crystallinity pattern, but introducing carbonyl and carboxyl groups changes the peak intensity of starches**.**

The structural characteristics of oxidized starch are influenced by various factors, including the source of starch, type, and concentration of oxidizer used. The difference in the degree of crystallinity depends upon the size of the crystals, the amount of amylopectin present, the length of amylopectin chains, how amylopectin is arranged within the crystal, and how double helices interact with each other (Hoover & Ratnayake, 2002). Although the crystallinity pattern of oxidized starch remains generally unaltered, the introduction of carbonyl and carboxyl groups can alter the peak intensity of the starch. The changes in the intensity of crystallinity have been reported for various types of starches. These include Sword bean (Adebowale et al., 2006), potato (Fiedorowicz & Para, 2006), banana (Sanchez-Rivera et al., 2009), Carioca bean (Vaniner et al., 2012), cassava (Klein et al., 2014), corn (El-Sheikh et al., 2010), and finger millet (Afolabi et al., 2012).

Vanier et al. (2012) reported peak intensity of carioca bean starches increases with concentration. However, the degree of crystallinity decreases at higher concentrations. The crystallinity difference between legume starches is affected by crystallite size, moisture content, polymer content, and the number of crystallites. The decrease in the degree of crystallinity at higher concentrations may be that the amylopectin chain was destroyed by active chlorine, which enhances the breakdown of the amylopectin chains. According to Sanchez-Rivera et al. (2009), the diffraction pattern of banana starch did not show any significant change. The chemical modification does not alter the crystalline region; all the modifications happen in amorphous areas. The peak intensity of starch increases after modification and increases in peak intensity due to the partial breakdown of the amorphous component of the starch molecules. Adebowale et al. (2006) reported that the peak intensity of sword bean starch was higher than its native form.

The effect of oxidation on the surface of different starch granules was reported for Carioca bean (Vanier et al., 2012), normal and waxy corn (Sandhu et al., 2008), sword bean (Adebowale et al., 2006), corn, pea, sweet corn (Zhang et al., 2009), potato (Castanha et al., 2017; Tolvanen et al., 2009; Ziegler-Borowska et al., 2018), Maize (Liu et al. 2014), wheat (Sandhu et al., 2012), Corn (Zuo et al., 2017; Ziegler-Borowska et al., 2018). Starch granules in storage tissue can be varied in composition, size, and shape. While there are no reports on changes in granule shape due to oxidation, differences in granule morphology have been observed under different oxidation conditions (Vanier et al., 2017).

3.4 Application

Nattokinase (NK) anionic starch-based nanospheres for treating thrombolytic disease were studied by (Huang et al., 2013). This study reported that starch-based nanospheres have high drug entrapment efficiency due to their net positive charge and weak ionic interactions. The hypochlorite oxidation method was used to create an anionic starch derivative and added functional copolymers with cationic amino acids. The oxidized starch-chitosan hydrogel was prepared using oxidized starch derivatives (Baran et al., 2004). Oxidized starch derivatives can conjugate and crosslink with other polymers due to the reactivity of groups like dialdehydes and ketones. As the amount of oxidized starch in the hydrogel increases, it swells more, and its viscosity decreases. In contrast, the tensile strength of oxidized starch decreases with a percentage increase in oxidized starch content.

The oxidized starches are primarily used in food applications like cream pudding, whipped cream, a mixture of dough powder, frozen food products, emulsifiers, conditioners for bread, and Arabic gum replacers (Chattopadhyay et al., 1997; Chung & Seib, 1991; Pietrzyk et al., 2012). Biodegradable films were prepared with different oxidized starch like barley, potato, and banana (El-Halal et al., 2015a; Hu et al., 2009; Fonseca et al., 2015; Zamidio-Flores et al., 2006). The oxidized barley starch biodegradable films show films' properties depending on the degree of starch oxidation. The morphologies of oxidized barley starches films were more uniform. The homogeneity of oxidized starch films relies on the depolymerization of starch molecules. The breakdown allows more significant interaction between the starch and the plasticizer (El-Halal et al., 2015a), . Hu et al. (2009) reported an improvement in the characteristics of the film prepared by the oxidation of potato starch. The film had better stability in acidic solution, improved transparency, and superior anti-crosslinking properties. In another study, biodegradable films were prepared with oxidized potato starches at 0.5–1.5% active chlorine (Fonseca et al., 2015). At high active chlorine concentrations, oxidized starch exhibited low water solubilities, low water vapor permeabilities, high tensile strength, and more transparent and flexible films than native starches. Furthermore, Zamidio-Flores et al. (2006) reported the presence of carboxyl and carbonyl groups in oxidized banana starch could quickly form hydrogen bonds with the hydroxyl groups and form a better tensile strength oxidized banana starch film.

Waxy maize starch nanocrystals were prepared by (Wei et al., 2016). The native starch nanocrystals self-aggregate, forming agglomerates at the micrometer scale. This aggregation behavior limited applications of starch nanocrystals as a homogeneous dispersion. They are modified with sodium hypochlorite to reduce the aggregation behavior and enhance the aqueous re-dispersibility of starch nanocrystals. The homogenous suspension of oxidized starch nanocrystals was stable for 20 days. Sodium hypochlorite induces hydrogen bonding and van der Waals forces, adding negative charges to starch nanocrystals' surfaces and improving water re-dispersibility.

Apart from food and pharmaceutical applications, oxidized starches are used in various industries like paper, textile, laundry finishing, sizing, and binding materials industries for their surface sizing, coating, binder, and film-forming solution (Sangseethong et al., 2010; Isah, 2018; El-Sheikh et al., 2010; SánchezRivera et al., 2005).

3.5 Summary

There is a wealth of existing literature on the production and properties of sodium hypochlorite-oxidized starches, which have been studied extensively over many years. Numerous studies have explored the effects of various factors, such as the type of starch used and the specific conditions of the oxidation reaction**.** In addition, hydrogen peroxide is an oxidizing agent, which can have similar effects to sodium hypochlorite if similar conditions are maintained. Moreover, hydrogen peroxide is a more environmentally friendly oxidizing agent than sodium hypochlorite, but it typically requires a catalyst to be effective. UV light is one safe catalyst that can be used to make the process eco-friendlier. There is very little research on other oxidizing agents, like periodic acid, sodium meta periodate, and periodate. More recently, a new method of ozone oxidation has been used for starch oxidation, but there is little information available on how direct and indirect methods of ozone affect the molecular, functional, pasting, and rheological properties of the starch. The way starch is structured, specifically, the amylose it contains plays a significant role in how it is affected by oxidation. Oxidized starches have potential applications in the food industry, especially in creating low glycaemic index foods. Oxidized starches are used in various other sectors besides food applications like paper, textiles, laundry finishing, surface sizing, coating, binder, and film-forming solution.

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Chapter 4
Hydrolysis of Starch

**Babatunde Olawoye, Olusola Samuel Jolayemi,
Babawande Adeboye Origbemisoye, Timilehin David Oluwajuyitan,
and Oyekemi Popoola-Akinola**

4.1 Introduction

Starch in plants and animals is stored as carbohydrates. Starch is used in food and other industries as a primary production material or as a processing aid like an additive. Starch in its native form has a limited ability to withstand some industrial and processing conditions, such as high-temperature processes, thus limiting its application as a food and industrial manufacturing ingredient. Some of these defects are evidenced as low solubility, ease of retrogradation, loss of viscosity, crystallinity, and birefringence, and inability to withstand different processing methods like high shear rate, pH, and temperature (Awolu & Olofinlae, 2016). In addition, starch is composed of long chains of glucose molecules linked together by glycosidic bonds, and the human digestive system cannot break down starch into glucose molecules without hydrolysis.

Hydrolysis of starch involves the addition of water molecules to break the glycosidic bonds between the glucose molecules, forming smaller polysaccharides, disaccharides, and, ultimately, glucose molecules. During hydrolysis, water molecules react with the glycosidic bonds that link the glucose units in the starch molecule, causing the bonds to break and resulting in the formation of smaller sugar units. This process is critical for the digestion and absorption of starch by the body and for producing glucose for various industrial applications. Hydrolysis of starch can be carried out through enzymatic or acid hydrolysis methods, each with its unique advantages and limitations. The specific method used depends on the desired end

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B. Olawoye (✉) · O. Popoola-Akinola
Department of Food Science and Technology, First Technical University Ibadan,
Ibadan, Nigeria

O. S. Jolayemi · B. A. Origbemisoye · T. D. Oluwajuyitan
Department of Food Science and Technology, Federal University of Technology Akure,
Akure, Nigeria

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product and the application. Hydrolysis is also used to produce various products, such as corn syrup, maltodextrin, and biofuels.

4.2 Methods for Hydrolysis of Starch

4.2.1 Acid Hydrolysis of Starch

Mineral acids like sulphuric or hydrochloric acids are used in treating starch to obtain acid-hydrolyzed starch. This process of acid-hydrolysis of starch results in the depolymerization of the starch polymer (De siqueira et al., 2017). Acid-hydrolysis of starch is performed to remove fatty substances from native starch and improve the starch's physical and chemical properties. Starch treated with acid shows several desired characteristics, such as increased gelatinization temperature, less retrogradation tendency, swelling power, and pasting thickness loss (Pratiwi et al., 2018).

Firstly, starch acid hydrolysis influences the amorphous structure of the starch granule, followed by gradual degradation of the crystalline region of the starch granule, resulting in the rise in peak gelatinization temperature. A surge in short chains amylose is achieved through this modification process which supports the retrogradation of starch that results in resistant starch formation. Acid-thinned starch is widely used during the manufacturing of sweet candies and gums. The tolerance of starch to refrigeration storage at 5 °C and freeze-thaw cycles can also be reduced through acid hydrolysis (Thys et al., 2013; Ulbrich & Floter, 2019).

4.2.1.1 Acid Hydrolysis Using Mineral and Organic Acid

Kirchoff, a German chemist discovered acid hydrolysis at the beginning of the nineteenth century. He discovered that boiling starch from wheat with dilute sulphuric acid produces sweet syrup (Dziedzic & Kearsley, 1995). Later, potato starch was used, sulphuric acids were replaced with HCl (Hydrochloric acid), and the reaction vessels were heated indirectly.

Bej et al. (2008) worked on acid hydrolysis of wheat flour in a batch reactor at varying temperatures using concentrated H_2SO_4. The maximum conversion of starch to reducing sugar (42%) was obtained at 95 °C and pH 3. In addition, Hoseinpour et al. (2010) carried out starch hydrolysis using dilute sulfuric acid. Under optimum conditions, the conversion of starch to glucose was completely attained at 1% acid, 130 °C, and 7.5% solids loading for ½ hour. The study showed that starch granules' amorphous regions are incompletely hydrolyzed, affecting the slowly digestible and resistant attributes of waxy maize starch. The number of slowly digestible and resistant starch decreased while the amount of rapidly digestible starch increased. Azmi et al. (2016) researched hydrolyzing cassava leaves with cassava native starch using nitric acid. The result showed that a known starch concentration is very important during hydrolysis compared to the acid concentration and temperature. Abdorreza et al. (2012) and Sunaryanto et al. (2013) studied the effect of acid hydrolysis on the rheological and physicochemical properties of sago starch with sulfuric acid to obtain maximum reducing sugar. Acid hydrolysis is one of the simple and convenient methods for native starch hydrolysis due to readily available resources at an economical price. However, it is not a flexible process and requires special equipment that can withstand high temperatures and acidic conditions. As a result, alternative methods such as enzymatic hydrolysis or chemical modification may be preferred in some cases to achieve greater flexibility and control over the resulting product. In addition, the method also forms undesirable by-products and relatively low yields (Ramprakash & Muthukumar, 2014). The degree of hydrolysis can be somewhat altered, but the end product will still be limited to a narrow range of oligosaccharide sizes. The removal of undesirable by-products, such as furan, is done if the product of hydrolysis is needed for further use (Klinke et al., 2004).

4.2.1.2 Effect of Acid Hydrolysis on the Chemical, Functional, Pasting, Thermal, Morphological Structure and Digestibility of Starch

Acid hydrolysis modification of native starch is an essential and effective method employed by starch industries as it does not destroy the granule morphology while changing the inner structure and functional properties (Wang et al., 2012). Shai et al. (2016), Wang et al. (2017) and Jiang et al. (2016) have studied the effect of acid modification on pea, corn, *Cynanchum auriculatum* and xanthan starch. The decrease in swelling power, water binding capacity and solubility index of starch from diverse botanical sources is achieved by acid hydrolysis (Kaur et al., 2011).

Primarily, the acid enters the starch granule with a preferential assault on the granule surface. At that point, the acid dissolves the loosely-packed undefined sections within the starch granule and hydrolyzes the starch atoms (Qiao et al., 2016). The particular hydrolysis of the amorphous sections leads to an increment within the relative crystallinity. It appears that the acid hydrolysis of waxy starch was quicker compared to G80 starch acid hydrolysis. The effectiveness of acid hydrolysis at the granule level may be impacted by the permeability of the starch granules (Huber & Bumiller, 2000). They also postulated that G80 starch is hydrolyzed from the exterior towards the insides of the granules by disseminating the acid into the granules at a moderate frequency. The contrast between the endo-corrosion design for the waxy starch and the exo-corrosion design for occurrence, acid hydrolysis beside hydrothermal modifications may be of advantage to the arrangement of resistant starch (Zavareze & Dias, 2011). Also, acid hydrolysis with autoclaving and subsequent β-amylolysis may decrease starch digestibility (Song, Janaswamy, and Yao, 2010). These collective modification strategies should assist in expanding industrial uses.

4.2.1.3 Acid Hydrolyzed Starch Applications Food and Industry

For industrial usage, the selection of starch is made based on its physicochemical characteristics and accessibility (Pascoal et al., 2013). The application of starch depends on the source from which they are extracted. Currently, a few applications of starch comprise the following: as a nourishment added substance to control the constancy and surface of soups and sauces, to resist gel breakdown during processing and to extend the shelf life of the food products; laundry measurement of fine textures and skin cosmetics within the cosmetic and textile industry; for improving paper quality and printing attributes within the paper industry; as sedate fillers within the pharmaceutical industry; as binders within the packaging industry (Falade & Okafor, 2013). Universally, thorough efforts have been directed toward creating polysaccharide derivatives of diverse sorts of starch for different applications in diverse industries (Lawal, 2011). Therefore, the far-reaching utilization of starch is vindicated since it is reasonable and accessible in huge amounts. In expansion, it is generally unadulterated and doesn't necessitate any serious refinement, as is regularly the situation with supplementary natural polymers, such as gums and celluloses (Daudt et al., 2014).

4.3 Enzymes Hydrolysis of Starch

4.3.1 Starch Hydrolysis Using Enzymes

In the last few decades, enzymes have been used to replace acid and alkaline hydrolysis of starch in the quest for a more environmentally friendly and greener way of starch processing. Amylase is the prominent enzyme in starch hydrolysis and the most frequently applied enzyme in starch hydrolysis and other amylaceous materials are α-amylase, β-amylase, and glucoamylase. Structurally, starch is a homopolysaccharide composed of repeating units of dehydroglucose linked together by α-1,4 linear chain called amylose and a branching chain by α-1,6 called amylopectin (Haq et al., 2019). Enzymatic hydrolysis of starch involves three important phases, gelatinization, liquefaction and saccharification that individually and collectively determine the properties of the resultant starch (Presecki et al., 2013).

Gelatinization Starch gelatinization is a process whereby starch granules lose their crystallinity under different temperatures and moisture conditions. During gelatinization, exterior amylopectin chains, packed together in double helix clusters, are uncoiled and melt (Miao et al., 2011a). Variations in amylose/amylopectin ratio, size, structure, and dispersal of starch granules, as well as the interior arrangement of starch segments within the granule, account for the variation in gelatinization temperatures for different starch origins. As earlier described by (Acosta-Pavas et al., 2020) on wheat starch, the gelatinization process includes starch heating in buffered solution (20% w/w) at slightly acidic pH from room temperature gradually till it attains a temperature of 90–95 °C for about 2 h. After the gelatinization temperature has been reached, the solution is allowed cool slowly to a temperature appropriate for the subsequent liquefaction step. During gelatinization, starch's native structure is transformed because of hydrolytic cleavage of its inter and intramolecular bonds in the presence of water and heat. This is accompanied by stretching of starch granules, swelling, leaching of the amorphous region and subsequent increase in viscosity (Fig. 4.1). The more defined crystalline region is weakened, thus causing the starch to be more vulnerable to enzymatic attack.

Liquefaction The second stage (liquefaction) involves activities of endo-enzyme amylase to randomly hydrolyze α-1-4 glycosidic linkages leading to loss of viscosity, and production of a mixture of glucose, oligo- and polysaccharide, and maltodextrin in the medium (Azmi et al., 2017). At the liquefaction temperature, the pH of the gelatinized starch is readjusted, an amylase enzyme is added at a predetermined E/S (enzyme: substate) ratio, and the mixture is agitated for a period. After the liquefaction step, the process is halted by the addition of HCl at pH 1.0.

Saccharification In the saccharification stage, α −1,4 and α-1,6 bonds of amylopectin – the main crystalline region of the starch is hydrolyzed by exo-enzymes such as glucoamylase/amyloglucosidase or pullulanase added at a specific E/S ratio and allow to agitate to produce glucose as a primary product. The reaction takes place at 60 °C and pH 4.3. Glucoamylase is capable of hydrolyzing both linkages in starch. It is more specific for α-1-4 and has negligible activity on α-1-6 bonds. Therefore, the theoretical >95% glucose yield reported in the literature (Govindasamy et al., 1995; Hii et al., 2012) may not be feasible due to some fundamental changes in reaction kinetics. For instance, solubilized polysaccharides can reassociate or

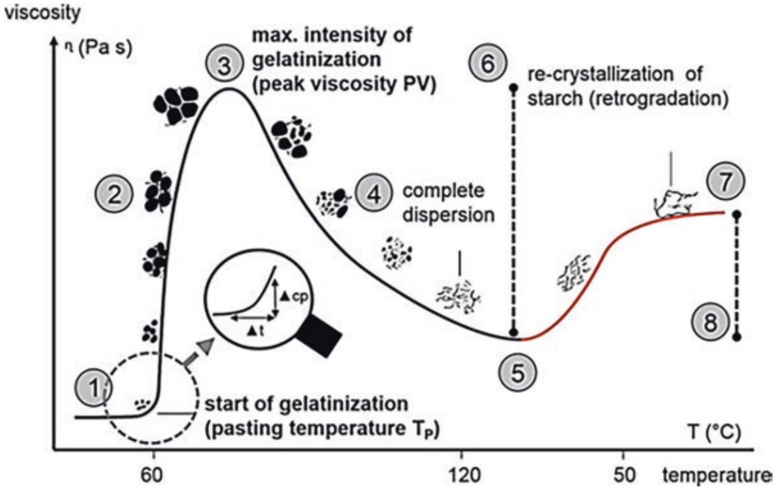


Fig. 4.1 Stepwise changes in viscosity of thermally treated starch. (Adapted from Schirmer et al., 2015)

retrograde, thus making the starch molecule increasingly resistant to the action of amylolytic enzymes as it undergoes retrogradation. Similarly, at high enzyme and product (D-glucose) concentrations, the reaction equilibrium can shift backward favoring glucose condensation (reverse reaction) hence preventing a continuous increase in glucose concentration (Bryjak et al., 2000). At the end of each enzymatic phase, the reaction must be halted by deactivating the enzymes by adding strong acid/alkaline or thermal shock (Morales et al., 2008).

Starch Amylolytic Enzymes Generally, endo-hydrolase, exo-hydrolase, debranching enzymes, transferase, and isomerase are the five main categories of starch-converting enzymes (Hua & Yang, 2016). Only endo-hydrolase, exo-hydrolase and debranching enzymes are involved in starch hydrolysis, as shown in Fig. 4.2.

Endo and Exo-Hydrolase Enzymes Endo-hydrolases, also known as endo-amylases, are enzymes that cut the α-1-4 glycosidic linkages at the interior section of the amylose or amylopectin polymer of starch, hence the name (endo-). α-amylase (EC 3.2.1.1) is a metalloenzyme endo-hydrolase enzyme whose activities depend on the presence of calcium as a cofactor (Sundarram & Murthy, 2014) and can be obtained from an exclusive range of Archaea and bacteria species, especially *Bacillus spp* (Konsoula & Liakopoulou-Kyriakides, 2007; Van Der Maarel et al., 2002). It can also be sourced from fungi and genetically modified microbial species. Bacterial α-amylase is classified as liquefying enzyme because they specifically cleave α-1,4 bonds at random. The fungal α-amylase supplementarily belongs to the saccharifying class and attacks the second linkage of the non-reducing termini, resulting in the severing of two glucose units at a stint (Azmi et al., 2017).

Supplementarily, exo-hydrolases (exo-amylases) either solely cut α-1-4 glycosidic bonds like β-amylase (EC 3.2.1.2) or may concurrently break both α-1-4 and α-1-6 glycosidic bonds in the case of glucoamylase (EC 3.2.1.3) and α-glucosidase (EC 3.2.1.20) (Van Der Maarel et al., 2002). Exo-amylases, as the name implies, exclusively catalyze hydrolytic cleavage of amylose or amylopectin external glucose residues to create glucose as the lone end product (glucoamylase and α-glucosidase), or maltose and β-limit dextrin (β-amylase). Glucoamylase preferentially hydrolyzes long-chain polysaccharides, whereas α-glucosidase preferentially hydrolyzes short-chain malto-oligosaccharides to release α-configured glucose (Carrasco et al., 2017). β-amylase and glucoamylase have also been sourced from different microorganisms (Horváthovái et al., 2000).

Debranching Enzymes Debranching enzymes catalyze the hydrolysis of branched-chain α-1-6 glycosidic linkages exclusively. Isoamylase (EC 3.2.1.68) and pullulanase (EC 3.2.1.41) type I are two examples of these enzymes. The most significant distinction between these two enzymes is their substrate selectivity rather than their reaction mechanisms (Hii et al., 2012). Pullulanase hydrolyzes pullulan, a polysaccharide containing an α-1-6 linked maltotriose repeating unit. Pullulanase can hydrolyze both pullulan and amylopectin's α −1-6 bonds; however, isoamylase can only hydrolyze amylopectin.

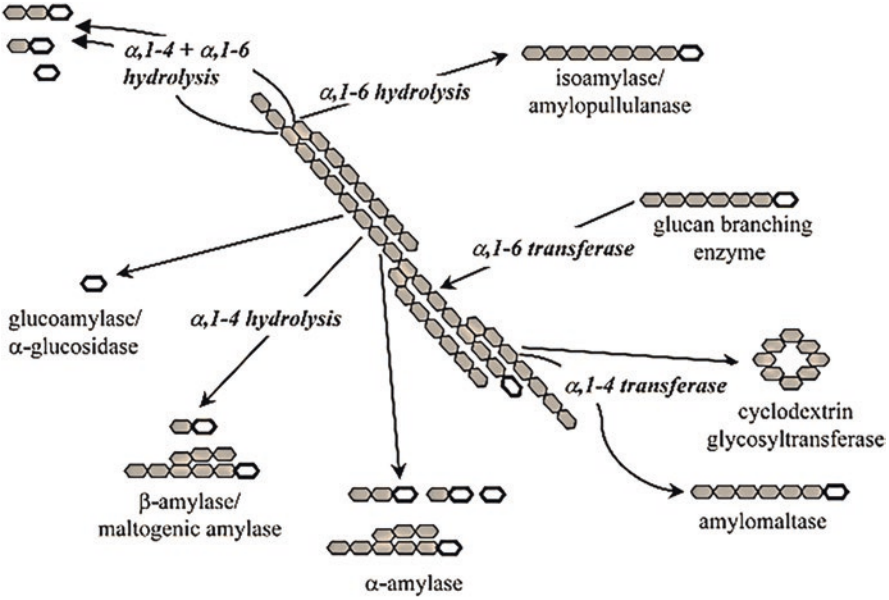


Fig. 4.2 Different starch degrading enzymes. (Adapted from Van Der Maarel et al., 2002)

4.3.2 Effect of Enzyme Hydrolysis on the Chemical, Functional, Pasting, Thermal, Morphological Structure and Digestibility of Starch

Effect of Enzyme Hydrolysis on Chemical Properties of Starch Enzymatic hydrolysis of starch confers a wide range of physicochemical changes on starch molecules depending on many factors, i.e., source of the starch, reaction conditions (E/S ratio, pH, temperature, and time), nature of the enzyme etc. In the work of Uthumporn et al. (2010), granular starch from different botanical origins was hydrolyzed by a mixture of amylolytic enzymes (α-amylase and glucoamylase) at sub-gelatinization temperature. The author observed that the susceptibility of the granular starch to the enzymes varied among different starch origins. Similarly, Zhang and Oates (1999) observed a substantial variation in the degree of susceptibilities of starch granules to enzyme attack among different potato varieties. There were significant reductions in the apparent amylose components of all the starch origins and corn starch was the most affected, followed by mung bean, cassava, andsago starch. This also agrees with the report on α-amylase-treated cassava starch (Salcedo-mendoza et al., 2018). Both amylopectin and amylose fractions are utilized concurrently in the amorphous regions, thus making the amorphous area of the starch more vulnerable to enzymatic degradation than the crystalline (Almeida et al., 2019).

The amount of glucose produced during hydrothermal enzymatic hydrolysis of starch is employed as an index of effective enzyme activity. In previous work on the optimization of starch hydrolysis by amylase (Agrawal et al., 2005), the amount of glucose generated increased with enzyme concentration up to a point. Another parameter used to express the extent of changes in starch chemical composition during enzymatic hydrolysis is the Dextrose Equivalent (DE), with a high dextrose equivalent indicating high hydrolytic conversion and a lesser average molecular mass (Takeiti et al., 2010). Tapioca and sweet potato starches experienced a remarkable increase in DE when hydrolyzed with a combination of glucoamylase and α-amylase below gelatinization temperature (Shariffa et al., 2009).

Effect of Enzyme Hydrolysis on Functional and Pasting Properties of Starch Paste clarity, swelling power and solubility index, are all influenced by enzyme hydrolysis to varying degrees depending on the nature of the enzyme, reaction conditions, and type of native starch. Increased paste clarity in amyloglucosidase hydrolyzed porous starch compared to α-amylase treated ones, was attributed to the absence of hydrolytic activities at the non-reducing end of the starch molecule (Dura et al., 2014). The solubility index of hydrolyzed starch measures the number of water-soluble compounds derived from starch conversion and molecular degradation and is related to the free sugar and other soluble components concentration. Amyloglucosidase cleaved the starch's intermolecular bonds to release more soluble compounds (Ye et al., 2018). Pasting temperatures of untreated and enzyme (α-amylase and glucoamylase) hydrolyzed cassava, corn, mung bean, and sago starches did not differ appreciably (Uthumporn et al., 2010). In contrast to this observation, pullulanase hydrolysis of potato, wheat, and pea starches, resulted in varying pasting temperatures (Liu et al., 2015).

After α-amylase hydrolysis, the viscosity of sweet potato, potato, Peruvian carrot and cassava starches reduced slightly (Rocha et al., 2010). The drop in peak viscosity can be accredited to the disintegration of some of the more delicate particles. Numerous pores observed on the surface of hydrolyzed starch granules indicated the breakdown of the starch, especially in the amorphous region (Chen & Zhang, 2012). These pores may have reduced the granule's water-holding capacity, resulting in a low peak viscosity. Furthermore, the degradation of amylose into low molecular weight soluble components further weakened the starch's structural integrity, making it more delicate, less viscous, and easily disordered. According to Uthumporn et al. (2010) after hydrolysis, the water binding capacity and swelling capacity of these starches are reduced. In contrast, the peak viscosity of mung bean starch increased dramatically, which can be accredited to its high amylose content after hydrolysis. Other authors have reported a slight increase in swelling power, amylose constituent, and solubility of some enzymatically treated starches (Shariffa et al., 2009).

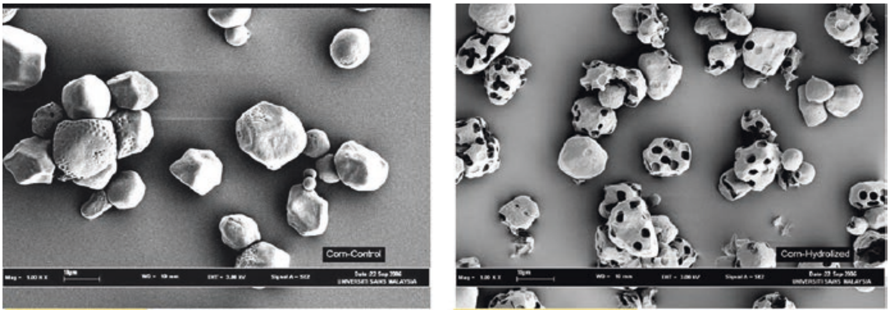
Effect of Enzyme Hydrolysis on the Morphological Structure and Thermal Properties of Starch Starch granules are divided into amorphous portions and sections of lower and higher crystallinity, with a gradual transition between them (Shariffa et al., 2009). These regions are susceptible to enzymatic attack to varying degrees. In addition, other factors influencing how amenable starch is to amylolytic enzymes are starch origin, granule size, reaction conditions and type of enzyme. Salcedo-mendoza et al. (2018) characterized morphological and physicochemical changes in enzyme-hydrolyzed cassava starch and observed no significant alterations in the structure and organization of the granules. However, the granule shape of hydrolyzed starch differed from that of native starch, and granule disintegration amplified with temperature and time. The native starch proved to be more vulnerable to retrogradation, syneresis, and post-thawing phase separation than enzyme-treated starch.

There are two ways by which enzymes interact with starch granules during hydrothermal hydrolysis: exo-corrosion, in which the enzyme disintegrates the external surface of the granules and produces characteristic pits and fissures (Fig. 4.3); and endo-corrosion, in which the enzyme forms a passage (tunnel) leading to the centre of the granule, that compromises the structural integrity of the granule, causing it to disintegrate (Lin et al., 2016). Cracks and pores appeared on the surface of corn starch granules treated with α-amylase or amyloglucosidase, but there were no visible changes in granule size as reported by Dura et al., (2014). Polymer leaching was also more noticeable in enzyme-hydrolyzed starch, possibly due to the surface pores. Other authors have linked granular pores, channels, physical permeability of the amorphous region, and enzyme accessibility of starch, with a rate of enzyme hydrolysis (Blazek & Gilbert, 2010).

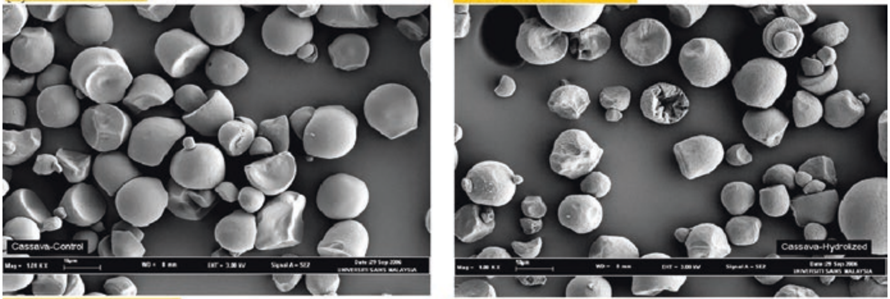
Effect of Enzyme Hydrolysis on Starch Digestibility Starch is divided into three categories based on the rate and degree of digestibility: rapidly digestible starch (RDS), resistant starch (RS) and slowly digestible starch (SDS) (Miao et al., 2011b). Studies have linked diabetes, cardiovascular disease, and obesity to long-term eating of foods with a high glycemic index (GI), that have been positively correlated with RDS content (Eleazu, 2016; Morris & Zemel, 1999). *In vitro* pancreatin α-amylase and amyloglucosidase partially hydrolysis of waxy maize starch exhibited an elevation in RDS and a corresponding decline in RS content of the starch residues (Miao et al., 2011b). There was no substantial change in SDS content of the starch despite a multiple-fold increase in average molecular weight components of the resultant mixture. In the work of Lin et al. (2016), high-amylose maize starch showed higher resistance to hydrolysis by amyloglucosidase and α-amylase and had a much lesser hydrolysis rate coefficient compared to regular maize starch.

4.3.3 Recent Advance on Enzyme Hydrolysis of Starch

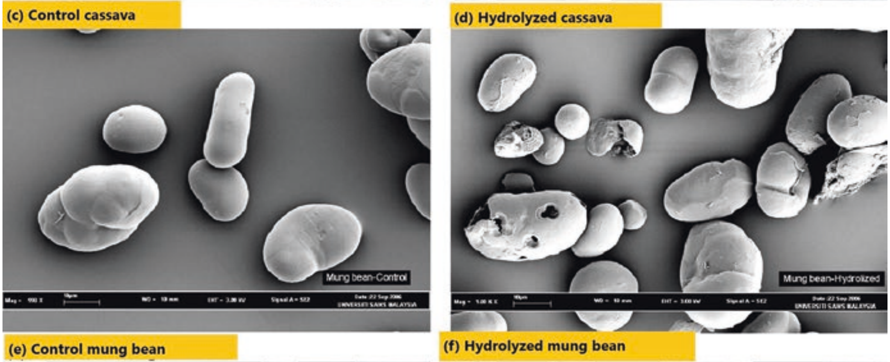
The two traditional unit operations (liquefaction and saccharification) involved in the conventional enzymatic hydrolysis of starch are highly energy-demanding and environmentally unfriendly. Most amylolytic enzymes are only amenable to this hydrothermal process for effective hydrolysis. However, a recent development has been proposed using a novel raw starch-digesting glucoamylase of high enzymatic



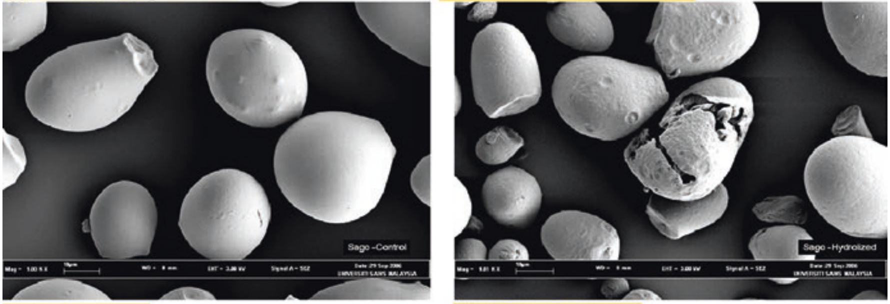
(a) Control Corn (b) Hydrolyzed corn



(c) Control cassava (d) Hydrolyzed cassava



(e) Control mung bean (f) Hydrolyzed mung bean



(g) Control sago (h) Hydrolyzed sago

Fig. 4.3 Scanning Electron Micrograms of enzyme hydrolyzed and control starch at sub-gelatinization temperature (Uthumporn et al., 2010) activity produced and purified from Penicillium oxalicum (Xu et al., 2016). Its ability to directly hydrolyze raw starch and simultaneously perform saccharification and fermentation makes it a more promising alternative in starch and alcohol production industries.

In a recent study (Zhai et al., 2022) using maltogenic α-amylase to hydrolyze wheat starch granule, the enzymes hydrolyzed both crystalline and amorphous parts of the starch correspondingly at sub-gelatinization temperature. In a recent work by Jung et al. (2022) and Zhang et al. (2019), the application of amylopectin chain elongating enzyme (amylosucrase) alone or alongside debranching enzyme (pullulanase) at different times was found to enhance the development of thermal stable starch, with declined contents of slowly digestible (SDS) and rapidly digestible starches (RDS) and improved contents of resistant starch (RS). This technology may be a more environmentally friendly way of generating modified starch with tunable physical and digestive properties. In the work of Zhao et al. (2021), the use of β-amylase and α-amylase in extruded barley flour resulted in a gelatinized product with a low peak viscosity, high water solubility and desirable alcohol level during whole barley beer production.

Gaquere-Parker et al. (2018) reported maximum hydrolysis of insoluble potato starch (Azure) at any temperature when the enzyme-catalyzed reaction is assisted with low-frequency sonication. Similarly, enhanced biocatalytic activities of amylolytic enzymes were achieved during a multi-enzyme (α-amylase and glucoamylase) co-immobilization (Salgaonkar et al., 2018). The high rate of starch hydrolysis, the thermal stability of the enzymes, and the retention of the enzyme activities after numerous recycling events were achieved. In the same vein, the application of immobilized fungal α-amylase to microgel system resulted in efficient starch-to-sugar conversion, greater enzyme activity at lower temperatures and high reusability (de Souza et al., 2019). In the place of expensive enzyme-supporting systems, the application of agro-industrial by-products as a cheap alternative for enzyme immobilization was recently proposed (da Costa Luchiari et al., 2021). Starch-to-glucose conversion of 75%, improved enzyme yield, thermal stability and overall efficient activities were achieved when glucoamylase was immobilized on corncob powder. Das and Kayastha (2019) revealed how a new β-amylase from peanut (*Arachishypogaea*) efficiently hydrolyzed native starch from potato and maize, leading to a remarkable increase in Dextrose Equivalent.

4.4 Other Methods of Starch Hydrolysis

4.4.1 Hydrolysis Using Ethanol and Microorganism

Hydrolysis of starch involves the breaking down of starch into smaller glucose molecules. Starch hydrolysis has traditionally been accomplished by several means of modification, such as physical, chemical, and enzymatic alteration. Amidst all these methods, enzymatic has been reported to produce better yield, structural, functional, rheological properties, and environmental adaptability. However, the high cost of enzymes limits their applications. Hence, the need for starch hydrolysis using other methods was felt. One such method is the use of ethanol/alcohol and microorganisms. Alcoholic hydrolysis of starch is mostly applied in conjunction with a small concentration of acid, popularly called “acid-alcohol” treatment, due to simultaneous hydrolysis and alcoholysis effects of the mixture at varying degrees on starch. Alcohol of varying molecular weights and forms, such as anhydrous methanol, ethanol, 2-propanol, 1-butanol etc., have been found suitable for this process (Chang et al., 2006; Lin et al., 2003). The technique involves suspending starch in alcohol, initiating the reaction with diluted inorganic acid, and allowing the reaction to proceed for hours in a shaking water bath at varying temperatures (Yiu et al., 2008; You & Izydorczyk, 2007; Singh et al., 2021). When the hydrolysis is completed, the mixture is neutralized with an alkali solution. This procedure confers some unprecedented modifications to the techno-functional properties of the native starch and the resultant modified forms have been found useful in various domestic and industrial applications.

In the developed community, the use of ethanol and microorganism in starch modification is becoming popular. An example is the use of Loog-Pang, which is made up of multi microorganisms such as yeast, moulds, and bacteria (Khamkeaw & Phisalaphong, 2016). Loog-Pang has the potential to produce carbohydrate hydrolyzing enzymes (α-amylase and glucosidase) which decompose starch into glucose and oligosaccharide to generate ethanol and other organic acids. Loop-Pang has been reported to be cheap and highly efficient in starch hydrolysis. Hence, the existence of amylolytic activity suggests that bacteria, moulds, or yeast can liquefy and transform starch and starch-containing plant materials into maltodextrins, sugar syrups, dextrose, maltose, glucose, and other products (Liu et al., 2011; Reddy et al., 2008). The microbes subsequently convert the hydrolysates into metabolites such as organic acids, ethanol, and aromatic compounds (Hittinger et al., 2018). Other recent advancements in microbial hydrolysis of amylolytic polymers are in producing poly-B-hydroxybutyrate (a biodegradable polymer substitute) from starch through bacteria saccharification and fermentation by Bacillus species. A blend of immobilized *Saccharomyces cerevisiae* and *Aspergillus awamori* was developed for starch bioprocessing, in which the fungus produces amylolytic enzymes while *S. cerevisiae* hydrolyzes starch and ferments it to ethanol in a consolidated single-phase process (Drosos et al., 2021). However, the overall quality of microbial starch hydrolysis is determined by the organisms’ nature, the hydrolytic potency of the enzymes inherent in the microbes, and the botanical origin of the starch. For example, the two glucoamylases isolated from *A. niger* degraded raw starch differently, with one being several folds more active against raw starch than the other (Xu et al., 2016).

4.4.2 Effect of Ethanol and Microbial Hydrolysis of Starch on Chemical, Functional, Pasting, Thermal, Morphological Structure and Digestibility of Starch

The hydrolysis of starch with ethanol changes some major intrinsic and extrinsic features of starch, influencing its chemical composition, functional and pasting qualities, thermal stability, and structural conformation. Ma and Robyt (1987) affirmed in an earlier study on acid-alcohol hydrolysis of potato and waxy maize starch using varying forms of alcohol (ethanol, 1-butanol, methanol, and 2-propanol) that all the alcohol-treated starch maintained high solubility in hot water and relatively retained their morphological appearances with varying degrees of granule damages. Generally, alcohol hydrolysis lowers the starch molecular weight (Chang et al., 2010) mainly due to the fractionation of resultant starch depending on the degree of alcoholysis, type of associated inorganic acids and process duration. Hydrochloric acid reduced starch chain length more than sulfuric or nitric acid in an alcohol medium (Chung et al., 2009). Methanol with about 50% alcoholysis proportion produced starch of higher molecular weights than other forms of alcohol such as 2-propanol and 1-butanol (Ma & Robyt, 1987). These claims were later confirmed by (Yiu et al., 2008), who produced modified sago starch of higher yield, desirable degree of polymerization, and minimal granular damages, from alcohol of varying molecular weights. With increasing duration of acid ethanol treatment, sorghum starch exhibited a gradual decline in amylose degradation, relative crystallinity, and pasting properties (Singh et al., 2021). The author attributed these physicochemical changes to a drop in molecular weight and a slow erosion of the amorphous area during hydrolysis. When compared with enzyme hydrolysis, alcohol/acid hydrolysis produced starch with a low degree of solubilization and practically intact granular morphology (You & Izydorczyk, 2007). However, the procedure results in substantially higher starch degradation than the enzymatic method.

In the case of microbial hydrolysis of starch, the rate of transformation of the starch molecules depends mainly on the amount of microbial starter culture. Reduced starter cultures caused starch structural breakdown evidenced by reduced crystallinity and compactness, surface erosion of starch granules, double helix and reduced molecular weight hence lowered digestibility (high slowly digestible starch content) (Tu et al., 2021). According to Situ et al. (2019) enzymatic hydrolysis of microorganisms during vinasse production, destroyed rice starch's morphological orientation. The use of an immobilized multiple-organism starch fermentation process resulted in an 82 percent yield, which was greater than other starch hydrolysis procedures. As a result, the technique has been recognized as an innovative technology that simplifies the brewing process by eliminating the malting stage (Drosos et al., 2021). After 120 h of hydrolysis of cassava starch employing Long-Pang multi-microorganisms at 35 °C, various advantages were seen, including enhanced glucose yield and purity, reduced temperature, and energy needs (Khamkeaw & Phisalaphong, 2016).

4.5 Conclusion and Future Perspectives

Modification phenomena are applicable to starch and are channeled toward reconstructing these intrinsic and extrinsic dysfunctionalities to make them appropriate for industrial application. Over the years, several physical, chemical, biological, and hurdle methods of starch modifications have proven successful. Over the last decade, there has been a flood of research on various types of chemical modification of starch, including oxidation, hydrolysis, etherification, esterification, cationization, and cross-linking processes. All these reactions are possible by the abundance of reactive hydroxyl functional groups on starch molecules, which makes them accessible to several redox processes.

In the case of starch hydrolysis, which could be chemical (acid-hydrolysis) or enzymatic (α-amylase, β-amylase, glucoamylase, and other debranching enzymes), there is partial depolymerization of starch by the breakdown of its amorphous or loosely packed region and subsequent increase in dextrose equivalent, decrease in resistant/slowly digestible starch and making it less susceptible retrogradation, syneresis and phase separation. Other forms of hydrolysis such as the application of alcohol of varying molecular weight, and microbial hydrolysis have also gained popularity. Alcohol hydrolysis produced starch that retained its natural granule morphology and low degree of solubilization when compared to other methods of hydrolysis. However, the microbial method of starch hydrolysis is comparatively more rapid, and less energy demanding in addition to higher glucose yield and purity, but its efficiency depends on the nature of microorganisms and their immobilization. Starch modification continues to be an intriguing area in food processing and industrial manufacturing. Research in this area is inundating but there are still other aspects of the discipline with a dearth of information and necessary attention.

For instance, modified starch has numerous advantages for food and several other industries in terms of nutritional, functional, and technical implications. It would be nearly hard to create an all-purpose starch that could be used as a component in any substrate given the number of modification techniques that are currently accessible. Therefore, more research and study should be put into comprehending and perhaps suggesting acceptable modification procedures and conditions suitable for starches obtained from various botanical sources.

While there have been a lot of successful studies on the physical, chemical, and enzymatic modification of starch, studies on the direct application of microorganisms in pure, starter form, or immobilized for molecular and structural alteration of starch are very scarce. More research attention should be focused on this area as the microbial technique could be more sustainable, eco-friendlier, and cost-effective in addition to being greener.

Finally, there is no mention in the literature of the modification of composite starch made from mixtures of several starches specifically for food applications. It is reasonable to suggest that a modified composite starch might have certain special and distinctive physicochemical, technological, and functional features that could be used for new food products.

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Chapter 5
Crosslinking of Starch

Nimish Shah, Mamta Saiyad, Milind Joshipura, Shibu Pillai, and Ankur Dwivedi

5.1 Introduction

In the last three decades, the polymer products derived from petroleum have increased many folds. The molecules with high molecular weight and hydrophobic nature provide high chemical stability and non-biodegradability. These non-biodegradable molecules make the disposal of products difficult, and it is a public concern. Nowadays, the focus of many research groups is on preparing new polymers without ozone depletion, even though it gives the same qualities of flexible rigidity and durability and remains lighter compared to solids. The polymers made from petroleum molecules degrade very slowly in the environment. The biodegradable polymer compounds are prepared by modifications in synthetic polymers with quickly degradable linkages.

On our earth, carbohydrates are more than all other organic materials combinedly. Starch is one of the cheapest carbohydrates, available abundantly. The use of starch raises the biodegradability of the polymer, and it makes it more affordable also. The alpha linkage of amylose starch provides flexibility and digestibility to the final product. In starch-based polymers, starch may range from 10 to 90%. As starch content increases, the product will become more and more biodegradable. Using biodegradable crosslink starch-based polymers would significantly improve the performance of effluent treatment plants and solid waste management issues. This starch-based material has a high heat value, is suitable for combustion, and is viable for enzymatic degradation to ethanol instead of landfilling (Ayoub & Rizvi, 2009, Ellis et al., 1998).

Starch is a widely used commodity for various applications, including food and non-food (cosmetic and medicinal). Starch is generally used for sizing, coating,

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N. Shah (✉) · M. Saiyad · M. Joshipura · S. Pillai · A. Dwivedi
Nirma University, Ahmedabad, India
e-mail: nimish.shah@nirmauni.ac.in

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specialty chemicals, fermentation, and substrates based on their lower cost and high purity. Starch offers many advantages in non-food applications. Its poly functionality and very high chemical reactivity make it an excellent polymer for many non-food applications in the industry. However, despite these exceptional properties, limitations like poor processability, mechanical properties, dimensional stability, and low moisture resistance make it difficult to use for many end products and challenge this promising candidate. In addition, starch contains many hydroxyl groups, which are responsible for the hydrophilic nature of starch; however, not all native starches are incompatible with many hydrophobic polymers. This also becomes a significant constraint in developing starch-based materials (Ayoub & Rizvi, 2009, Entwistle 1998).

Native starch has severe limitations, such as the granules rupturing, dehydrating, and swelling rapidly (Ayoub & Rizvi, 2009; Shah et al., 2016; Wolf et al., 1999). Its paste loses viscosity to produce a weak, stringy, cohesive body. In addition, water solubility and brittleness of native starch film limit its applications. Therefore, starches are modified for the desired temperature viscosity relationship, gelatinization, cooking characteristics, stable paste against acid or heat or mechanical shear, retrogradation tendencies, ionic character, and hydrophilic character.

Many chemical and physical processes are developed for the modification of starch. The chemical modification produces water-resistant starches that will not degrade quickly. One can convert hydrophilic hydroxyl groups by reacting with carboxylic acids to solve this hydrophilicity problem. For the development of starch acetate, the cost of the method is a significant obstacle. For example, compared to native starch, the price will become ten times higher with this modification. However, crosslinking of starch is one of the most common polysaccharide chemistry methods, offering low-cost structural transformation. Crosslinking of starch is carried out with crosslinking agents, establishing intermolecular bridges between polysaccharide molecules. These bridges are responsible for changes in the characteristics of native starch and offer resistance against high shear, acidity, and temperature. It shows improved textural properties and viscosity of native starch. The reagents used for crosslinking are bifunctional or multifunctional, capable of forming ester or ether links with hydroxyl functional starch groups. Other chemicals include epichlorohydrin, sodium tripolyphosphate, sodium trimetaphosphate, monosodium phosphate, phosphoryl chloride, adipic and acetic anhydrides, succinic anhydride, and vinyl acetate, have been reported for crosslinking of starch. The multifunctionality of reagents results in a variety of products. For example, the reaction between epichlorohydrin and starch can produce two molecules: crosslinked starch with the reaction between epoxides and glucose units and epichlorohydrin homopolymer. Because of hydroxyl groups and bonds between glucose units, the structure of crosslinked polymer become interconnected and complicated in three dimensions (Ayoub & Rizvi, 2009; Guarás et al., 2017; Hamdi et al., 2001; Masuda et al., 1978; Woo & Seib, 1997).

Various forms of crosslink starch material are available, such as gels, polymers, microbeads, films, composites, and hydrophilic networks of crosslink starch material. Different industries use them as an excipient or hydrogel for other purposes, such as biomedical and pharmaceutical research for transporting molecules. In wastewater treatment, it is used for decolorization, chelation of pollutants, or extraction of metals. The crosslinked polymer and its structures can be characterized by different characteristics such as retention of water, density and its variation with temperature, mechanical & chemical stability, and thermal transition through several methods such as FTIR, XRD, DSC, fluorescence, and enzymatic degradation. High-resolution solid-state NMR can provide excellent information about this material, mainly its structure and molecular motilities (Ayoub & Rizvi, 2009). This chapter provides the numerous methods/routes reported for synthesizing and characterizing crosslinked starch, along with their advantages and limitations.

5.2 Technologies for Crosslinking of Starch

Crosslinking is one of the most used methods for modifying native starch. Due to crosslinking, the starch granules show reduced swelling under cooking conditions resulting in limited gelatinization. The degree of crosslinking of starch depends on factors like pH, temperature, reaction time, the extent of substitution, crosslinking reagent composition, and starch source (Ayoub & Rizvi, 2009; Shah et al., 2016). crosslinked starch possesses a three-dimensional structure. The formation of crosslinking between the macromolecule chains is the main aim of these reactions (Hennink & van Nostrum, 2012). During the primary stage of the crosslinking reactions, the branches on the macromolecule chains are initially formed. The length of these branches develops gradually and produces a 3D structure.

Researchers proposed different processes for the development of these crosslinked structures. Physical characteristics of starch-based crosslinked polymers mainly depend on the degree of crosslinking (Akhtar et al., 2016; Kuo & Ma, 2001). Crosslinked starch-based polymers can either be synthesized by physical crosslinking (Kamoun et al., 2015) or chemical crosslinking (Hennink & Nostrum, 2012; Chung & Park, 2009). Researchers have reported/ proposed various methods for the synthesis/crosslinking of starch (Bhatia et al., 2013; Vieira et al., 2008). Crosslinked starch polymers prepared via physical blending are found to have poor stability but show insolubility in water. Physical blending is a reversible process (Aly, 1998; Nguyen & West, 2002). Chemically crosslinked polymers are formed via covalent bonds and are found to be more stable (Connell, 1975).

Chemical crosslinking of starch can be carried out via (I) reaction with different reagents (bi or multifunctional), (II) end-functional macromonomers polymerization, and (III) click reactions between different polymeric groups (Imamura et al., 1989; Liu et al., 2010; Miyata et al., 2002; Lu et al., 2009; Lu et al., 2011; Martin & Avérous 2001).

5.2.1 Crosslinking Starch/Polymer Reactive Systems

Starch is hydrophilic, and most polymers are hydrophobic and thermodynamically immiscible, with starch causing phase separation and incompatibility, resulting in poor mechanical properties. That can be improved by molecule bonding for the most promising results. Narayan et al. (1999) reported the preparation of a starch-PCL (polycaprolactone) blend. They reported the three different routes of ε-caprolactone monomers polymerization in the presence of starch.

1. The polymerization reaction was carried out between 100–150 °C in the molten state in the presence of stannous octoate (a Lewis acid catalyst). With this method, monomer conversion can be achieved very high (>98%). Still, the limitations are longer reaction time (3 h), formation of oligomers (both linear and cyclic), and lower grafting efficiency.
2. PCL polymerized with starch in the presence of alkoxide. Aluminum alkoxide results in a conversion of 30%, and if the same reaction is carried out in the presence of titanium oxide, it can achieve a 98.5% conversion. With a molecular weight ranging from 25,000 to 4,00,000 of PCL, high mutations can be acquired by a reactive extrusion process for 1–3 minutes residence time. Some patents mention a temperature of 180 C during the extrusion process and discuss the effects of the acid value of monomer, residence time, and screw configuration on molecular weight. It is reported in one patent that the starch PCL blends were synthesized in 3 extrusion steps: (1) homopolymerization of ε-caprolactone in the presence of aluminum alkoxide, (2) extrusion of starch plasticizer pallets, (3) extrusion blending of materials from previous steps. These films are currently marketed under the name 'Envar.'
3. PCL polymerization with starch in the presence of catalyst triethyl aluminum. It is reported as the fastest batch reaction with grafting efficiency (up to 95%) and higher conversion (>99.9%). In situ, the triethyl aluminum reacts with starch to form starch–aluminum alkoxides, which act as the initial species for this polymerization (Ayoub & Rizvi, 2009)

5.2.2 Crosslinked Starch/Starch Systems in Aqueous Suspensions

Hydrogen bonding and chemical reactions can make bonding two starch molecules possible. When starch molecules are heated in water, hydrogen bonds may get destroyed or weaken, and the molecules depart. Crosslinking bridges the molecules, so molecules will remain intact. The crosslinking reactions are carried out in a granular state, so the chemical bonds introduced will be much less than the anhydrous glucose units present or the amount of starch used. Usually, these reactions are carried out at 50 °C in atmospheric conditions except a few. Different researchers have reported so many bi or polyfunctional agents. Only bi and polyfunctional groups carry out the reaction between polymer chain functional groups during crosslinking. Bi-functional will connect two polymer chains, whereas polyfunctional would be able to react with more than two, generating a higher degree of crosslinking. In many reactions, an alkaline medium like sodium hydroxide solution is used to promote crosslinking. The alkaline conditions are maintained below the level that peptizes or swells starch. The exception is crosslinking with aldehydes and citric acid, which is carried out with an acidic medium (Ayoub & Rizvi, 2009). After achieving the desired degree of crosslinking, the reaction chain can be stopped by changing the pH of the mass. After neutralizing the mass, the starch is filtered and washed with solvents or water to remove unreacted reagents, salts, byproducts produced by the side reactions, etc.

The characteristics of crosslinked starch to pH variations and shear highly depend on the type of crosslinking agent used. However, from reagent to reagent, this variation is quite considerable. Crosslinking with distarch, glycerol will attach with hydroxyls of starch via ether linkages and produce crosslinked starch with high resistance to acid, alkali, or enzyme attack. It can withstand pH variation and mechanical shear. In crosslinked starch, it becomes difficult to analyze the substitution. Mitchell et al. (1982) have developed a gas-liquid chromatographic method for determining adipate levels in distarch adipate. Koch et al. (1982) have developed an improved method for detecting the levels of phosphorus associated with distarch phosphate. Researchers also developed some methods where no reagents are used (Ayoub & Rizvi, 2009)(Wurzburg, 1986).

The functional groups exist in polymer precursors or can be added to the chain to prepare it to react. Different higher and lower molecular weight crosslinking agents (such as dialdehyde, epoxy compounds, formaldehyde, glutaraldehyde, etc.) are used (Denizli et al., 2004; Gulati et al., 2011; Ramamurthi & Vesely, 2003; Zhu, 2010). When crosslinking is carried out physically, the interaction between the opposite charge or hydrogen bonding between macromolecules is incorporated (Hoare & Kohane, 2008). In chemical crosslinking, the product macromolecules show uniform composition and perfect structure and composition, but most crosslinking agents are toxic. Comparatively mild conditions are utilized in physical crosslinking. For this reason, they could be utilized in drug delivery or other pharmaceutical applications (Hoffman, 2012; Jobling, 2004).

5.2.3 Physical Crosslink Starch

One of the easiest ways to synthesize crosslinked polymers is physical crosslinking. Ease of method and no need for any additional crosslinking agent is attracting researchers. These polymers are also known as self-assembled polymers. The macromolecules participating in physical crosslinking may show various interactions like H-bond, ionic & electrostatic interaction, polyelectron complexation, hydrophilicity, hydrophobicity, etc., reversible interactions depending on physical conditions (Hennink, 2002; Rosiak & Yoshii, 1999; Singh et al., 2017). The crosslinked starch or its copolymers prepared via this method are not using any toxic crosslinks, making it suitable for biomedical applications like drug delivery, tissue engineering, etc. (Xiao, 2013; Xiao & Yang, 2006; Xiao et al., 2010). Different processes are used to prepare physically crosslinked starch and its copolymers, like heat-induced aggregation, complex coacervation, freeze-drying, freeze-thawing, etc. Bhatia et al. (2013). Not only starch but starch copolymers are synthesized by physical crosslinking by different researchers with various biodegradable synthetic macromolecules. Mehling et al. (2009) prepared a starch-based aerogel microsphere by crosslinking. Different researchers have observed that the gelation of native starch is simple Shen et al., (2012), By heat treatment, hydration begins in starch, bringing about gelation (García-González et al., 2012; Glenn et al., 2008). Tan et al., (2012) and García-González et al. (2012) combined gelating conduct with emulsion for starch aerogel synthesis. Roy et al. (2009) and Calinescu et al. (2012) noted that various properties (e.g., release properties, swelling, etc.) of crosslinked starch can be alterable by adjusting the non-ionic groups. Peppas et al. (2006) and Kim et al. (2015) prepared starch-PVA ecofriendly macromolecules by repeated freezing/defrosting and physical blending processes. Ishigaki et al. (1999) blended PVA–starch plastic film and studied its biodegradability. Jayasekara et al. (2004) also prepared the same blend and studied its surface and other characteristics. Bourtoom and Chinnan (2008) prepared rice starch–chitosan biodegradable film by physical blending. One of the main aims of the physical blending method is to enhance biodegradability by maintaining other properties along with cost-effectiveness (Mani & Bhattacharya, 2001; Ratto et al., 1999). In respect of biodegradability, other than starch, from synthetic polymers, PLA (polyacetic acid) is a good polyester and has wide use in biomedical sectors. Research has attempted to produce hydrogel by blending starch polysaccharides and PLA (Jun, 2000; Wang et al., 2007).

Because the nature of PLA molecules is hydrophobic and starch is hydrophilic, their interfacial interaction will be lower. So, the mechanical properties of the blend will be poor (Wang et al., 2008). It can be enhanced by using plasticizers like formamide, sorbitol, glycerol, etc. (Li & Huneault, 2011). A suitable compatibilizer may also be applied to raise compatibility among starch and PLA. PVA can also be used as a compatibilizer, developing a continuous phase between starch and PVA during blending because both are polyol (Ke & Sun, 2003). It has been reported that the interfacial interaction between starch-PLA is poor. Starch works as a filler and is loaded in a PLA matrix without PVA. One more method used to raise interfacial affinity is gelatinization. Park et al. (2000) noted that starch granules get dissolve easily and make good dispersion.

One more water-soluble synthetic polymer holding good biodegradability and is compatible with starch is Polyvinyl alcohol (PVA). The bonds between the –OH groups of PVA and the chain of starch result in excellent processability and mechanical properties (Haschke et al., 1998; Lawton, 1996). Tudorachi et al. (2000) reported that the tensile strength is improved with increased PVA concentration. All these characteristics make PVA more suitable to blend with starch (Kato et al., 2003). Jang et al. (2007) studied that PVA/starch blend polymer is completely biodegradable.

Starch was blended with polycaprolactone (PCL, a biodegradable and eco-friendly polymer), and the degradation of blends was studied by Di Franco et al. (2004). Vertuccio et al. (2009) reinforced PCL/starch blends with nano-clay and fiber reported improved biodegradability and hydrolytic stability. Some biopolymers are also studied blended with starch to synthesize physically crosslinked macromolecules. Polysaccharides such as chitosan and cellulose are blended with starch to get physically crosslinked. These polysaccharides are readily available, renewable, non-toxic, and inexpensive (Zhai et al., 2004). Starch contains many -OH groups, and chitosan contains -NH_3 and -OH groups. Due to bonds between these groups, blends got crosslinked. The blended copolymers show superb film forming and mechanical properties. Many researchers studied starch-chitosan blends (Arvanitoyannis & Biliaderis, 1998; Garcia et al., 1999; Lazaridou & Biliaderis, 2002; Lourdin et al., 1995; Mali & Grossmann, 2003). The properties of these blends depend on the ratio of starch and chitosan or other polysaccharides (Bourtoom & Chinnan, 2008). Psomiadou et al. (1996) prepared edible films from microcrystalline cellulose (MCC) and methylcellulose (MC) with corn starch and polyols using different plasticizers such as glycerol.

5.2.4 Synthesis of Crosslinked Starches by Radiation Techniques

Many researchers have applied radiation methods such as electron beam or gamma irradiation to modify starch in liquid or solid-state, especially in grafting. Ionizing radiation may degrade starch molecules and process yield by chain scission and crosslinking. It is reported that if scission has been predominant by crosslinking, the high-purity insoluble microscopic gel is formed. This is an advantage of the radiation method over the traditional one. Many novel hydrogels are developed by researchers with radiation methods, which do not need any additives. It is also observed that crosslinking can be enhanced by incorporating water in radiation techniques. Hydrogels are formed by crosslinking of starch by radiation. The essential feature of hydrogel is to swell and hold water (or sometimes other solvents) in its network structure. This swelling characteristic of hydrogels is a significant property in deciding its applications. The swelling property is measured using deionized water and 0.9% NaCl, which decreases with increased irradiation dose and polymer concentration. It is reported that the hydrogel prepared with 40% crosslinking, with 2 kGy energy, absorbed about 500 g of distilled water. The structural changes are not affecting swelling properties only but also biodegradability (Ayoub & Rizvi, 2009). Biodegradability decreased, which is an advantageous feature for medical and agriculture applications (Zhao et al., 2003). It has been reported that the biodegradation of unirradiated and crosslinked starch by controlled compost was about 43.3% and 39.5%, respectively. Compared to cellulose powder, starch, even crosslinked starch, shows degradation faster. So in other words, crosslinked starch, when discarded, biodegradation occurs naturally in the soil, so this material becomes more acceptable even after crosslinking (Fei et al., 2000).

5.3 Crosslinking Agents for Starch

There are a variety of reagents reported for crosslinking of starch. United States FDA permitted some food-grade crosslinking agents: adipic acetic mixed anhydride, phosphoryl chloride, sodium trimetaphosphate (STMP), and mixtures of STMP and sodium tripolyphosphates (STPP). Previously used epichlorohydrin for crosslinking starch is out of use nowadays due to carcinogenic properties. Food and packaging industries are showing interest in citric acid and malonic acids for crosslinking starch (de Oliveira et al., 2014; Shah et al., 2016; Singh et al., 2012).

Starch contains two types of –OH groups, primary and secondary. Primary hydroxyl groups are 6-OH, and secondary are 2-OH and 3-OH. For crosslinking of starch, these hydroxyl groups are to be reacted with multifunctional reagents. Typical starch molecules have many closer and entangled chains, so reactions occur between single or side chains hydroxyl groups. Ether or ester bonds are formed by crosslinking reagents between hydroxyl functional groups of starch chains. Significantly less quantity of multifunctional agents is enough for the crosslinking reaction between starch chains (Koo et al., 2010; Shah et al., 2016; Singh et al., 2012; Whistler, 2012; Whistler et al., 2012).

5.3.1 Crosslinking with Phosphorous Oxychloride

Crosslinking of starch with phosphorous oxychloride is a rapid crosslinking that results in distarch phosphate. The efficiency of this reaction is highly dependent on pH. The most efficient crosslinking can be achieved with an alkaline medium with a pH above 11. It is reported that using 2% w/w sodium sulfate may improve the rate and efficiency further (Gao et al., 2014; Shah et al., 2016; Singh et al., 2010).

In phosphoryl chloride, there are three chlorides. Phosphoryl chloride will produce phosphorous dichloride immediately with water by one chloride ion (hl (half-life) 0.01 s), which is likely crosslinking agent. The remaining two chloride ions (hl = ~4 min) of phosphoryl chloride will react with water simultaneously. All three phosphoryl chloride ions may not have enough time to diffuse into the starch molecules. So practically, phosphoryl chloride should generate phosphorous dichloride as fast as possible in starch solution in situ, which can diffuse into starch to crosslink it (Ayoub & Rizvi, 2009; Cui, 2005; Delley et al., 2012; Shah et al., 2016; Singh et al., 2012).

5.3.1.1 Crosslinking of Corn Starches with $POCl_3$ Under Ultra-High Pressure

$POCl_3$ is reported as a crosslinking agent by Kim et al. (2015), and the method is as follows: suspend 20 g starch in 10% w/w solution of anhydrous sodium sulfate in 80 ml double distilled water with rapid stirring. After adding 0.1% $POCl_3$ (w/w), add 1 M NaOH to adjust pH to around 11.5 and maintain 45 °C for 120 min. to complete the reaction, add 1 M HCl to bring the pH of the mass to 5.5 and separate the polymer. It has also been reported that ultra-high pressure crosslinking reaction completed within 15 min at 400 MPa (Shah et al., 2016).

5.3.2 Crosslinking with Sodium Trimetaphosphate

The reaction for crosslinking of starch by sodium trimetaphosphate is shown in Fig. 5.1. Sodium trimetaphosphate (STMP) is a non-hazardous reagent for crosslinking, whose reaction rate can be enhanced with rising pH or using sodium sulfate (Cui, 2005; Singh et al., 2012).

5.3.2.1 Preparation of Crosslinked Corn Starch

The use of co-reagents STMP/STPP (99/1% w/w) for crosslinking of starch is reported by shi et al. (2013). The co-reagent mentioned above is added to an aqueous starch solution, and pH is adjusted to 11.0 using. 1 N sodium hydroxide. A shaking water bath maintains the mass at 45 °C for 3 h. With the help of 0.1 N HCl, crosslinked starch suspensions are neutralized before washing and drying at 40 °C for 24 h in the oven (Shah et al., 2016; Shi et al., 2013; Zhang et al., 2014).

5.3.3 Crosslinking with Adipate

Figure 5.2 shows crosslinking of starch with adipate, a rapid reaction in mild alkaline conditions (pH 8). A mixture of acetic acid/ adipic acid anhydride is used to maintain the pH of the solution. In this reaction, along with crosslinking, hydroxyls

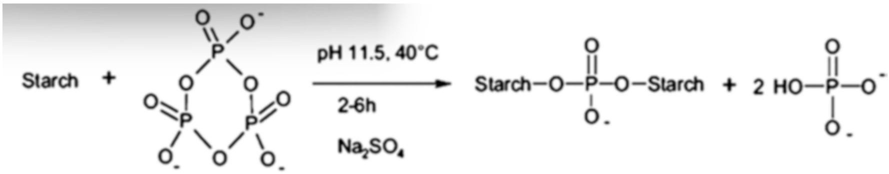

Fig. 5.1 crosslinking of corn starch with sodium trimetaphosphate

are acetylated. These simultaneous reactions prepare double-modified starch. As this reaction is rapid and needs very few qty. of reagents, so challenging to measure the extent of crosslinking. If a phosphorylating reagent is used, the ^{31}P-NMR methods can help find a crosslinking degree (Cui, 2005; Salar et al., 2013; Shah et al., 2016).

5.3.4 Crosslinking with Epichlorohydrin

Zhang et al. (2014) carried out crosslinking of starch with epichlorohydrin. Epichlorohydrin is taken in a small ratio with starch in solution and stirred for 17 h at 40 °C. The reaction can be captured by adding 3% HCl solution to raise pH to 5.25. this reaction occurs in a series of steps. Starch can produce multifunctional products with epichlorohydrin. As the reaction proceeds, one or two molecules of epichlorohydrin will be consumed by every single link (Shah et al., 2016; Zhang et al., 2014).

5.3.5 Crosslinking with Malonic Acid

Malonic acid is a crosslinking agent in the presence of the catalyst sodium hypophosphite monohydrate (Nahyp). Corn and potato starch is used for crosslinking following gelatinization. Ghosh reported that the degree of substitution (DS) increases with residence time and temperature. Compared to corn starch, potato starch was found to be crosslinked.

5.3.6 Crosslinking with Citric Acid

To develop biodegradable crosslink starch, citric acid is under focus as a crosslinking agent for various applications like packaging and food. The concentration of citric acid is selected based on the requirement; however, the maximum

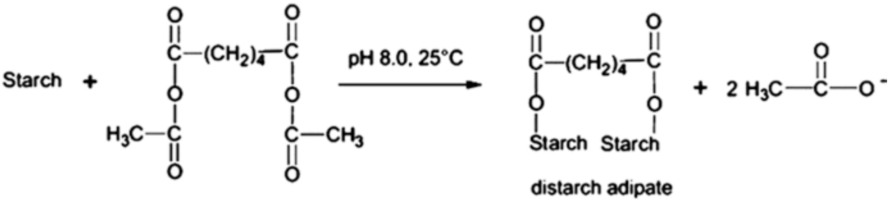

Fig. 5.2 Crosslinking of starch with adipate

concentration reported for starches is 60%. In brief, citric acid (based on dry starch weight) is dissolved in distilled water, and the pH is adjusted to 3.5. Once pH is stabilized, a weighted quantity of starch is mixed manually with the solution and left for 16 h for reaction at 30 °C. The treated starch is then centrifuged, dried, and left at 130 °C for 2 h for crosslinking. The crosslinked and dried starch is finally washed to remove the yellowish color of unreacted citric acid.

Many researchers report citric acid for self (photopolymerization or self-polymerization) and crosslinking with other polymers like chitosan, cellulose, etc. The crosslinking process remains the same, like gelatinization followed by crosslinking. Citric acid has been reported for photopolymerization and to react with other polymers. The reaction mechanism is the same when a reaction is carried out because polysaccharides have similar functional groups. Their crosslinking degree can be measured using viscosity behavior.

5.4 Effect of Crosslinking on Starch Properties

The degree of crosslinking of starch depends on many parameters such as reaction time, temperature, pH, type and concentration of the crosslinking agent, the treatment of raw starch, preparation of starch for the final application, and many more. When the degree of crosslinking is shallow, the extent and yield of crosslinking of starch become challenging to measure chemically; hence, there is a need for physical property measurement (Zhang et al., 2014). Researchers observed the connection of changes in swelling, optical clarity, pasting curves, and fluidity of granules with the degree of crosslinking, so these properties can be utilized to judge the extent of crosslinking (Singh et al., 2012).

Among various properties, viscosity and swelling are the most useful properties for estimating the extent of crosslinking. Increase the level of crosslinking, and the swelling of granules will be reduced. The crosslinks prevent granules' swelling, so the starch molecules cannot even gelatinize (Chantaro et al., 2013; Cui, 2005). The viscosity of starch can be measured/plotted with the help of RVA (Rapid Viscosity Analyser). On RVA, Chantaro et al. (2013) noted that the peak viscosity of native starch rises with crosslinking. The presence of additional covalent bonds in crosslinked starch enables the starch molecules to resist a temperature of 95 °C and not dissolve before 30 min (Shah et al., 2016; Zhang et al., 2014).

In RVA, the viscosity breakdown of native starch is found more than crosslinked starch. Due to crosslinking, the bonding between molecules increases, increasing mechanical strength and keeping the swollen granules intact. Hence, the molecules resist mechanical shear and show less reduction in viscosity. (Cui, 2005). Peak viscosity was found to lower with an increasing crosslinking degree. It may be due to an increase in crosslinking agent and bond concentration. It also depends on time and temperature during crosslinking reactions. As crosslinking degree rises, the granule volume may reduce, and the viscosity also reduces. So higher crosslinked granules show lower peaks compared to lower crosslinked starch.

Amylograms of corn starch (native and crosslinked) are shown in Fig. 5.3. Cui, (2005) noted that the highest peak viscosity could be achieved for starch crosslinked with phosphoryl chloride, even with the lowest concentration. A steady decline in peak and excellent paste viscosity is observed with an increasing quantity of phosphoryl chloride.

At higher swelling, the starch molecules become fragile. Upon heating or agitation for a prolonged period, swelled granules fragments. Due to the fragmentation of granules, a rapid fall of viscosity can be seen on the RVA plot. Acid treatment of starch shows the rapid breakdown in viscosity in RVA. Kahraman et al. (2015) observed that crosslinking bonds would help resist this structural damage. And so, crosslinked starch can withstand acidic conditions and will not get rupture. During the study of the effects of pH, the RVA viscosity curves studied for crosslinked and native waxy corn starch cooked at pH 5 and 7 are the same. Upon cooking, crosslinked waxy starch shows a stringy, rubbery, and cohesive texture, whereas crosslinked corn starch shows a paste-like structure with good rheological properties, making it more suitable for applications as a thickener in food (Shah et al., 2016).

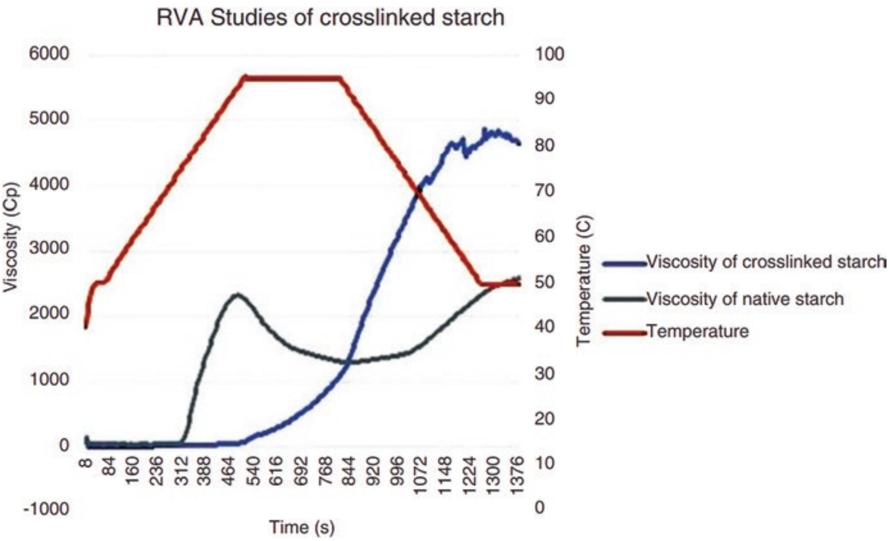

Fig. 5.3 Amylograms of native and crosslinked corn starch

5.5 Determination of the Degree of Crosslinking

For measurement of the degree of crosslinking of modified starch, a viscosity-based method is reported by (Kaur et al., 2004). RVA is used to measure the viscosity of 25% starch solution. In place of RVA, starch pasting cells and a controlled stress rheometer can also be used. One can get a degree of crosslinking as follows:

$$Degree\,of\,crosslinking = \frac{(A - B)}{A} \times 100$$

where A is the peak viscosity of the control sample (without a crosslinking agent), and B is the peak viscosity of the crosslinked starch.

To study the viscosity behavior of native and modified starch, RVA was used. In RVA, heating and cooling cycles are controlled via programming. A measured concentration of the starch solution is placed in RVA. Starch solution temperature increased from 50 to 95 °C at a constant rate of 11 °C/min and was maintained at 95 °C for 2 min. Then cool of paste to 50 °C at the same 11 °C/min rates and maintain 50 °C for 2 min. During the complete cycle, viscosity is monitored. The peak viscosity of the sample is also shown in the amylograph of RVA (Shah et al., 2016; Chan et al., 2012).

5.6 Application of Crosslinked Starches

There are many applications suggested by researchers for crosslinked thermoplastic starch-based materials. But in the polymer market, it is used as loose filler material, not in other applications. Possible reasons for the scenario are shorter shelf life, poor mechanical strength, and dependency of swelling on relative humidity. The ageing of hydrophilic polymers is well known; at lower and higher partial water pressure, the crakes and crazes are developed, resulting in damage to the structure. Due to this phenomenon, this polymer shows crystallization above the glass transition and physical ageing below it. During storage, the crystallinity type may also get change. The kind of crystallinity was revealed by X-Ray diffraction. It has been reported that the origin of starch, amylose contents, and the cross-linking transformation process affect crystallinity. To overcome this issue, some researchers have reported the influence of photo-crosslinking on the degradation of crosslinked starch (Nagasawa et al., 2004).

Processes for crosslinking starch without using crosslinking agents consist of two steps: starch film extrusion followed by UV exposure, which will crosslink the film. Here homogeneous crosslinking is claimed by researchers. It is an innovative product to produce modified starch to use as an excipient for the controlled release of drugs, thickeners, and neutral hydrogels; by variation in exposure time to UV, a variety of crosslinking densities can be obtained. The process is crosslinking starch in solid form; it is a specialty. Crosslinking densities of the prepared starch are a function of various parameters like destructuring agents (like NaOH), plasticizers, and macromolecule accessibility (Seow et al., 1999; Clerici, M. T. P. S. 2012; Zeleznak & Hoseney, 1987). The following are the observations:

1. The kinetics acceleration is a function of plasticizers used
2. A higher concentration of plasticizers used conventionally reduces the probability of macromolecular interactions resulting in the reduction of crosslinking densities.
3. The reactivity of crosslinking reaction increases in an alkaline medium. In the case of an alkali like NaOH can enhance the accessibility of starch to photo additives, such as samples plasticized with sorbitol.
4. Starch is grafted with part of sensitizer derivatives
5. Young modulus and ultimate strength of the crosslinked films are enhanced. However, the crosslinking rate governs this enhancement. The insolubility and variation in viscosity are exciting properties of these newly developed modified starches.

Nowadays, advancements in processing and applications are leading to a rising requirement for additional item transformations and refinement, albeit perhaps of the earlier application, the adhesives from starch have generally kept up with their situation. The paper industry has explored the huge scope of cationic starches as a wet-end additive. This advancement has been emphatically upheld by reusing and recycling wastepaper. Starch as a part of food items is an everyday encounter for everybody. For modified starches, consideration these days is centered around such properties as expansion, texture, shelf-life, stability, and thickening. In the textile industry, starch and its derivatives are used as an easily removable temporary processing aid for principal applications such as fabric painting and warp sizing. Over and above these four areas, some more application areas where starch and its products are applied as a flocculant in the process for potable water purification, oil and gas deep-well drilling to control fluid loss, paste clarity improvement, adaptation in ionic character, alteration in solution consistency, improvement of stability, adjustment of viscosity, etc. Modified or Crosslinked starch is used to introduce properties like hydrophobicity, thermal stability, chemical reactivity, salt tolerance improvement, resistance to fermentation, rate of dissolving, etc. As an adhesive, it is used in the winding, laminating, formulators, wallpaper, paper bags, gumming, corrugated boards, etc. Paper industries use Crosslinked or modified starch for coating, surface sizing, wet end, etc. Crosslinked starch is used in various food items such as dairy & bakery products, infant foods, extruded products, confectionary items, convenience products, and preserved foods. Modified starch is widely used in many other industries like ceramics, personal care, biodegradable plastics, dry batteries, soil erosion prevention, agglomeration, mining operations, construction, water treatment, oil & gas industries, etc. (Kraak, 1992; Reddy & Yang, 2011; Reddy & Seib, 2000; Rouse et al., 1996; Sreedhar et al., 2006).

5.6.1 Crosslinked Starch in Pharmaceutical Applications

Controlled release of the drug pre-loaded in nano molecule was observed by involving indomethacin as the model drug. Starch nano molecule grafted on fatty acid was viewed as a decent vehicle for controlled oral drug conveyance. Simi and Emilia Abraham (2007), in one study, metformin hydrochloride was added to the CHI-ST solution to synthesize drug-loaded beads prior to expelling into sodium hydroxide-methanol solution. Drug release studies in the acidic and alkaline medium were carried out by utilizing UV-VIS spectrophotometer, demonstrating that the release is sustained over a long time. The results recommend that CHI-ST crosslinked beads can be used to control drug release (Kumari & Rani, 2011). Kenawy et al. (2014) reported that the release profile study, degradation, thermal properties, mechanical, morphological, and physicochemical, and showed that the expansion of hydroxyethyl starch (HES)- PVA hydrogels are supposed to work on utility as hydrogel film for biomedical applications, explicitly for wound dressing application. Cross-linking philosophy has been applied to foster another controlled-release drug delivery system. Cross-linking on soft gelatin capsules (SGC) was carried out by cross-linking agent into the gelatin shell or fill material. Disintegration studies were performed to exhibit various degrees of cross-linking. Chitosan films were crosslinked with dialdehyde starch as a crosslinking agent. It is reported that the water-swelling and mechanical properties of the films were enhanced. The crosslinked films were analyzed by FTIR and Xray, which showed that Schiff's base formed, obliterating chitosan's crystallization.

Additionally, the antimicrobial effects of all the crosslinked films on *S. aureus* and *E. coli* remained unaffected (Tang et al., 2003). Various crosslinked waxy-corn starches and pregelatinized crosslinked waxy-corn starches were assessed as binding agents in a conventional tableting wet granulation process. The crosslinked starches perform the same as native corn or waxy-corn starch (Visavarungroj et al., 1990a, b; Visavarungroj et al., 1991). Various kinds of crosslinked starches and pregelatinized-crosslinked starches were assessed for their utilization as hydrophilic matrices. A crosslinked-starch film with gelatin containing ZnO-loaded halloysite nanotube and bacterial nanocellulose for wound healing applications (Farnaz Jaberifard et al., 2022). The tensile strength of crosslinked starch fibers improved by almost 10 times. The membranes of crosslinked starch fibers are non-cytotoxic. So, they might find applications in tissue designing, drug delivery, clinical treatment, etc. (Wang et al., 2016).

5.6.2 Crosslinked Starch in Flocculation Application

A novel flocculant (CATCS) from corn starch and chitosan was synthesized, and its flocculation performance was examined (You et al., 2009). Wang et al. (2017) studied Crosslinked starches as an effective and recyclable adsorbent for wastewater treatment.

5.6.3 Crosslinked Starch in Food Applications

Crosslinked, etherified, and/or esterified starches have been broadly utilized for different food items, such as breads and cakes, snack foods, etc., to enhance their quality. In bread making, the quality and texture of dough and bread are affected by starch (Miyazaki et al., 2006; Omoregie Egharevba, 2020). Crosslinked starch is applied as a thickener and texturizer to prepare dairy and bakery products, gravies, sauces, and soups. It is also used to expand bread and mixture items, fill in canned foods and fruit pies, and enhance rheological properties (Korma et al., 2016; Abbas et al., 2010; Van Hung & Morita, 2004; Omoregie Egharevba, 2020). Crosslinked starches show quite high resistance to oven-baking temperatures. High-density bakery products like cakes, bread, pies, biscuits, wafers, and sausages use crosslinked starches as heat resistant. Gelatinized starch influences pasta delicateness and versatility, edibility, and tastiness. Crosslinking gives the required primary immovability to the past (Omoregie Egharevba, 2020). Pregelatinized starches are utilized to retain water or accomplish thickening without heat in instant breakfast foods, puddings, ready-to-use bread mixtures, and instant lactic mixtures (Michael & Brown, 1968; Omoregie Egharevba, 2020).

5.6.4 Crosslinking-Modified Starch as Fat Replacer

Fat replacer is a development created over the most recent couple of many years, which is utilized as a substitute for fat, partitioned into two sorts, to be specific fat substitutes and fat mimetics. Crosslinked starch is fascinating to keep on being grown, particularly as a fat replacer in different food items. Rice starch is utilized in Low-fat cream, sweet potato starch in Low-fat ice cream, corn starch in Low-fat mayonnaise, and cassava starch is utilized in Low-fat biscuits (Subroto et al. (2020).

5.6.5 Crosslinked Starch in Some Other Applications

A biodegradable fertilizer made of blends of starch and chitosan is proposed (Perez & Francois, 2016). Valodkar and Thakore (2010) studied isocyanate crosslinked reactive starch nanoparticles for thermo-responsive conducting applications. El-Tahlawy et al. (2007) prepared starch microcellular foam (SMCF) from crosslinked native corn starch with glutaraldehyde using a solvent exchange technique. Im et al. (2021) studied Crosslinked starch for numerous geotechnical fields like road construction and pavement. One such model would be in the development of unpaved roads in non-industrial nations. The binding strength of starch could be improved for soil reinforcing with the utilization of crosslinking. Shogren et al., (2003); Valodkar & Thakore, (2011); Dou et al. (2015) prepared a dialdehyde starch crosslinked feather keratin film to be used in food packaging applications. Many of the less recognizable applications get away from consideration due to their low-volume, solitary, or obscure nature. In synopsis, obviously due to the extraordinary variety of its modern applications, starch – and not least potato starch – has a place with perhaps the most flexible and versatile polymer.

5.7 Conclusions

Starch is one of the most promising carbohydrates. By crosslinking it, many properties can be varied, makings it more applicable. There are many crosslinking agents used by different research groups. Still, some of the properties make starch not usable. Further research in this direction may open doors for many applications in various areas. Many groups are working on the modification of starch by various methods. Nowadays, nano starch is used in many applications. It is showing promising results. It can be studied by crosslinking how the properties can vary to make it more suitable. Soon, nano starch modification and crosslinking will be studied.

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Chapter 6
Octenyl Succinic Anhydride Modification of Starch

Babatunde Olawoye, Oyekemi Olabisi Popoola-Akinola, Timilehin David Oluwajuyitan, Deborah Bolutife Adetola, Adenike Esther Adeloye, and Adedotun Alexander Ayodele

6.1 Introduction

Starch comprises macro-constituents of several food products as well as its properties coupled with its association with various constituents majorly lipids and water which are of interest in the food as well as pharmaceutical industries and human nutrition. Their functionality as well as form varies amongst botanical species, hence, providing starches having widespread properties and can as well be detrimental during processing as a result of raw materials inconsistency (Tang & Copeland, 2007). The prediction of the functionality from the structural knowledge and the interaction of starches with other food components is still a challenge in food science as well as nutrition in addition to industries producing starch. The suitability of starch for a specific food processing and/or its nutritional characteristics is dependent on the understanding of the environment as well as genetic factors controlling the biosynthesis of starch as well as the morphology of granules and also on the process of the material. In diets, starch makes up 50 to 70%, as it is an essential component of the brain as well as blood cells which are utilized in the generation of energy. The derivation of starch from products of agriculture is regarded as an important criterion in developing humans (Gamage et al., 2022), even though excessive intake of Rapidly digestible starch (RS) may be associated with some illnesses. Several isolations have been done to extract starch, root and tuber crops from various cereals (for instance, products from bakeries, snacks, baby

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B. Olawoye (✉) · O. O. Popoola-Akinola · D. B. Adetola · A. E. Adeloye · A. A. Ayodele
Department of Food Science and Technology, First Technical University Ibadan,
Ibadan, Nigeria

T. D. Oluwajuyitan
Department of Food Science and Technology, Federal University of Technology Akure,
Akure, Nigeria

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foods, meat products, replacers, confectionaries, beer, soups, sauces, snack foods, sugar syrups, coffee whitener and soft drinks constituting roughly 60% of these products and constitutes about 40% in non-edible and pharmaceuticals such as seed coatings, fertilizers, textiles, adhesives, diapers, fibres, paint and paper industries, bioplastics, cement, building materials as well as drilling of oil (Burrell, 2003). There is a need for ensuring fitness for purpose for starches having a great range of functional properties needed to produce a wide range of end products. Starch exists in discrete particles called starch granules, and these granules are of different shapes and sizes depending on the source of the starch, consisting of concentric shells often known as alternating crystalline and amorphous growth rings. The crystalline region is composed of amylopectin, which constitutes 75% of the starch, and the amorphous region is composed of amylose, which constitutes 25% of the starch. Starch has unique chemical and physical properties and is a predominant food reserve in plants providing 70 to 80% of calories consumed by humans worldwide. It is not sweet, not readily soluble in cold water and forms paste and gel in hot water. Starch functions as a thickener in tomato paste, an adhesive in the paper industry, a dusting agent in the dough, a stabilizer or emulsifier, a bulky agent in ice cream and yoghurt, gelling material in ham and a binding agent in extruded products. Native starches produced from various plant sources possess functional deficiencies like insolubility in cold water, poor colour, and sensitivity to temperature, pH, shear and retrogradation, limiting their industrial application. Due to these shortcomings, native starch is modified to make it more applicable in the food industry. For modification, starch may be treated chemically, physically, or enzymatically to produce a paste that can withstand heat, shear and acid associated with a particular processing condition and to introduce specific functionalities like the increase in solubility, enhancement of pasting clarity, improvement in the interaction with other substances, enhancement of gel formation and gel strength, etc. Also, two or more modification methods can be combined to produce modified starch with certain functional properties.

Esterification is a chemical method of modifying starch, and it has been found that with the use of dicarboxylic acids, starch can impart hydrophobic properties but can be better obtained with the use of octenyl succinic anhydride (OSA), which is known as OSA starch. The presence of hydroxyl (OH^-) groups of glucose units is modified by octenyl succinic anhydride (OSA) in an aqueous dispersion. An amphiphilic property is obtained, constituting both hydrophobic and hydrophilic groups by esterification when octenyl succinic anhydride (OSA) is introduced in a starch chain. Octenyl succinic anhydride starch is produced by partially replacing the hydroxyl (OH^-) group in starch with the hydrophobic components, thereby providing the starch with amphiphilic and interfacial properties. This reaction is commonly carried out in an aqueous state under a moderately warm alkaline condition.

Structure and pasting property of oat starch

6.2 Synthesis of OSA Starch (with an Emphasis on Recent Advances in All the Forms of Modification)

6.2.1 *Conventional Method of Synthesis (Aqueous Medium)*

Using OSA for starch esterification entails partially substituting hydroxyl groups of starch with hydrophobic octenyl group imparting starch molecules an amphiphilic nature. The esterification of starch using OSA yields octenyl succinylated starch. (Caldwell & Wurzburg, 1953) originally patented starch esterification to introduce hydrophobic groups, producing derivatives have emulsifying characteristics. These authors demonstrated the chain reaction of polysaccharides (such as dextrins, cellulose and starch) esterified by substituting cyclic dicarboxylic acid anhydride. They described three routes including the technique using an aqueous medium, a dry technique involving a reaction in the absence of water between starch that was previously treated with alkali and an organic suspension technique, which utilizes a liquid (organic) in place of water as its medium of dispersion. Synthesis of starch derivatives using OSA has been done traditionally in an aqueous medium under mild alkaline conditions. It involves action in an aqueous slurry with pH varying between 7 and 11. Table 6.1 illustrates the value of reaction conditions used for OSA modification of starch under mild alkaline conditions. After the reaction is completed, it undergoes filtration or centrifugation, and products are washed in water and ethanol or acetone and then dried and ground. The digestible starch (DS) s utilized in expressing the modification extent. It is the average number of hydroxyl groups per repeating unit that have been replaced by a given substituent, which is defined by the number of hydroxyl groups in the repeating unit, which can be

Table 6.1 Values of reaction conditions used for OSA modification of starch under mild conditions of alkaline

Starch type	(%w/w, starch basis)	pH	Temp (°C)	OSA	Time (h)	DS	Reference
Corn	30	8.5	253		6	0.018	Simsek et al. (2015)
Wheat	30	8.5	25	3	6	0.022 0.0181	Eliasson et al. (2013)
Potato	35	8.5	35	3	2	0.017	Wang et al. (2016)
Unripe plantain	30	8.5	25	3	6	0.011 150.038	Bello-Flores et al. (2014)
Pearl millet	35	5	35	3	2	0.016	Sharma et al. (2016)
Cassava	30	8.5	35	1.5 3 6 9	3	0.008 0.018 0.029 0.035	Zhang et al. (2017)
Ginkgo	30	8.5	35	1.58 3		0.006 0.017	Zheng et al. (2017)

chemically modified. The value of DS is determined using titration (Sharma et al., 2016). However, quite several authors have exploited nuclear magnetic resonance spectroscopy for applications in physical sciences as well as in the food industry. It is used when structural information about a molecule is required. OSA-modified starch is allowed by the Food and Drug Administration as an agent in food. Modifying starch with 3% OSA gives a theoretical degree of substitution of 0.0231, (the theoretical degree of substitution is obtained assuming that the total amount of reagent added (epoxide and chlorohydrin) reacts completely with the polymer) provided that there is a reaction of starch with the entire OSA. However, the theoretical degree of substitution is not reached easily with most authors reporting lower values. The reaction efficiency expresses the substitution proportion achieved and is the value achieved when DS obtained is divided by the theoretical degree of substitution.

As represented in the Table 6.1, the degree of substitution achieved by OSA-esterified starch with the conventional aqueous medium method depends on the source of starch utilized and the reaction conditions. As regards the outcome of the concentration of OSA on the DS, starch DS usually increases with the OSA molecules available close to starch molecules. However, a rise in DS is usually not proportionate to the increase in the concentration of the reagent, hence reducing RE. Using this method, various studies over the years have been published, with some studies having their reaction conditions optimized to maximize the efficiency of the reaction (Xu et al. (2012). Over the years, several authors have investigated OSA starches synthesis after the invention of (Caldwell and Wurzburg (1953). Presently, the majorly used method for OSA starch synthesis comprises the suspension of starch (granulose) in the water and then adding OSA in drops while stirring, with the maintenance of pH (around 8) with sodium hydroxide. The reaction takes place at room temperature of between 25 to 35 °C and it continues up till there is the stabilization of the slurry pH or termination by reducing pH together with hydrochloric acid. As a result of the low water solubility of octenyl succinic-anhydride, poor efficiency of reaction often results in addition to an uneven octenyl succinic group distribution (Chen et al., 2014a). The pathway for esterified OSA starch has undergone extensive investigations with continuous reactions being optimized.

In food systems, OSA starch finds extensive application as an encapsulating agent, a fat replacer and an emulsifier (Mason, 2009). Starches modified with OSA usually display a reduction in the temperature of gelatinization as well as enthalpy, increased swelling power, lower digestibility and an increased paste clarity and paste viscosity. These occur due to the introduction of hydrophobic groups, which are new and concurrent changes which are yielded in the starch granules structure. The physical and chemical characteristics of OSA starch are dependent upon the starch characteristics of the raw material, the conditions of processing utilized before (if pre-treatments are applied), during and after (if post-treatments were applied), the starch modification, the derivatization extent as well as the introduced group's distribution within the granules. Hence, different properties can be used to synthesize varieties of OSA starches and are useful for diverse applications.

6.2.2 Variations in the Traditional Synthesis Method

In the conventional synthesis method, the solubility of octenyl succinic anhydride in water is characterized by low solubility. This makes the reaction medium contain droplets of OSA having restricted accessibility into the inside of the semi-crystalline granules of starch. Hence, the reaction of starch and OSA using the aqueous medium method is believed to take place on the surface of the granules, which has often resulted in a poor RE and not even OS group distribution. Chen et al. (2014b) have reported higher concentrations of groups of octenyl succinate in comparison with the bulk value that corresponds. These have been targeted towards an increased degree of substitution as well as reaction efficiency, a reduction in time of reaction, a more balanced organization of groups of esters within the starch granules or/and the production of OSA starches having specific or enhanced functional characteristics. Various contributions have also focused their studies on getting to the interior regions of the starch granules with the replacement since a greater apportionment of the octenyl succinic groups in the interior of the granules is said to produce enhanced emulsifying properties (Wang et al., 2016). In situ mechanical as well as ultrasonic treatments, chemical, hydrothermal in addition to enzymatic pre-treatments modifications that were introduced a few years back, as demonstrated in Table 6.2. Zhang et al. (2010) reported that mechanical treatment such as ball milling starch granules functions as a means of making starch more liable to modification by a decrease in the crystallinity of granules. Increasing the mechanical activation time to 10 h caused a progressive reduction in the starch crystallinity with a concomitant increase in values of DS as well as RE. This behaviour was linked to an easy starch dispersion in water and an increased amount of hydroxyl groups with its availability for nucleophile attack for the anhydride moieties.

Recently, in situ (mechanical) method was proposed.at constant reaction conditions, an increase by 11% of DS as well as RE (from 78.45 to 86.86) was reported by Wang et al. (2015) when shear treatment with high speed (10,000 rpm) was utilized compared to mechanical stirring at 500 rpm in the reaction. Reports by authors claim that the effect of cavitation of the method of treatment elevated the starch area by a reduction in the surface of the granule and a reduction of OSA in terms of drop size, which facilitates penetration in the granules, this treatment uses high speed resulted in the starch granules rapturing as seen with a scanning electron microscope. With the same aim, a preparation of OSA starch using ultrasonic-assisted treatment was prepared by Chen et al. (2014a). They discovered 600 W to be the minimum power used in the maximization of DS, having a value (15%) higher than the control and RE having values which increase from 75.78 up to 87.63%. Also, when the reaction was aided using ultrasonic power, there was a drastic reduction from 6.5 h up to 1.5 h. The results were linked to a reduced and homogenised size of droplets of OSA by the forces generated as well as the effects of captivation, which may have caused an increase in the surface area resulting from the destruction of the surface of granules as well as pore creation in addition to grooves. Moderation in the reduction of uneven OS group distribution by ultrasonic

Table 6.2 Conventional method variations of synthesis of OSA starches

Variations	DS (3% OSA)			RE (%)[a]		Advantages/Disadvantages	References
	Control	Modified	Increment	Control	Modified		
Elevated speed shear (corn)	0.0182	0.0202	11%	78.45	86.86	Better OSA penetration/plausible granule rupture	Wang et al. (2015)
Hydrothermal pre-treatment (35% starch suspension, 60 °C, 3 h)	0.0164	0.0190	16%	72.20	81.76	Enhanced OSA penetration	Chen et al. (2014b)
Ultrasonic power (corn)	0.0176	0.0203	15%	75.78	87.63	Lower time of retention, better OSA penetration	Chen et al. (2014a)
Heat-moisture pre-treatment (25% moisture, 100 °C, 5 h)	0.0154	0.0181	18%	66.49	78.09	Granules are strengthened	Jiranuntakul et al. (2014)
Debranching (pullanase) pre-treatment (waxy rice)	0.0130	0.0202	55%	561	874	Low recovery yield	Klaochanpong et al. (2017)
Chemical pre-treatment (hydrolysis with HCl)[b] (high amylose maize	0.0131	0.0152	16%	56.5	65.5	Better emulsifying properties	Sweedman et al. (2014a, b)

[a] RE values correspond to data reported by authors

[b] Results varying with the botanical source of starch

assistance, because of small OSA droplets, was as well reported, which might have gotten to the interior regions of the granules of starch by the grooves generated.

Using an approach dedicated to enhancing the accessibility of granules, a pre-treatment using hydrothermal was performed by Chen et al. (2014b) on starch before esterification with OSA. The process involved the starch at varying temperatures of between 48-62 °C having contact with water and stirring at 3 h. These authors reported an increase in DS and RS values when the temperature of pre-treatment was brought to 60 °C. DS increased by 16%, while an increase from 77.20–81.76% was observed for the RE and a more uniform OS group distribution. The improvements were observed by the author to be associated with increased porosity and swelling power of the granules that are pre-treated with increasing accessibility which must have enabled OSA droplets migration present in the starch to be easier. A hydrothermal technique was also performed by Jiranuntakul et al. (2014) on starch to improve its octenyl succinylation. Results obtained showed that starch treated that OS heat-moisture (25% moisture content, at 100^0Cfor 1–5 h) revealed a DS in addition to a RE that was higher compared to the non-pre-treated one DS increased by 18% while an increase from 66.49–78.09% was observed for RE for a condition that optimum implying a heat-moisture starch granules pre-treatment of 5 h. The results were characteristic of an alteration/reduction of crystallinity of starch and channels, hollows, and voids generation in the granules, facilitating the entrance as well as OSA reaction present in the granules.

Using enzymatic pre-treatment, channel generation in the granules has been proposed. Using an α-amylase, just before OSA modification, (Huang et al., 2010) examined the effect of granular corn starch pre-treatment. Treatment using α-amylase is believed to ease the formation of spores in the granules of starch, hence, their surface area is increased and the promotion of access of chemical reagents to their internal region. However, OS values reported for pre-treated starch were significantly lower compared to the controls that were not pre-treated. Results were linked to more prepared amylase access to the regions of the starch, which are amorphous, in which removal gave rise to granules which were less susceptible to the reaction with the OSA. However, even the values of OS reached a lower value upon the pre-treatment with α-amylase, an increase in OSA group distribution was observed in the inside of the starch granules pre-treated with enzymes (observation was derived with X-ray photo-electron data). This suggested enhanced access of droplets of OSA to the interior part of the granules of starch through the pores that were produced. Using the same approach, granules of waxy maize starch were pre-treated by Ye et al. (2014) with α-amylase as well as glucoamylase, which aimed to proliferate the surface area of the granules by the creation of numerous pinholes, with the ground surface being the starting to the inside. Increases in DS values were only noted at concentrations of OSA greater than or equal to 9% resulting from a surface area that was higher with more diffusion of OSA in the granules. A relatively low RE was, however, observed. The authors concluded that only a substitution limit is obtainable for the modification of OSA starch granules using the aqueous slurry method. The elevated DS as well RE attained were highlighted when maltodextrin (water soluble) was utilized here was also the availability for hydrolysis reaction among the oligomers. Higher access to OSA starch hydroxyl groups which permitted the acetyl to react directly when dispersed/hydrolysed molecules of starch was recently proposed. Klaochanpong et al. (2017) using this method made a comparison of the esterification of OSA efficiency of starches (waxy) in the granular form together with debranched form, implying total starch gelatinization which was followed by treatment with pullulanase. This was attributed to the reaction occurring efficiently on chains of dispersed starch in comparison with chains which were packed inside the granules of starch, resulting from the difficult state of OSA in getting to the crystalline granular areas. However, the recovery of OS dispersed starch molecules by its formation of a complex with ethanol to form precipitate was not entire and a vital part of the debranched starch was not able to be recovered following modification. OSA resulted in yields that were lower compared to granular starch. Sweedman et al. (2014a) concerning pre-treatment using acid hydrolysis hydrolysed several sources of starch with hydrochloric acid before its esterification using OSA to determine how the OS starch structure affects its characteristics. Majorly, those that are correlated to emulsification. The hydrolysis by acid adversely affected OSA substitution of normal and waxy starches, having high amylose content. In contrast, maize content attained an increased DS upon pre-treatment with acid hydrolysis.

6.2.3 Alternative Method of OSA Starch Synthesis

Over the years, much attention has been placed on alternate methods as well as modifications of conventional methods such as hydrothermal, chemical, in situ mechanical as well as ultrasonic assistance, mechanical and enzymatic starch pre-treatments (Wang et al., 2015). Chemical and enzymatic post-treatments of conventional OSA starch have also been studied. In addition, there has also been the development of OSA starch having several chemical bases such as esterifying starch using OSA with acetic acid being present in the absence of water under high temperature and pressure (Wang et al., 2016), microwave-assisted esterification at pH of 8.5 (Rivero et al., 2009) and pyridine esterification at 110 °C. Irrespective of the method of synthesis used, some characterisation techniques have often been used for describing the physical and chemical, functional in addition to morphological properties of synthesized OSA starches, hence making it possible to evaluate and predict their response in several applications. Table 6.3 illustrates other recent alternative methods which have been proposed.

Potato starch was esterified with OSA (Sandhu et al., 2015) in an aqueous slurry by utilizing a high concentration of starch (100 g of starch in 120 ml of octenyl succinate anhydride water solution). In addition, the authors ascertained the outcome of pH on the dextrose equivalent (DE) as well as the RE. Drying of starch without washing was done after an hr of reaction up to $\leq$5% moisture and then heated for 2 h at 130 °C. For an electric oven, a maximum DS was reported by the authors for

Table 6.3 Alternative methods to OSA starch modification

Benefits	Outcomes	Reference
Aqueous media; pH, higher DS 4 + dry heating	Damage of granules	Sandhu et al. (2015)
Dry milling of media NaOH	Simple technology, low Granule damage, cost and environmental protection	Sandhu et al. (2015)
Dry milling of media Na_2CO_3	Technology is simple, Granule damage, environmental protection, loss of crystallinity	Hu et al. (2016)
Ionic liquids (IL) media with lipase as a catalyst	IL are lowly toxic, Granule damage, flammable and crystallinity loss, recyclable, and reduced structure. Reduced thermal stability, green catalyst, lipase inactivation possible	Li et al. (2016)

the reaction carried out at acidic pH. At this reaction condition, changes on the surface of granules and their edge definition loss were observed using a scanning electron microscope (SEM), particularly in small granules.

Recently, methods of dry milling have also been given much attention. Using the dry milling approach, rice starch (waxy) was modified with OSA by Chen et al. (2014a) with the aid of a dry mill where NaOH (0–11%), OSA (0–9%), as well as starch, were incorporated in the absence of water (except NaOH solution containing water (4%)). There was mechanical activation of the mixture in the mill at 450 revolutions per minute during varying time intervals when varied from 0–70 h. Optimum NaOH value (0.9%) together with OSA value (4%) were reported by these authors with values of DS as well as RE increasing with reaction time. The results obtained had a correlation with the force (mechanical) generated by the dry milling process diminishing the size of the granules, hence, increasing the surface area. In addition, the damaging of the starch crystalline structure resulted in reactive site exposure which is encapsulated in the region (crystalline) of the granules of starch, hence providing additional positions for the esterification (Chen et al., 2014a). Recently, (Hu et al., 2016) observed the dry milling process of OSA synthesis. A reaction between starch from cassava, OSA (8%), as well as sodium carbonate, was carried out by these authors using a ball mill (customised) at 60 °C at varying time intervals of between 30–120 min. They observed a high DS of 0.0295 in 30 min, maximum DS of 0.0397, following 90 min of the reaction. Damage of granules was also observed by Zhang et al. (2013) with the starch crystallinity reducing with the time of milling resulting from the continuous destructive event of hydrogen bonds present in between the molecules of starch by severe ball milling. These changes brought about by the reaction's mechanical activation can cause an increment in the starch accessibility and reactivity, with an improvement between starch as well as reagents contacts in the solid state. Reports by both (Chen et al., 2014a, b; Hu et al., 2016) using dry milling had the advantages of uncomplicated technology, low cost as well as protection of the environment as a result of the operation being carried out with added solvents absent.

The usage of enzymatic catalysts in liquids (ionic) media is also a method that has been reported recently. Li et al. (2016) in their report made use of a technique called two (2) step technique in which maize starch was initially dissolved in 1-butyl-3-methylimidazolium chlorine [BMlmCl] to eradicate its semi-crystalline structure in addition to proliferating the surface area of the granules, then esterifying using OSA in 1-octyl-3-methylimidazolium nitrate with Novozyme 435 as a catalyst. Starch concentration (5%), lipase load (1%), time of reaction (3 h) and temperature (50 °C) were optimized. As expected, the granules of starch were distorted by [BMlmCl] pre-treatment, appearing as clusters with the use of a scanning electron microscope, as well as showing a total amorphous pattern of X-ray diffraction. However, 0.0130 was the maximum degree of substitution achieved which was lower compared to the DE by the conventional OSA protocol, possibly because of the low action of lipase even though the liquid (ionic) utilized as a medium of reaction was designed to be suitable to enzymatic activity (Agama-Acevedo & Bello-Perez (2017).

6.3 Characterization of OSA-Starch

OSA starch is a food hydrocolloid derived from starch esterification with OSA which contain amphiphilic properties. This characteristic makes it hydrophobic and lipophilic and being a food additive, it is a modification process that is getting more attention due to its usage as an encapsulating agent, fat replacer and most especially as an emulsifier in the food industry. Characterization of the starch modified with octenyl succinic anhydride is a very important aspect to be studied and its properties have been critically examined. Altuna et al. (2018) reported that OSA starch portrays hydrophobic and amphiphilic properties, hence, making it suitable for applications in emulsions.

Modification of starch chemically, physically, or enzymatically is gaining advantage because unmodified starch lacks functional and physicochemical properties which are essential in its application in various food products (Olawoye et al., 2022). Using chemical modification, the main amylopectin structure of unmodified starch has an impact on the result of OSA starch structure. The physicochemical properties of OSA starches are influenced by the processing conditions utilized before, during and after modification of starch, derivatization amount, introduced group distribution within starch granules and properties of the starch type. Synthesis of OSA starches having various properties can be employed in a variety of applications depending on the products used. OS-starches have been derived by esterifying octenyl succinic anhydride in an aqueous slurry under somewhat acidic conditions. OSA minimal solubility in water leads to unequal distribution of OS groups with poor reaction efficiency (Olawoye et al., 2021).

Over the last few years, investigations have been made on modification of traditional approaches, to find shorter reaction times, enhanced reaction efficiency, a higher degree of substitution, improvement of functional properties and OS groups homogenous distribution within starch granules. Retrogradation (partial recrystallization of gelatinized starch), pasting, swelling, and gelatinization are all essential features of starch in diverse applications when studying its functional characterization. The gelatinization process involves mixing starch with water in the presence of increasing temperature, causing the starch granules to inflate and rupture as the crystalline structure is destroyed (Chen et al., 2021).

OS-starches with enzymes and chemicals have also been investigated by Wang et al. (2019). OS-starch obtained with various chemical approaches such as starch esterification of OSA with acetic acid (Quintero-Castaño et al., 2020), microwave (Tong et al., 2019) and pyridine (Liu et al., 2022) have also been developed. Regardless of the synthesis method chosen, a variety of characterization approaches have been employed to define the functional, morphological, and physicochemical properties of synthesized OS-starches, permitting their assessment, analysis and behaviour when applied. Characterization techniques are utilized to check the qualities of starch derived from the synthesis pathway, as a result, studying their behaviour, structural molecular alterations, degree of substitution and branching is critical for the characterization of OSA starch.

6.3.1 Branching Degree

Points of branching average number which is regarded as the total number of the average percentage of glycosidic connections per molecule of starch are called the degree of branching (DB). It can be quantified as a characterization approach, Nuclear Magnetic Resonance (one of the reliable techniques), so far, the sample is completely dissolved and homogeneously dispersed**.** It is noted that all samples of starch cannot be completely dissolved by water, especially those with high amylose content (Gao et al., 2021). DMSO-d6, which is widely used is shown to provide 100% starch-dissolving properties. Groups with protons that are exchangeable like the OH group with labile protons, on the other hand, have signals, which is broad and can obscure other interested peaks and make DB calculations more difficult. There have been several approaches documented for eliminating these unwanted signals. A mixture of 80/20 DMSO-d6/D2O was introduced by Jiang et al. (2016), in place of DMSO-d6, assuming the exact ratio of D20 was not added. Solubility difficulties and insufficient spectrum clarity were observed, leading to a technique that was restricted. Though an effective process, predeuteration of starch was carried out by Punia et al. (2019) before dissolving in boiling D2O when solubilized in DMSO-d6 and then freeze-dried. This makes it time-consuming because of numerous repetition times until there is achieved significant degree of starch deuteration. Recent researchers devised a simple procedure involving TFA-d1 (deuterotrifluroacetic acid), which results in a rapid move to OH groups of starch by which exchangeable protons of high frequency emanated, resulting in spectra of 1H NMR, which is well-defined and clear.

6.3.2 Degree of Substitution

The substituents' average number per unit of anhydro-glucose, which expresses the extent of the change is called the degree of substitution (DS). Titration is the most common method for determining this value (Zheng et al., 2017). The degree of substitution obtained by the theoretical substitution degree is the calculated reaction efficiency (RE). It represents the proportion of substitution achieved. The FDA has approved OSA-modified starch (3% w/w basis of starch) as a food additive. When 3% OSA is added to starch, 0.0231 is the theoretical substitution degree, assuming the starch reacted with all the octenyl succinic anhydride. This degree of substitution (theoretical), however, is difficult to achieve and minimal levels have been reported by most authors. There is an increase in the starch degree of substitution with a 3% OSA percentage added, due to more OSA molecules being made available which are close to molecules of starch. However, the degree of substitution and concentration of reagent rise ratios are not the same (not proportional), resulting in lower reaction efficiency. Gao et al. (2021) used α-amylases and glucoamylases to pre-treat waxy maize starch granules to increase the surface area of granules. In the forms of debranched and waxy starch granules, the efficiency of esterified octenyl succinic anhydride was compared by Krolikowska et al. (2019). Treatment with the enzyme pullulanase was done, which attained full gelatinization. It was observed that before octenyl succinic anhydride modification during starch debranching by the enzyme pullulanase hydrolysis, there was an increase in the degree of substitution derived because octenyl succinic anhydride struggled to get to the starch granular crystalline regions. It was concluded that chains of starch when dispersed compared to chain-packed granular starch portray more reaction efficiency attributes. Olawoye et al. (2022) studied how modified starch impacts its features, especially emulsification-related ones by HCL hydrolysis of various sources of starch before being esterified with octenyl succinic anhydride. The octenyl succinic anhydride replacement of waxy and regular starches was negatively influenced by acid hydrolysis. In contrast, pre-treated acid hydrolysis increased the DS obtained for starch with a high amylose concentration. Analyses of OSA starches were performed to see if substitution occurred. Substitution extent in OSA starch is determined using three ways; Fourier transform infrared (FT-IR), Titration and Nuclear Magnetic Resonance (NMR).

6.3.2.1 Titration

To determine the extent of substitution in OSA starches, two (2) types of titration procedures are used. The first method involves saponifying in a solution of alkali and then titrating the surplus alkali. In a solution (basic), there is a suspension of octenyl succinic anhydride starch to saponify the groups of OS in a general procedure (Nhouchi et al., 2022). A solution of HCL is used to titrate the excess alkali in the medium. Since the backbone of starch seems to degrade during this process, in the same way by treating the starch (void of modification), a blank value must be obtained. The starch of octenyl succinic anhydride is disseminated in a solution of isopropanol or HCL in the second titration procedure (Yao et al., 2020). The washing of isopropanol on the derived solid is done until no Cl is identified, and then a solution of AgNO3 is introduced and redispersed in distilled water. After boiling, titration of the solution is done with sodium hydroxide solution until neutralization is achieved. It should be noted that the approach of titration shouldn't be used to measure the products substitution extent made with the starch-chain-degrading method because there could be changes in the result of titration when comparing modified and unmodified starch, due to starch degradation by chemicals or the presence of OS group.

6.3.2.2 Nuclear Magnetic Resonance (NMR) Method

Chemical alteration of polymers such as OSA starches is commonly quantified using NMR. The DS can be determined as the anomeric protons ratio of the starch (Sweedman et al., 2013), utilized as a reference from the intensity of the group signals of OS. Starch and OS group signals, as seen in (Shah et al., 2018) approach, require very controlled circumstances to be detectable in the same study. Per characterization, tiny amounts of product (usually approximately 2–5 mg) is the minimum requirement in preparation of the sample, which is a significant benefit when compared to approaches of titration. Furthermore, nuclear magnetic resonance allows values of branching degree and substitution extent emanating from the starch sample of octenyl succinic anhydride to be determined in the same experiment. However, NMR is not commonly used in the industry. In deuterium oxide (D20), nuclear magnetic resonance was used to dissolve samples of starch partially (Altuna et al., 2018). Problems of water solubility can be solved by lowering the starch chain molecular weight via hydrolysis (basic) (Shogren, 2003) or via pre-digesting the product with amylase (Bai et al., 2011). As already mentioned, OSA starch is amphiphilic in nature (the hydrophilic component being the starch backbone and the hydrophobic component is octenyl), capable of forming combinations through the interaction of hydrophobic aqueous medium resulting in characterization by nuclear magnetic resonance through reduction of significant signal and as a result, there is an inaccurate estimation of substitution extent. Regardless of their content of amylose, solutions of lithium bromide (LiBr) / DMSO were observed to dissolve different samples of starch completely (Mora et al., 2020) and also that DMSO can mix with octenyl succinic anhydride, which happens to be the best system of solvent for the characterization.

6.3.2.3 Fourier Transform Infrared (FT-IR) Method

The FT-IR technique is widely regarded as the most effective method for the determination of the chemical structure of polymers and functional groups. When comparing spectra of Fourier transform infrared and starches of OS, a new band of absorption (around 1725 cm^-1) is often observed, which is ascribed to the longing vibration of the group of carbonyls added. The appearance of these two peaks supports the OS group introduction. Furthermore, with DS, several authors have observed an increase in their intensity (Zheng et al., 2017). Unfortunately, for high DS (≥ 0.3), FTIR has only been found to be effective (Olawoye et al., 2020), making it inappropriate for most applications in industries where only a lower substitution degree is allowed.

6.3.3 Changes in Molecular Structure

The size of structural properties can be used to calculate the molecular weight of OSA starch. Utilization of size exclusion chromatography with a refractive index detector and multi-angle light scattering has been routinely made to analyze changes in starch molecular weight after the starch has been esterified with octenyl succinic anhydride. With the addition of OS groups, the molecular weight increases, nevertheless, the loss has been attributed to starch degradation due to the reaction conditions utilized in previous (traditional) procedures. Octenyl succinate takes place predominantly in the sectional amorphous starch granules, which were frequently related to the branched regions of amylopectin, also in certain cases to molecules of amylose (He et al., 2008). The structural features of OSA starch have been occasionally documented. Evaluation of structural features of branching polymers which were polydisperse involves numerous diverse criteria to offer significant information on the molecular size.

In a study by Chen et al. (2021), the average degree of polymerization was used to measure the number of manganese (Mn) molecular weight average in samples of rice, starches of the waxy corn and tapioca starches before octenyl succinic anhydride treatment with a chemical. To determine Mn content, the content of reducing sugar was measured due to hydrolysis via pre- and post-treatment with a concentrated solution of alkaline. Why this type of method is commonly used and simple to use, free or connected groups of OS are likely to engage with a test of lowering sugar. Furthermore, the approach is only correct for compounds like amylose (unbranched starch), having a lower weight of molecular properties. Other advanced approaches can give more helpful information regarding the starch structures of octenyl succinic anhydride.

6.3.3.1 Size Exclusion Chromatography (SEC)

This kind of approach can be used to determine the size distribution of starch, which is completely branched, and variants of modified octenyl succinic anhydride as well as the hydrodynamic size distribution of individual branches after enzymatic debranching. In addition, thorough dissolving of molecules should be done in the eluent to effectively quantify size characteristics. Water is not the greatest solvent for complete unmodified starch as previously noted, however, some writers have claimed success using aqueous eluent phases. Based on a previous review and study by Shah et al. (2018), debranching of modified maize starches with octenyl succinic anhydride with enzyme pullulanase and exposing them to the chromatography of size exclusion by hydrolysis or pH change of solvent makes them soluble in an aqueous medium, breaking them down into smaller components. Introduction of DMSO was made by Pérez-Gallardo et al. (2012) to pre-dissolve maize starches which were modified with the hydrolysis of acid before performing SEC characterization using a buffer of sodium nitrite at 40 °C, 0.02 percent of sodium azide and an aqueous mobile phase. It is yet to be proven that in an aqueous eluent, SEC characterization of entire molecules of starch, regardless of pre-treatment, results in the real dissolution of molecules with degradation or aggregation.

6.3.3.2 Asymmetric Flow Field-Flow Fractionation (AF4)

Sweedman et al. (2013) found that size-exclusive chromatography causes degradation by shear of molecular branching-like amylopectin, in which its molecular mass is very high. Hence, AF4 was investigated as an alternative. However, it has been observed that DMSO has noise signal issues when detected in differential refractive index and has been shown that it cannot be employed for full native starch (Altuna et al., 2018). In the study by Nhouchi et al. (2022), AF4 was used to estimate the octenyl succinic anhydride of a large size of starch. The study was reliable because, in an aqueous condition, the starch was degraded enough, therefore the noise problem from signal DMSO was avoided. There is also a comparison of ferritin standards to sodium nitrite aqueous solutions characterization. The results of their AF4 information showed that homogenization by high pressure severely destroys chains of starch (Nhouchi et al., 2022) and the amount of molar weight loss was related to conditions of turbulent flow.

In conclusion, it is observed that the characterization of modified starch of octenyl succinic anhydride and its area of application (based on the extent of substitution, structural molecular change, behavior, and branching degree) depend on the starch type, conditions, interactions of octenyl succinic anhydride and on different methods and approaches.

6.4 Effects of OSA Modification on Functional Properties

6.4.1 Pasting Properties

The pasting properties of starch refer to the transformation that takes place on starch when in contact with water under a controlled temperature. This transformation affects the starch structure, digestibility, texture and food application/ end use. The pasting properties of starch are usually measured using rapid viscoanalyzer (RVA). The three major parameters of starch studied under RVA test include peak viscosity (PV), hot paste viscosity (HPV) and cool paste viscosity (CPV). PV indicates the strength/ power of the paste after gelatinization which shows the swelling capacity of the starch on heating, while HPV and CPV refer to the resistances to flow, shear or agitation of starch paste at high or low temperatures respectively. OSA modification of starch has been reported to influence PV, HPV and CPV respectively. Carlos-Amaya et al. (2011) reported that OSA modification of starch has a great impact on the pasting properties of native starch. They further establish that banana starch modified using OSA experiences higher PV, HPV and CPV than their native starch counterpart. A similar trend of observation was reported by Song et al. (2010) for corn starch-modified using OSA. Furthermore, Zhu et al. (2011) affirmed the report of Song et al. (2010) stating that corn starch modified with OSA had higher PV, HPV and CPV.

On the other hand, a variation from the aforementioned has been reported due to OSA modification crop starch. For instance, a decrease in PV, HPV and CPV has been reported in OSA-modified potato starch with variation in the degree of substitution above 0.016 (Wang et al., 2010). Likewise, (Thirathumthavorn & Charoenrein, 2006) also reported a decrease in PV, HPV and CPV because of OSA modification on tapioca starch as well as rice grain starches with a decrease in the degree of substitution. Furthermore, (Sweedman et al., 2013) establish that the relationship between RVA parameters (PV, HPV and CPV) is inversely proportional to the degree of substitution of OSA starch molecular weight and this reduces the solubility in water for amylose chains. Finally, it could be deduced that OSA modification of starch could either, reduce or increase RVA parameter as influenced by either degree of substitution or molecular weight of PV.

6.4.2 Thermal Properties

OSA modification involves the introduction of octenyl molecule into native starch which transform native starch hydrophilic behavior to hydrophobic. This transformation affects the thermal properties of the starches (Bajaj et al., 2019). A significant difference was recorded between native and OSA-modified starches. A significant change/ increase was observed in the enthalpy of gelatinization (EG) and transition temperature (TT) simultaneously with a decrease in the pH of the reaction. The changes in OSA EG and TT were attributed due to the introduction of octenyl succinate into starch molecules causing the breaking down of hydrogen bonds of starch long chains, removal of the crystalline structure and which is more energy-demanding (Bajaj et al., 2019). Hui et al. (2009) also established that an inversely proportional relationship exists between the degree of substitution and reaction temperature of OSA modification. They also concluded that better solubility of OSA modification is achieved when the temperature is increased which enhances the breaking down of starch molecules. Furthermore, some publications have also attributed the thermal effects OSA modification on native starch to include reduction in TT and elasticity of starch structure (Thirathumthavorn & Charoenrein, 2006; Bhosale & Singhal, 2007; Punia-Bangar et al., 2021).

6.4.3 *Starch Digestibility*

Starch digestibility refers to the rate at which starch molecules are being broken down into smaller molecules of starch in the gastrointestinal tract in line with their rate of absorption in the body system. One major constraint of native starches is due to their semi-crystalline structure which renders them difficult to digest. Starch digestibility can be categorized into three (3) major groups namely slowly digestible starch SDS, rapidly digestible starch RDS and resistant starch (RS). SDS and RS consumption are preferable for human health as the rate of release of glucose into the bloodstream is slow and this does not trigger hyperglycemia linked with insulin sensitivity and type 2- diabetes (Oluwajuyitan et al., 2021). Meanwhile, RDS rate of release of glucose into the bloodstream is faster and as such may not be suitable for diabetic patients as it may result in prospandial hyperglycemia.

OSA modification plays a crucial role in starch digestibility of native starch, modified starch or both. Sweedman et al. (2013) reported that OSA modification has a couple of effects on starch digestibility. They conclude that OSA modification increases the proportion of RS and SDS more than other modes of chemical modification (cross-linking, acetylation, hydroxypropylation) of starch which is beneficial for human health. Juansang et al. (2012) in a similar study, modified canna starch using OSA modification method. They also report a significant increase in SDS and RS than chemical modification compared with native canna starch which gives a better nutritional benefit. Using human subjects, (Wolf et al., 2001) reported that modification of maize starch using OSA significantly increase maize SDS and resulted in low glycemic index response by subjects compared to subjects administered with pure glucose. An earlier report of (Kelly, 1991) also confirmed that developed infant formula with a high proportion of OSA-modified starch resulted in a decrease in blood glucose levels after consumption which may be attributed to slowly digestion of large starch molecules in formulated infant formula. The mechanism of increase in SDS, RS and decrease in RDS in OSA-modified starch has been reported by (Zhang et al., 2011) and (Sweedman et al., 2013) to be attributed to the increase in amylose content in OSA starches, destruction of native starch crystalline structure and low degree of substation (0.009). Hence, OSA modification increases SDS and RS content in native starch thereby reducing RDS content which is of health benefits for human consumption in reduction of risk of high blood glucose levels.

6.5 Application of Octenyl Succinic Anhydride (OSA) Starch

The amphiphilic properties of OSA-modified starch have a wide diversity of applications and its application is not limited to the food industry. It is used as coatings and films, in gel formation, as an encapsulating and emulsifying agent, in the production of juice, carbonated drinks, energy drinks, salad dressings, creams, and alcoholic beverages and in non-food industry as adhesives, paints and biodegradable chemicals that have low influence in the mining and extraction of oil (Crossman et al., 2004; Eoff et al., 2005). Its application will depend on the functional prerequisite for a specific application which is dependent on the fundamental substrate formation of the modified starch.

Octenyl succinic anhydride starch is applicable in diverse ways and one of them is its application as an emulsifier. An emulsion is made up of two immiscible liquids in which one of the liquids is dispersed as minute orbicular droplets in another liquid. Most emulsions found in food are either oil in water (as in milk) or water in oil (as in mayonnaise). The applications of emulsion are not only limited to food but also applicable in the nutraceutical, medical, cosmetics, detergent and pharmaceutical industries. The process of conglomeration and coalescence takes place in emulsion leading to the cause of separation of the phases in the cause of storage thereby bringing about a reduction in its functional properties and level of acceptance. Surfactant agents also known as stabilizers are thereby introduced to the medium to stabilize its emulsion. An emulsion can be stabilized by artificial surfactants or through animal source emulsifiers, but some shortcomings have been found which may be associated with the surfactants like frothing, biological reactions, air entrapment and irritancy and all these have some adverse effects on the functional properties of the medium. However, this led to sourcing emulsion stabilizers from plants also known as Pickering emulsion Berton-Carabin and Schröen (2015). This tends to be more stable compared to artificial surfactants and animal-based emulsifiers. Solid particles derived from plants such as starch and cellulose have been used to produce Pickering emulsions and they are stable in contrast to Ostwald ripening and coalescence (Matos et al., 2018).

Octenyl succinic starch is a hydrocolloid which possesses both hydrophobic and hydrophilic properties and can thereby be applicable in some processing as an emulsifier. The hydrated units of colloidal proportion disperse readily because of their high solubility in water and interact intensely through hydrogen bonding alongside the boundary water molecules and the relatively near hydrocolloid molecules. For the emulsifying agent to be effective, the hydrocolloid constituents need to have some interface activity connoting that the main hydrophilic macromolecules should have some hydrophobic groups which would be available in such a way that it will allow them to attach to and diffuse at the surface thereby making the emulsion agent to be stabilized. In reference to past work that was done, it was revealed by Song et al. (2015) that the increase in the emulsion viscosity could slow down the unrestricted motion of oil droplets which delay creaming, ccoalescence,and flocculation; thus, providing different techniques for the stabilization of emulsion which is pertinent in food applications. It was also revealed that Pickering emulsion from plant sources was polymerized to produce a series of polymers used virtually in the application of food packaging (Nikfarjam et al., 2014). The amphiphilic property of the octenyl succinic starch enables it to be used as an encapsulating agent for ingredients that are hydrophobic but biologically active. This method enables the bioactive substance to be included or incorporated into food and also to guard against air, water and high temperature. The encapsulated compound is gotten as a powder form after which the octenyl succinic starch is dispersed in water and the hydrophobic substance is incorporated to form an emulsion which is then spray dried to produce capsules which are the bioactive compound used in the formulation of foodSantos).

Octenyl succinic starch is also used in food formulations. It is used as an ingredient in food processing due to its functional properties such as its pasting properties, slow rate of digestibility and as an emulsifying agent. It is being used in the production of fortified food or a dietary supplement which is needed in healthy living and guards against obesity, diabetes, and other types of diseases. It can also be used in replacement of fat for egg yolk in the production of mayonnaise with low fat as it has been found and concluded that a satisfactory emulsion could be obtained if 75% of the egg yolk is replaced with octenyl succinic anhydride starch. It is also applicable in the production of bread dough in which it can be substituted for starch or fat. Ghazaei et al. (2015) substituted 10% of wheat flour with octenyl succinic starch in the production of the bread and the best product emerged from the dough made with the pregelatinized octenyl succinic starch. This reduced the ability of the dough to retain moisture.

6.6 Conclusion and Future Perspective

OSA starch is a food hydrocolloid derived from starch esterification with octenyl succinic anhydride containing amphiphilic and lipophilic properties, hence, making it suitable for use in emulsions. In food systems, it functions as an encapsulating, emulsifying and stabilizing agent. Starch modified with OSA has been shown to display a reduction in the temperature of gelatinization and enthalpy, an increased swelling power, paste clarity and paste viscosity and a reduction in digestibility. Owing to the versatile applications of OSA starch, its synthesis has recently increased. Over the years, various studies on the variations of OSA starch have been carried out to overcome the shortcomings of the conventional method of synthesis. Some of the variations have been targeted towards optimizing reaction conditions, maximizing reaction efficiency and degree of substitution, reduction in time of reaction, a more balanced organization of a group of esters within the starch granules, a homogenous distribution of OS groups, in addition to producing OSA starches with improved functional properties. With the constant expansion of the starch industry, further studies should be carried out to explore the functionality of OSA starches.

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Chapter 7
Dry Heat Treatment of Starch

Arun Kumar Pandey, Sheetal Thakur, and Dev Kumar Yadav

7.1 Introduction

The physical modifications of starches are preferred as the safe method to avoid environmental pollution and other health hazards. In the physical modification, pregelatinization, annealing, steam treatment, dry heat treatment (DHT), etc., are prectised to alter starch granules' molecular structure and properties. The multifunctional properties of starch can be achieved through precise control over dry heat treatment conditions. Chiu et al. (1998) were the first who use a dry heating process to formulate physically modified starches and reported that the starch functionality on heat treatment is similar to that of the chemically modified starches. In continuation to these claims, several researchers illustrated similar reports that the heat-treated starches could be a better substitute for the starches modified by chemical cross-linking methods like chlorination, to manufacture hydrophobic starch with enhanced oil-binding properties (Ozawa et al., 2009; Seguchi, 2001). Due to the release of toxic and reactive components during chemical treatment methods that may travel to food products during processing, components made from physical methods have a preference for food producers as well as consumers

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Arun Kumar Pandey and Sheetal Thakur contributed equally with all other contributors.

A. K. Pandey
Amity Institute of Biotechnology, Amity University, Jaipur, Rajasthan, India

S. Thakur (✉)
University Centre for Research & Development, Department of Biotechnology, Chandigarh University, Gharuan-Mohali, Punjab, India

D. K. Yadav
Grain Science and Technology Division, DRDO-Defence Food Research Laboratory, Mysore, Karnataka, India

V. S. Sharanagat et al. (eds.), *Starch: Advances in Modifications, Technologies and Applications*, https://doi.org/10.1007/978-3-031-35843-2_7

in recent times. Commonly, the temperature used during dry heating varies between 110 and 150 °C with low humidity conditions for 1–4 h (Bae & Lee, 2018).

The moist-heat treatment has been studied extensively as a physical modification method of starch compared to the dry heat treatment method. But, nowadays, dry heat treatment (DHT) has received greater attention for modifying starch, among other methods, due to its simplicity and safety. The structure of starch granules remains unaffected during physical modification through DHT, but physicochemical properties change (Sun et al., 2014b). Several studies reported the change in structural and functional properties of starch derived from sources like waxy corn, waxy rice, potato, sweet potato, taro, and cassava starch (Sun et al., 2014b; Li et al., 2013; Pramodrao & Riar, 2014). The origins of starch and the treatment conditions (temperature and time) significantly influence starch's structural and physicochemical properties. DHT of starch in combination with ionic gums at high temperatures for a predetermined time could be used to modify starch with functionality equivalent to that obtained through chemical modification. DHT can be used as an effective alternative to chemical modification for tailoring starch. The alteration of physicochemical characteristics of starch (viscosity, swelling power, solubility, colour, etc.) through DHT can improve its functional characteristics like coating, filler, texturizing, and whitening properties as per the requirement for specific food applications (Chandanasree et al., 2016).

Su et al. (2018) studied the effect of DHT and xanthan gum on rice starch of different amylose content (high 37.85%, medium 27.55% and low 9.98%). The physicochemical, pasting and rheological properties of xanthan gum-added native starches with different amylose content were significantly influenced after DHT compared to the native counterpart. The absence of breakdown indicated that the samples pretreated with xanthan gum achieved higher pasting stability under shear force and high temperature during pasting compared to the untreated native starch. The pasting property of xanthan gum pretreated low amylose rice starch was more impressive than high and medium amylose rice starches. The morphological evaluation of modified starch samples after gelatinization shows that DHT induced some interactions between xanthan gum and rice starch molecules resulting in restricted granule swelling. The modification of starch not only enhances the paste clarity and sheen, film formation, texture of paste and gel, and adhesion but also reduces the retrogradation, pastes gelling tendency and syneresis of gel (BeMiller, 1997). Several starch based food items like, bakery goods, vermicelli, syrups, jellies and edible coating are having starch as a prime component (Abegunde et al., 2013). Hence, DHT of starches is a budding method for modification without any harmful impact of chemical treatments. In this chapter, we further discuss the process of DHT, effect of DHT on different properties of starch, applications of dry heated starches and types of dry heat treatment methods.

7.2 Process of Dry Heat Treatment of Starch

For the DHT of starch, it is important to extract and purify the starch from plant/food sources. Several starch extraction methods are reported in different research studies, which might vary depending on the nature and the type of raw material. However, the most common way of extracting starch from the plant/food source includes the following steps: cleaning, slicing, drying, crushing and sieving. The obtained powder of the sample source must then be suspended in water and repeatedly centrifuged up to a certain time to remove impurities. The precipitate is further suspended repeatedly with 95% alcohol to remove the remaining impurities from the starch. The finally obtained starch pallet should be dried at a temperature of up to 40 °C for sufficient time to reduce the final moisture content <10%, followed by crushing and sieving to obtain a high-purity starch sample. The purity of obtained starch sample can be determined using the starch purity analysis kit (Liu et al., 2019, 2022a). The dehydrated starch sample (moisture content <10%) can be kept for dry heat treatment (repeated/continuous) in a hot air oven at a temperature between 110 and 150 °C for a predetermined time. The treated sample is then cooled for 1 h, packed and stored under ambient conditions for further use or studies (Liu et al., 2022a).

7.3 Mechanish of Dry Heat Treatment

DHT is a physical modification of starch which involves relatively very low moisture content, <10% and heating at temperature around 130 °C for varied time interval. The mechanism of DHT lies on movement of heat from the outer surface to the core of starch granules through conduction mode which damages granular surface as well as internal chemical structure to a certain extent. The crystalline structure and amorphous regions of starch greatly varies with the nature of DHT. High temperature accelerates the movement of molecules which might lead to rupturing and collapsing the starch granule and results leach out of amylose. The breakdown of hydrogen bonds promotes the swelling of starch granules and decreases the pasting onset temperature.

7.4 Effect of Dry Heat Treatment on Various Properties of Starch

Nowadays, several studies have been performed to understand the effect of dry heating on various characteristics of starch with and without treatment of ionic gums (Carboxymethyl cellulose, Guar gum, Xanthan gum, Sodium alginate, etc.) (Gou et al., 2019). The DHT of starches may harm the native starch granules to a certain extent depending on the treatment conditions that result in certain structural changes in the amorphous and crystalline regions of the starch granule (Lim et al., 2006). To elaborate, the DHT of rice starch at varied time-temperature combinations showed that the native crystalline structure was unaffected upto 150 °C for 4 h; however, X-ray diffraction patterns showed the decline in peak intensities with an increase in heating time further (Bae & Lee, 2018). Parboiled rice showed a noteworthy decline in the crystalline behavior of starch on treatment with dry heat and a slight restructuring of crystalline polymorphs at some point during cooling and storage (Dutta et al., 2016). In addition, when potato starch was heat treated (130 °C for 2–4 h) along with carboxymethyl cellulose, the high compactness in the colloidal structure was noticed (Sun et al., 2013). Moreover, physicochemical behavior, *viz.* swelling-solubility and viscosity, were influenced by dry heating action, enhancing starches' functional characteristics. The dry heat treatment is considered to have non-toxic behavior, and the non-generation of any detrimental by-product allows the food industry to have an increased preference for this method (Zhou et al., 2019). In recent studies, several physicochemical variations have been claimed to occur in the starches modified by dry heating. The lower starch crystallinity, decreased gelatinization temperature and increased oil binding capacity are various changes described in smaller molecular fractions of rice starch treated with dry heat (Zhou et al., 2019). Similar changes were observed in the waxy corn starch, where the dry heating method modified the starch structure with a significant impact on the physic-chemical properties, and the aggregation of starch granules was noted (Zou et al., 2019).

The change in temperature and time during DHT has a significant change in gelatinization temperature and enthalpy, as well as the semi-crystalline region of starch. Starch's pasting property and gel strength are directly proportional to the heating temperature, whereas, indirectly, to heating time (Table 7.1). An i*n vitro* study reported that DHT of starch lowers the rapid digestibility and glycemic index compared to native starch. However, *in vitro* starch digestibility, pasting viscosity, and gel strength negatively correlate with DHT heating temperature. It has also been observed that DHT could be useful for enhancing starch's physical and nutritional properties (Oh et al., 2017) (Fig. 7.1).

7.5 Properties of Dry Heated Starch

7.5.1 Moisture Content

The changes in molecular structure and granule size of starches occur on heating; these modifications depend upon moisture availability. If the moisture is inadequate, a temperature for the heating is selected to modify the physical properties without starch's gelatinization (Hoover & Manuel, 1996). A certain amount of free water in flour is relatively easy to remove by vaporization at temperatures below 100 °C. In contrast, H-bond formation between starch chains and water molecules has been reported due to the high affinity of starch chains toward water molecules

Table 7.1 Effect of dry heat treatment on physico-chemical properties of starch

Raw material	Treatment conditions	Physico-chemical parameters										Reference
		Solubility	Crystallinity	Enthalpy	Gelatinization temperature	Gel strength/ hardness	Pasting property	Paste clarity	Viscosity	Swelling power	Starch digestibility	
Chestnut starch	Continuous dry heating and repeated dry heating at 140 °C for up to 12 h	↑				↓	↓	↑	↓	↓		Liu et al. (2022a, b)
Waxy corn starch	Continuous dry heating and repeated dry heating at 140 °C for up to 12 h	↑	↑ (up to 5 h treatment); ↓ (after 5 h treatment)	↓	↓		↓	↑	↑ (up to 4 h treatment); ↓ (after 4 h treatment)	↓		Zou et al. (2019)
Corn starch	Corn starch with lysine admixture was continuous dry heated at 130 °C for 4 h		↓	↓	↓		↑		↑			Ji et al. (2016)
High amylose rice starch	Hydrocolloids pre-treatment followed by dry heat treatment at 130 °C for up to 4 h	↑				↓			↑	↓	↓	Oh et al. (2017)

(continued)

Table 7.1 (continued)

Raw material	Treatment conditions	Physico-chemical parameters										Reference
		Solubility	Crystallinity	Enthalpy	Gelatinization temperature	Gel strength/ hardness	Pasting property	Paste clarity	Viscosity	Swelling power	Starch digestibility	
Mung bean starch	Continuous and repeated dry heat treatments at 130 °C for up to 18 h	↑	No change	↑				↓	↓	↓	↓	Liang et al. (2021)
Proso millet starch	Dry heat treatment at 130 °C for up to 4 h		↓	↓	↓	↑	↓		↑			Sun, Gong et al. (2014a)
Glutinous rice starch	Dry heat treatment at 130 °C for up to 4 h		↑			↑			↑			Qiu et al. (2015)
Quinoa starch	Dry heat treatment at 150 °C to 200 °C for up to 2 h	↑					↓	↑	↑	↓		Rao et al. (2022)
Normal maize starch	Repeated and continuous dry heat treatment at 140 °C for up to 20 h	↑	↑ (up to 12 h); ↓ (after 20 h)	↑ (up to 12 h); ↓ (after 20 h)				↑	↓	↑	↓	Zou et al. (2019)

Raw material	Treatment conditions	Physico-chemical parameters										Reference
		Solubility	Crystallinity	Enthalpy	Gelatinization temperature	Gel strength/ hardness	Pasting property	Paste clarity	Viscosity	Swelling power	Starch digestibility	
Discorea starch	Sodium alginate modified starch treated with dry heat treatment at 130 °C for up to 4 h	↑						↑		↓		Vashisht et al. (2017)
Waxy potato starch	Dry heat treatment at 110 °C for up to 2.5 h	↑	↓	↓	↓				↓	↑		Liu et al. (2019)
Sweet potato starch	Repeated and continuous dry heat treatment at 130 °C for up to 18 h	↑	↓ (upto 3 h of CDHT and upto 9 h of RDHT and then increased)	↓	↓			↓	↓	↓	↓	Gou et al. (2019)

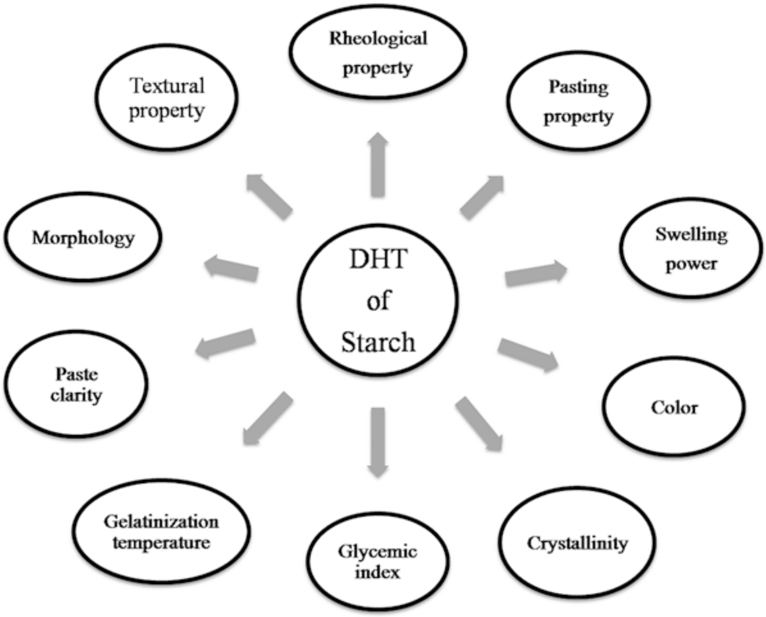

Fig. 7.1 Effect of dry heat treatment on quality parameters of starch

(Trommsdorff & Tomka, 1995). Based upon this observation, it was suggested that on DHT of wheat flour above 100 °C, the water exclusion might relate to the water that was bonded to the components of flour, *i.e.* proteins and starch (González et al., 2021).

7.5.2 Color

The effect of DHT on the color of wheat flour (González et al., 2021) and whole grain barley (Boyd et al., 2017) was studied on a Lab scale, and an increase in darkness, redness and greenness was observed in both types of flours. The results indicated that DHT caused browning of the flour, possibly due to caramelization and Maillard reactions stimulated by flour constituents (Chung et al., 2012).

7.5.3 Structural Characteristics

Mobility of starch units is directly related to the temperature (Chen et al., 2015) and the heat treatment showed increased starch mobility, which eventually aids the interaction of amylose and amylopectin units. An enhancement in the mobility and deduction in the amount of water molecules resulted in the reorganization as well as precision of crystalline sections of starch granules after cooling. Similar findings were claimed with FTIR analysis of millet flour, where the potency of FTIR band (1047 cm^{-1}) was perceived after the dry heating method (Sun et al., 2014a). Hence, an augmentation of this band up to 150 °C indicated that the water loss performs a major function in rearranging and recrystallization of starch chains.

The relative crystallinity of different dry heat-treated starches from cereals and millets was studied using XRD, and a significant change was observed with the increased temperature of dry heating. A decrease in relative crystallinity of high amylose rice starch was noticed on treatment with dry heat (110, 130 and 150 °C for 1, 2 and 4 h) (Bae & Lee, 2018). Similar results were observed for proso millet starch, where a reduction in relative crystallinity was seen after DHT at 130 °C for 2–4 h (Sun et al., 2014a). This trend might be due to the distortion of the crystalline state or amplification of the amorphous area. Also, the decreased crystallinity may be because of the binary helical shift throughout the treatment, due to which there might be an interruption or alteration in starch crystalline region or orientation, respectively (Singh et al., 2011). Moreover, the alterations in the amorphous region during heating and the presence of non-starch ingredients may also decrease the crystallinity of DHT starches. However, an opposite trend has been observed for waxy rice starch, where the crystallinity was increased after DHT which might be due to the partial restructuring of the amorphous region during dry heating (Li et al., 2013). The researcher also postulated that the crystallinity might also be raised under depleted moisture heat treatment, suggesting that the amorphous region was more susceptible to heating methods than the crystalline area, irrespective of the moisture availability.

7.5.4 Morphological Characteristics

The morphological characteristics of starches after DHT were studied for various categories of sources. Researchers developed micrographs from scanning electron microscope (SEM) and studied the same for the analysis of starch granule structure. Starch granule distribution and size are considered to be the significant properties imparting a substantial impact on starch functionality (Rasper, 1971).

Liu et al. (2019) studied the micrographs of waxy potato starch using SEM and observed that native starch granule surface was smooth without the presence of fracture or pores. However, DHT caused the changes in the starch surface. The umbilical point (reffered as the initial point for the development of starch granules) was observed to be easily destructed by DHT and the potholes were developed. This showed that DHT loose the structure of starch molecules to some extent. DHT of Dioscorea starch at 130 °C for 2 h and 4 h showed the ruptured starch granules (Vashisht et al., 2017). Similarly, chestnut starch showed the loss of smooth surface and the occurance of cracking was observed after DHT (Gul et al., 2014). Also, an increase in the fractures on granule surface was observed with an increase in temperature. This cracked appearance might be because of the amylose leaching and increased heating effect that fasten the movement of molecules. The SEM analysis of sweet potato starch using (continuous dry heat treatment) CDHT and (repeated dry heat treatment) RDHT showed that both the treatment methods caused the surface rupturing of few starch granules which was comparable to popcorn (Gou et al., 2019). Moreover, there was non-significant variation in the appearance of ruptured starch granules with an increase in RDHT cyclying and CDHT time. The SEM results indicated that the DHT may influence the starch structure as well as the order of starch crystallites.

Lei et al. (2020) studied the morphological structure of native and dry heat treated maize starches and observed the significant changes in the starch granule structure after DHT. The polygonal and irregular shapes along with smooth surfaces, without any pores and fractures have been observed in native maize starch granules (Liu et al., 2016). The granular shape and appearance of dry heat treated maize starch was retained during DHT, indicated that the typical granular structure was not destructed. This indicated that the DHT caused the accumulation of starch granules into big lumps that might be due to the reduced moisture and association between the particles at elevated temperatures (Zou et al., 2019). SEM of millet starch granules (native and dry heat treated) was also studied by Sun et al. (2014a) and it was observed that the surface of DHT starch granules was smoother and plumer as compared to that of native starch. Moreover, no lump formation after DHT was noticed that might be due to the interaction of starch molecules with other components of non-starch part during DHT and the similar notion was claimed by Chiu et al. (1998, 1999). The DHT of starches above 100 °C for a longer period of time presented the functionality similar to chemical cross-linking (Chiu et al., 1998, 1999; Sun et al., 2013).

7.5.5 Solubility and Swelling Power

Starch's solubility and swelling power are used to define the degree of amylose leaching out and the water absorption capacity of starch during processing. Studies show that the DHT treatment increased the leaching property of the amylose content of starch. The increase in DHT treatment temperature positively correlated with starch's solubility and leach out. The amylose content of starch has a great association with the solubility of starch, while amylopectin is responsible for the influence on the swelling power of DHT starch. Starch's solubility and swelling power also vary with the kind of treatment conditions used during DHT, i.e., CDHT or RDHT. The change in treatment temperature and time also influences the solubility and swelling power of starch. Liu et al. (2022a) found that the solubility of starch was increased while swelling power decreased during both CDHT and RDHT conditions. Similar results of increase in solubility of quinoa starch after RDHT and CDHT were claimed by Zou et al. (2020). The DHT promotes the leaching of amylose while inhibiting its swelling power. The studies show that at low DHT temperature (50 °C) the swelling power of starch granules is found to be the lowest, while the increase in treatment temperature leads to a slight increase in swelling power. The morphological changes in starch granules, especially, increased aggregation of starch granules at low temperatures could be a probable reason for decreased starch swelling power at low-temperature DHT. However, the increase in DHT temperature cause an increase in starch solubility which might lead to partial gelatinization, and hence results in a slight increase in swelling power (Vashisht et al., 2017; Liu et al., 2022a).

7.5.6 Paste Clarity

DHT influences the paste clarity of native starch, and the addition of ionic gum to starch prior to DHT further restricts the swelling property of starch granules. This can be assessed in terms of the change in % transmittance of starch gel. For instance, when the sample of native starch is modified at a certain temperature, the starch granules are ruptured and dispersed, followed by leaching out of amylose in the suspension with the increase in temperature. However, ionic gum pre-treatment of starch prior to DHT restricted the complete gelatinization and swelling of granules leading to relatively high integrity compared to native starch and forming a heterogeneous suspension. This change in suspension results in light scattering when a beam of light passes through the heterogeneous suspension and leads to the non-availability of % transmittance of ionic gums-modified samples (Su et al., 2018).

7.5.7 Pasting Properties and Gel Strength

The effect of the dry heat treatment method on the pasting behavior of different cereal starches was studied by various researchers. Li et al. (2013) studied the pasting profile of waxy rice starch using a dry heating technique and observed a decrease in onset temperature after dry heating at 130 °C for 4 h. This may be due to the breakdown of hydrogen bonds, resulting from which the distension of starch granules could be endorsed, hence the decline in onset temperature. Also, a decrease in peak viscosity of the starch extracted from millet was examined after dry heating (130 °C for 2–4 h), whereas an increase in final viscosity and trough viscosity was noted with the same DHT (Sun et al., 2014a). Continuous and repeated dry heating methods showed a decrease in pasting viscosities too. The highest swelling rate of the starch before breakdown is considered peak viscosity, which is indicative of the swelling power of starches (Zavareze & Dias, 2011). Millet starch showed a decline in the peak viscosity after DHT (130 °C at 2-4 h) (Sun et al., 2014a). Similar changes in peak and final viscosity of corn and pea starch were observed on DHT under similar conditions of temperature-time combination (Sun et al., 2014b). By using different DHT methods on sweet potato starches, the reduction in peak viscosity was calculated with an increase in CDHT time and RDHT cycles (Gou et al., 2019). This decline might be attributed to the improved alliance between the starch chains and strong forces among intra-granular bonds during the equilibrium period of DHT. The minimum viscosity in the late heating phase is represented as the Trough viscosity (TV). DHT showed a decrease in TV, indicating the rupturing of swollen starch granules (Gong et al., 2017). Also, this decline could be due to the strengthened configuration of starch after treatment. This indicated that the thermal stability of sweet potato starch could be improved by DHT (Gou et al., 2019).

Final viscosity (FV) is indicative of retrogradation, gel hardness and the amount of leached amylase (Xu et al., 2018a). Irrespective of the type of DHT, a significant decrease in FV was observed, possibly because the DHT could reduce the shortened amylopectin structure and help in précised crystalline arrangement. Setback viscosity is indicative of the possible deterioration of starches, and the inferior setback signifies that starch would be hard to depreciate. This trend of setback was observed for RDHT and CDHT treatments on sweet potato starch. The heat-paste stability is referred to as Breakdown viscosity (BV), and it is calculated as peak viscosity minus final viscosity (Xu et al., 2018b). An enhancement in the paste stability is revealed with a reduced breakdown viscosity after DHT, which showed that the starch turned out to be more resistant to elevated temperature and shearing after DHT (Sun et al., 2014b).

The peak time is indicative of the time period needed to achieve the highest viscosity, and the temperature at which the paste viscosity of starch starts amplifying is called as pasting temperature; both suggest the gelatinization behavior of starches (Kim et al., 2007). The peak time of sweet potato starches decreased with increased time and cycles of dry heating treatment, indicating that starch swelling could occur in less time. This might be because DHT could damage the surface configuration of starch, making it easy for the water molecules to move into the granules of starch effortlessly.

Sun et al. (2014a, b) compared the pasting curve of native and DHT starch with the viscosity curve of native and DHT flours for Proso millets. No significant change was observed in the starch curve after DHT, whereas the viscosity curves of DHT flour were near that of native and DHT starches. The results suggested that in the high-viscosity products, the dry heated flour could be the better choice than that of starches because flour remains a rich source of nutrients and also overcome the problem of starch extraction. However, The gelling property of starch is greatly associated with their amylose content as well as the kind of treatment. The gelling strength of DHT starch is also associated with storage temperature. Gel stored at a low storage temperature (4 °C) shows higher strength as compared to that stored at room temperature (25 °C) (Su et al., 2018).

7.5.8 *Starch Digestibility and Glycemic Index*

The *in vitro* starch digestibility of wheat bread showed significant changes in the digestibility behavior at varied dry heat treatment temperatures. A significant decrease in RDS (Rapidly digestible starch) and an increase in SDS (Slowly digestible starch) with the increase in DHT temperature was recorded by González et al. (2021). The researcher claimed that the trend might be due to the ease of access of amylolytic enzyme to starch molecules getting destabilized by the structural alterations of starch as persuaded by DHT. Bae and Lee (2018) reported similar evidence of an increase in SDS and RS (Resistant starch) in rice starch with distinctive heat treatment methods, and it is apparent to have desired nutritional attribute of starchy foods.

Resistant starch (RS), considered dietary fiber, primarily gets fermented in the gut by colon microbes after passing unhydrolyzed from the upper gastrointestinal tract (GIT). It is evident that RS provides health benefits to humans by producing short-chain fatty acids and modifying the composition of the gut microbiome (Gu et al., 2020). An enhancement in the level of RS in the sample of rice starch was observed, and it stated that the dry heating could be responsible for the reorganization between starch molecules and the restructuring of starch crystallites which ultimately improved the integrity of starch crystallization and hence the RS level (Bae & Lee, 2018). Therefore, the increase in RS can be achieved using the dry heating method as a promising processing means.

7.6 Effect of Repeated and Continuous Dry Heat Treatment

The effect of repeated and continuous DHT on starch properties was studied by various researchers, and significant results were observed. The time required to reach an equilibrium point between the situation of treatment and the degree of alteration in physicochemical and structural behavior of starch might be reduced in continuous DHT. Moreover, the effect of continuous treatment was observed to control the degree of alterations. On the other hand, if the cooling of granules of starches at room temperature is done after the point of stability is reached and then the reheating for numerous succession phases, the more significant modifications would be recorded. The pasting behavior of CDHT and RDHT sweet potato starches was also compared and it was observed that all the pasting viscosities and peak time of CDHT starches were lesser than those of RDHT starches (Gou et al., 2019). Although the difference was insignificant, the lower paste viscosity of CDHT starch signifies the high potency of intramolecular bond forces, which was more effective during CDHT than RDHT. Conversely, it was also noticed that during RDHT cycles, the structure of starch granules might be partially damaged, which is indicative of a reduced amount of energy required to paste RDHT starches. The effect of CDHT and RDHT was studied on physicochemical and digestive properties of mung bean starch by Liang et al. (2021) and the results showed that the alteration in swelling and solubility, amylose content and pasting parameters of mung bean starch was more prominent in CDHT as compared to that of RDHT. Zhou et al. (2021) performed CDHT and RDHT on quinoa starch and reported the effect on physicochemical and structural properties. A significantly higher water absorption index, relative crystallinity and RDS were examined in RDHT quinoa starch samples than that of CDHT for the similar period of time. However, RDHT starch had lower swelling power, solubility and paste viscosities as compared to that of CDHT sample. Overall, it was concluded that RDHT had superior performance in changing the physicochemical and structural characteristics of quinoa starch than CDHT.

7.7 Role of Ionic Gums on Starch Modification

Ionic gums play an important role in strengthening the gel-forming ability of DHT starch (Liu et al., 2022b). Su et al. (2018) found that the gel strength of DHT starch was influenced significantly by their native counterpart when pretreated with xanthan gum. The starch with high amylose content was found to have significantly higher gelling strength as compared to native starch as well as the starch with low and medium amylose content. Xanthan gum pretreated DHT-modified low amylose starch gel shows higher flowability due to low amylose content. Li et al. (2013) found that the swelling of the granules was restricted, and viscosity was increased continuously during pasting due to DHT of waxy rice starch impregnated with xanthan. After pasting, the ability of gel formation of the treated starch was strengthened, and the particle size was increased due to the cross-linking of starch granules with xanthan polymer. The crystallinity of DHT starch was also increased, but the amorphous region of treated starch granules became more resistant to DHT when impregnated with xanthan. However, crystallinity was found to be correlated with melting enthalpy. Furthermore, Chandanasree et al. (2016) studied the effect of DHT with and without ionic gums on various properties of cassava starch. Ji et al. (2016) investigated the properties of corn starch modified with lysine using dry heat treatment. Their study shows that dry heat treatment of starch with lysine reduced the pasting temperature and degree of crystallinity while increased viscosity and particle size of the starch granule due to the formation of a cross-link between native starch granule and lysine.

7.8 Applications of Dry Heat Treated Starch

7.8.1 Dry Heat Treatment and Starch-Based Bakery Products

The impact of DHT on wheat flour and the products formed from it, *viz.* bread, cakes etc., is another remarkable study investigating wheat characteristics. Several studies claim improved changes in whole wheat flour, wheat dough, bread etc., on treating with dry heat. As an example, the enhancement in the functionality of whole wheat flour to use in bread making was observed by Sudha et al. (2016). DHT of wheat dough at 100 °C for 12 min showed an improvement in mechanical stability, especially the viscosity of the dough (Bucsella et al., 2016). Further, an interesting change in the composition of volatile components was observed when the wheat flour was treated at temperatures more than 120 °C range. At elevated temperatures, an increase in the amount of furans, pyrazines and sulphur-containing components is claimed to improve flours' aroma (roasty) (Xu et al., 2017).

DHT has been observed to have a potentially beneficial impact on the shelf-life augmentation of different flours by way of decreasing enzyme activity and moisture content of fat-rich compounds (Purhagen et al., 2011). Along with such biological outcomes, DHT has a noteworthy influence on the physicochemical and functional behavior of flours. Starch molecules and gluten proteins are the major determinants of such properties, where the gluten proteins show unfolding above 50 °C. Due to this, the hydrophobic regions of protein fragments get more revealed, allowing the reorganization of disulfide bonds (Jeanjean et al., 1980). This leads to the formation of gluten aggregates, the alteration in molecular weight dispersal, and reduced extractability. These changes may lead to a durable dough production that can endure an elevated sugar content which is appropriate for cake/biscuit manufacturing (Nakamura et al., 2008; Chesterton et al., 2015).

Nakamura et al. (2008) observed that the DHT of wheat flour showed an increment in the springiness of pancakes as well as the volume of bread and sponge cake. This increase was claimed to be related to the enhanced hydrophobic behaviour of wheat starch which makes it available to bind to the surface of air bubbles and ultimately adds stability to the dough and batter of bread and cake, respectively (Seguchi, 2001). Based upon this, it was suggested that cereal flours' increased oil binding capacity was due to the modifications in the starch surface protein to hydrophobic from hydrophilic via DHT.

7.8.2 Dry Heat Treatment in 3D Food Applications

Three-dimensional printing is an innovative technique which is now used for attaining ingenious and individualized food items. In order to achieve this, the food formulations generally called as "inks" needs to be studied thoroughly before being processes through 3D printing. Maniglia et al. (2020a) used DHT to enhance the wheat starch characteristics with a motive to develop hydrogels to be utilized as “inks” for 3D printing. The method was observed to be easy and safe. DHT of wheat starch was done at 130 °C for 2 h and 4 h and an increase in granule size was recorded without any alteration in granule surface and shape. No change in the functional group of starch after DHT was witnessed which is an indicative of negligible oxidation. Further, the developed hydrogel “inks” from modified starch had more resistance to stress, more gel firmness, more structural strength at rest and lower synersis as compared to those based on native starch. Also, the hydrogels based on the starch dry heat treated for 4 h displayed the best results for printability and better reproducibility.

Similarly, the preparation of hydrogels was done by using cassava starch for the application in 3D printing (Maniglia et al., 2020b). DHT was conducted using similar time-tempeature combinations (130 °C for 2 h and 4 h) as used for wheat starch hydrogel preparation. An increase in granule size and carbonyl content was observed with an elevation in DHT time. Hence, the hydrogels obtained from the starches after DHT had improved gel texture, pasting behaviour and pritability that supports the usage of cassava starch for 3D printing.

7.8.3 Dry Heat Treatment in Functionality Enhancement of Dry Milk

DHT was used in the improvement of functional properties of low heat nonfat dry milk with the results of enhanced foaming properties and heat stability (Alan et al., 2019). When compared with high heat dry milk, low heat product had the improved functionality in context to emulsification and heat steadiness and the results were comparable or surpassed the properties of high heat products. The use of radio frequency dielectric heating technique alongwith dry heating could be a promising approach for the production of certain food items that can utilize the low heat nonfat dry milk, like, bakery, ice cream and meat products.

7.8.4 Dry Heat Treatment in Shelf Life Enhancement

The storage stability of pearl millet flour is a big challenge with respect to rancidity and organoleptic acceptability. In order to retain the quality of pearl millet flour during storage, DHT was performed before storage (Arora et al., 2002). It was observed that the rise in free fatty acid content and lipase activity was reduced in DHT flour during storage when compared with untreated flour. Also, the acceptability study revealed that the flour as well as chapatti made from heat treated pearl millet were organoleptically acceptable throughout the storage period. The bitterness and

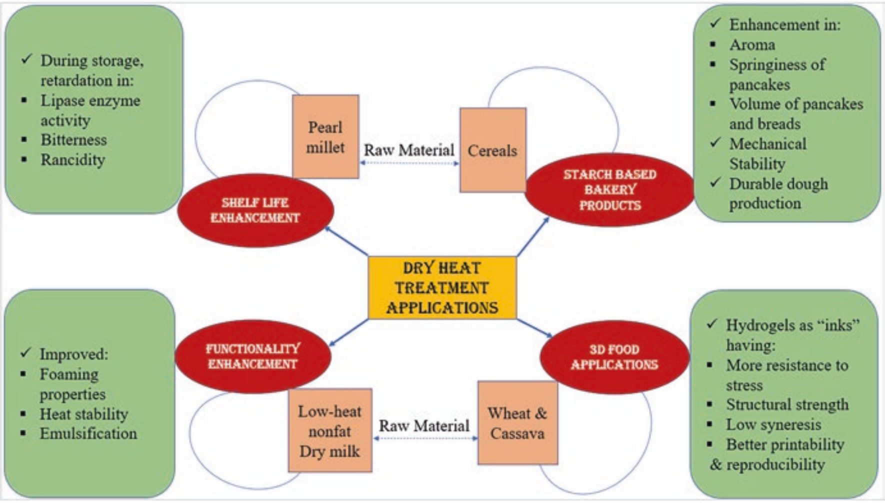

Fig. 7.2 Application of dry heat treatment

rancidity of pearl millet flour during storage is caused by the activity of lipase enzyme which caused the breakdown of glycerides and subsequent increase of free fatty acids (Pruthi, 1981). Hence, in order to retard the lipase activity before grinding, DHT of pearl millet grains for 2 h was found to be the most efficient for the reduction of the activity of lipase enzyme during storage (Kadlag et al., 1995). Singh and Goyal (2005) used DHT as one of the pre treatment methods for the processing of pearl millet to yield the flour with more shelf stability (Fig. 7.2).

7.9 Conclusion

A positive influence of DHT on various properties of diverse varieties of starch isolated from different food sources gives rise to a reliable and safe method of starch modification. Also, the variations in the treatment conditions with respect to time and temperature under CDHT and RDHT show significant variations in various properties of starch. Hence, DHT may prove to be an effective method for starch modifications. However, most studies investigated DHT modification of starch under limited treatment conditions such as heating temperature, reaction time and origin characteristics of starch. Therefore, extensive investigations need to be conducted under DHT conditions to understand the phenomenon precisely. Nevertheless, DHT is an effective technology that can be used in the starch processing industries to provide benefits to human health and the environment.

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Chapter 8
Heat-Moisture Treatment of Starch

Renuka Singh, Yogesh Kumar, Yograj Bist, Partiti Pamecha, Munit Shukla, and Anamika Singh

8.1 Introduction

Heat Moisture Treatment (HMT) is a well-established starch modification technique that involves subjecting starch to moisture levels of less than 35% w/w at temperatures ranging from 80–140 °C for 1–24 h (Aaliya et al., 2021). Various methods, such as oven heating, extrusion, autoclaving, and microwave heating, can be used for HMT. The process parameters, such as temperature, time, and moisture content, can be adjusted to achieve the desired modifications. During HMT, the modification is achieved due to increased starch chain interaction, leading to the rupture of the crystalline structure and subsequent disruption, rearrangement of the double-helix structure, and perfection of the small crystalline regions of the starch granule (Schafranski et al., 2021; Collado & Corke, 1999). However, these changes in the structure and properties of starch vary with the starch source and amylose content (da Rosa Zavareze, Pinto, et al., 2012a).

HMT significantly affects the morphology, crystallinity, double helix arrangement, and amylose-lipid complexes to change starch's in-vitro digestibility, pasting, and gel properties (Maniglia et al., 2021). HMT-modified starches' film-forming ability can be exploited to develop packaging material (Singh et al., 2009). The

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R. Singh
Department of Food Engineering and Technology, SLIET, Longowal, Punjab, India

Department of Food Technology, Parul Institute of Applied Sciences, Parul University, Vadodara, India

Y. Kumar · Y. Bist (✉) · A. Singh
Department of Food Engineering and Technology, SLIET, Longowal, Punjab, India

P. Pamecha · M. Shukla
Department of Food Technology, Parul Institute of Applied Sciences, Parul University, Vadodara, India

V. S. Sharanagat et al. (eds.), *Starch: Advances in Modifications, Technologies and Applications*, https://doi.org/10.1007/978-3-031-35843-2_8

studies on various starches indicate that HMT reduces the swelling of starch granules, increases the gelatinization temperature, and produces stable paste viscosity with less breakdown. These characteristics of HMT-modified starches resemble the typical characteristic of chemically modified crosslinked starch (Watcharatewinkul et al., 2009).

In recent years, physical methods like HMT have gained significant attention for modifying various starches, including corn, potato, rice, and wheat. It is more environmentally friendly than other starch modification techniques and requires lower cost and energy, making it an attractive option for food manufacturers (Ali et al., 2020). Recently, HMT starches have become popular among health-conscious people as they have relatively high resistant starch content and a lower glycemic index (Alimi et al., 2017). Resistant starch is a type of starch that resists digestion in the small intestine and passes to the colon, serving as dietary fiber (Mudgil & Barak, 2013). The production of resistant starch by HMT involves breaking down the starch molecules into smaller, more resistant molecules that are not easily digestible in the small intestine (Dupuis et al., 2014). Resistant starch has several health benefits, including improving bowel health and reducing the risk of colon cancer (Birt et al., 2013). Therefore, HMT-modified resistant starch has been used in several food applications, such as bread, pasta, and dairy products, to improve their nutritional value. Additionally, using HMT to produce resistant starch has the added benefit of not requiring chemicals, making it a more environmentally friendly and cost-effective option for food manufacturers.

8.2 Methods and Modification Mechanism

The HMT process involves the treatment of starch with moisture and heat under controlled conditions. Samples are prepared by mixing starch with water to obtain a specific moisture content. The moisture content depends on the desired degree of modification and can range from 15 to 35% (Schafranski et al., 2021). The samples are hermetically sealed and equilibrated for uniform moisture distribution and for restricting evaporative moisture loss. The pressure developed helps to increase thermal energy, leading to the kinetic energy of water molecules and causing large-scale segmental motions that enable the amorphous domain of starch to become more flexible. In the next step, the starch slurry is heated and incubated at a specific temperature, usually between 100 and 130 °C, for a specific time, typically between 2 and 24 hours (Lv et al., 2022). After the incubation period, excess moisture is removed from the modified starch through a drying process, and then the starch is milled to produce a fine powder. The extent of HMT modification can be intensified by prolonging the heating time, adding cycling times, and increasing the treatment temperature, promoting additional re-ordering between the starch. The increase in the interactions of the starch chains ruptures and separates the crystalline double helical structure during HMT. Further, the broken crystals subsequently rearrange themselves due to retrogradation during the cooling of treated samples. Figure 8.1

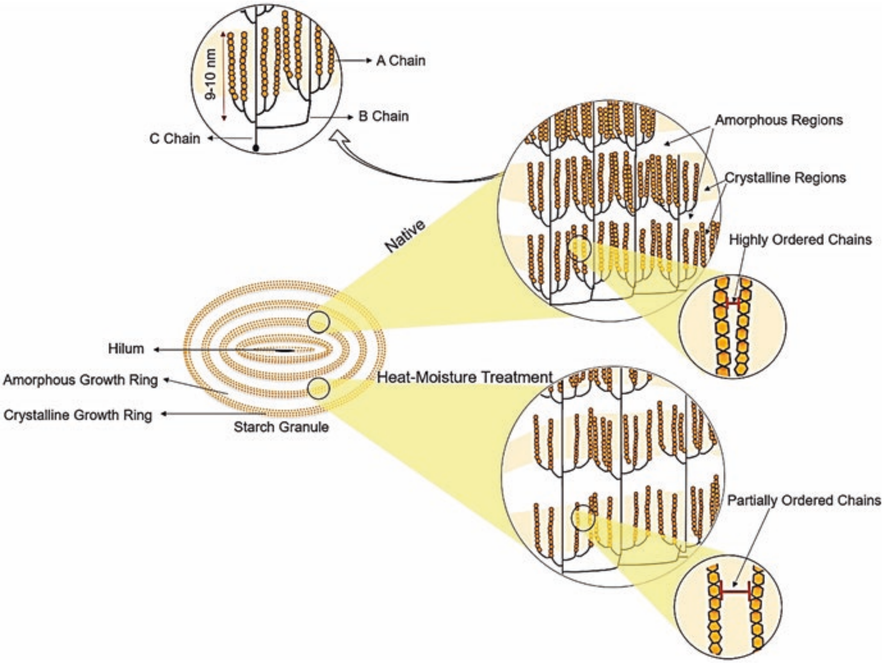

Fig. 8.1 Structure of native starch granules and after HMT-modified. (Reprinted from Schafranski et al. (2021) with permission from Elsevier)

shows the structural changes due to HMT modification. The HMT parameters, such as temperature and treatment time for the different methods, can be optimized based on the type of starch and the desired degree of modification. The extent of modification can be measured by parameters such as the degree of crystallinity, amylose content, and gelatinization properties using techniques such as X-ray diffraction, differential scanning calorimetry, and rapid visco analyzer (Schafranski et al., 2021; Wang et al., 2021).

8.2.1 HMT with Autoclave

The autoclave method is a common method for the HMT and involves subjecting the starch slurry to high temperatures and pressures in an autoclave. Starch is generally mixed with water to form a slurry (15–30% moisture content) with a desired consistency. The slurry is loaded into the autoclave and heated to a specific temperature (120–150 °C) and pressure (1.5–3.0 atm) for a predetermined time (30 min to 2 h). After reaching the desired time and temperature, the autoclave is cooled down, and the modified starch is collected. The exact treatment conditions can be varied depending on the type of starch and the desired extent of modification.

During the autoclave method, the high temperature and pressure cause the starch granules to swell and gelatinize, changing physical and chemical properties. As a result, autoclaved starch may have improved properties such as increased solubility, improved viscosity, and reduced retrogradation. More cycles of autoclaving can further increase the extent of modification. For example, Khurshida and Deka (2022) used cassava starch (Manihot esculenta) at moisture levels of 10% and 20% w/v and autoclaved for 30 min at 120 °C and a pressure of 15 psi. The samples were stored for 24 hours at 4 °C and underwent additional autoclaving and cooling cycles for 3 days. After the HMT treatment, the starch was dried at 40 °C until a constant mass was obtained.

8.2.2 HMT with Extrusion

HMT of starch with extrusion is carried out using an extruder, which applies heat, pressure, and high shear forces to change the starch properties. Firstly, starch is mixed with water to obtain desired moisture content. The moisture content is generally fixed below 40% to promote partial gelatinization (Singh et al., 2011). The mixture is fed into an extruder, passing through the extruder barrel under heat and mechanical shear. The high temperature and pressure break down the swollen starch molecules, leading to partial gelatinization. In addition, the high temperature and shear forces can also lead to the formation of new structures and complexes within the starch, resulting in modified functionality and properties. The mixture expands rapidly as it exits the die plate, forming a porous and fibrous structure and appearing as various cracks, pits, and holes on the surface of starch granules. Lastly, the expanded starch is dried to remove any residual moisture. The extruded starch has lower crystallinity and is resistant to digestion (Shrestha et al., 2010). However, the content of resistant starch formation depends on feed moisture and extrusion temperature. In addition, the partially gelatinized starch has increased solubility and viscosity when mixed with water, even at low temperatures. Conclusively, the extrusion significantly reduced the amylose content, darkened the color, increased solubility, and decreased gelatinization properties.

The method for modifying starch using extrusion is explained by Yan et al. (2019), who adjusted the moisture of corn starch to 30% for 24 h. A twin-screw laboratory-scale extruder having a screw diameter of 24 mm, a length-to-diameter ratio of 25:1, and a die-hole diameter of 6 mm was used to extrude the starch. The twin screw was segmented into four zones. In the first zone, liquid feeding was performed at 1.5 kg/h. In zones II-IV, three different temperature segments were heated by an electric cartridge with temperatures of 50 °C, 65 °C, and 85 °C. The temperature of these zones can be increased or decreased depending on the extent of modification. For example, Wang et al. (2021) set the barrel temperatures for three zones at 70, 90, and 100 °C. After extrusion, the corn starch was dried at 40 °C for 24 h.

8.3 Effect of Modification on Different Properties

8.3.1 Functional Properties

Many researchers reported that HMT reduces the swelling power (SP) of different starches like potato (Guanaratne & Hoover, 2002), kithul (Sudheesh et al., 2020), buckwheat (Goel et al., 2020), and rice (Hormdok & Noomhorn, 2007). The swelling power reduction is due to increased amylose-lipid interaction, amylose-amylopectin interactions, rearrangement, and association of starch chains. Moreover, the reorganization of starch chains promoted interactions among the starch functional groups resulting high ordered helical amylopectin side chain clusters, which inhibits the swelling power. Furthermore, the increase in moisture during HMT increases the extent of modification resulting in reduced swelling power and solubility. Sharma et al. (2015) reported a similar finding for pearl millet starch, the swelling and solubility decrease with increases in moisture levels. The rearrangement of the starch component restricted hydration which decreases the swelling power, and amylose dissociates from starch granules when heated in water, reducing solubility. White sorghum starch's swelling power and solubility decrease with increased moisture content after HMT modification. HMT increases the granule's rigidity and reduces the granular stability due to double helices disruption from the crystalline regions (Olayinka et al., 2008). The different sources of HMT-modified starch like cocoyam, African yam, and mung bean starch. A similar reduction in swelling and solubility was reported. The structural reorientation of starch crystallinity augmentation limits the swelling and solubility (Lawal, 2005; Adebowale et al., 2009; Barua & Srivasta, 2017).

However, Balasubramanian et al. (2014) reported that HMT-modified pearl millet has higher swelling and solubility. The possible reason may be that the impact on starch hydration is source-dependent, and discrepancies still occur among the same species. Zeng et al. (2015) also reported that HMT improves the swelling power of waxy rice starch due to the partial transformation of crystal into the loose, amorphous state of waxy starch, which favors more hydration and swelling.

8.3.2 Thermo-Pasting Properties

The effect of HMT on pasting properties varies with treatment temperature, starch moisture, and duration of the treatment. In general, HMT can reduce the peak viscosity, increase the breakdown viscosity, and delay the setback viscosity of starches. These changes can result in a smoother texture and improved mouthfeel in certain food products, such as bakery products, dairy products, and snacks. The rearrangement of starch chains due to HMT limits its hydration capacity and increases the gelatinization temperature, which can benefit products that require high-temperature processing, such as extruded snacks. The possible reason may be that HMT decreases the swelling capacity and reduces the destabilization effect of the amorphous region of crystallite melting. The limited granule swelling and a small-leached fraction reduce the paste viscosity. A similar increase in the pasting temperature of taro starch reported by Deka and Sit (2016), indicates higher mobility and conformational reorganization of the starch molecules after modification. Hormdok and Noomhorm (2007) reported that rice starch's pasting and gelatinization temperature increases after HMT, which shows that the starch becomes more stable during cooking. The HMT treatment forms a new superficial layer that restricts water penetration and surface transformation of starch granules by direct exposure to heat and moisture, thereby limiting water entry into the starch granules. These changes have also been confirmed by thermal analysis with an increase in gelatinization temperatures with the degree of HMT. In addition, HMT also reduces the enthalpy of gelatinization by increasing the mobility of double helices to form the crystalline structure, disrupting the hydrogen bonds in the double helices and between adjacent double helices (da Rosa Zavareze et al., 2012b). However, Hormdok and Noomhorm (2007) reported that the reduction in enthalpy might be due to the partial gelatinization of amylose and amylopectin molecules. In general, HMT-modified starches give single endothermic; however, some studies have also detected two endothermic peaks, especially at starch having moisture greater than 20% before treatment (da Rosa Zavareze et al., 2012b). The presence of two endotherms suggests that crystallites formed upon treatment may have been more heterogeneous (differences in crystalline size and stability) than the crystallites of low HMT starch. The two endotherms represent the melting of crystallites of widely differing stability. Hormdok and Noomhorm (2007) also observed two peaks in the gelatinization endotherm of HMT rice starch and suggested that it was due to the presence of two types of starch crystallites that caused a biphasic uptake of heat upon gelatinization.

8.3.3 Structural and Morphological Properties

Studies confirm that HMT does not alter granule shape and the integrity of starch granules (Fig. 8.2). However, the granule's surface experiences change like cavities, pitting, and indentation due to the re-association of the starch chains within the granule where the tissue structure was weak (Li et al., 2011a). Moreover, the pressure and heating outside the starch granules can cause the loosely packed granules to become more compact and tighter due to recrystallization. The more the interaction of starch chains, the tighter the structure will be within amorphous regions. However, Deka and Sit (2016) reported that autoclaved taro starch loses its physical integrity due to swelling, breakup, and aggregation of the granules.

The autoclaved taro starch showed a typical A-type pattern and a reduction in the peak intensity pattern of XRD. The reduction in intensity pattern is due to the loss of the crystalline array, which breaks the hydrogen bond and displaces the neighboring double helices. The morphological properties of autoclave-treated

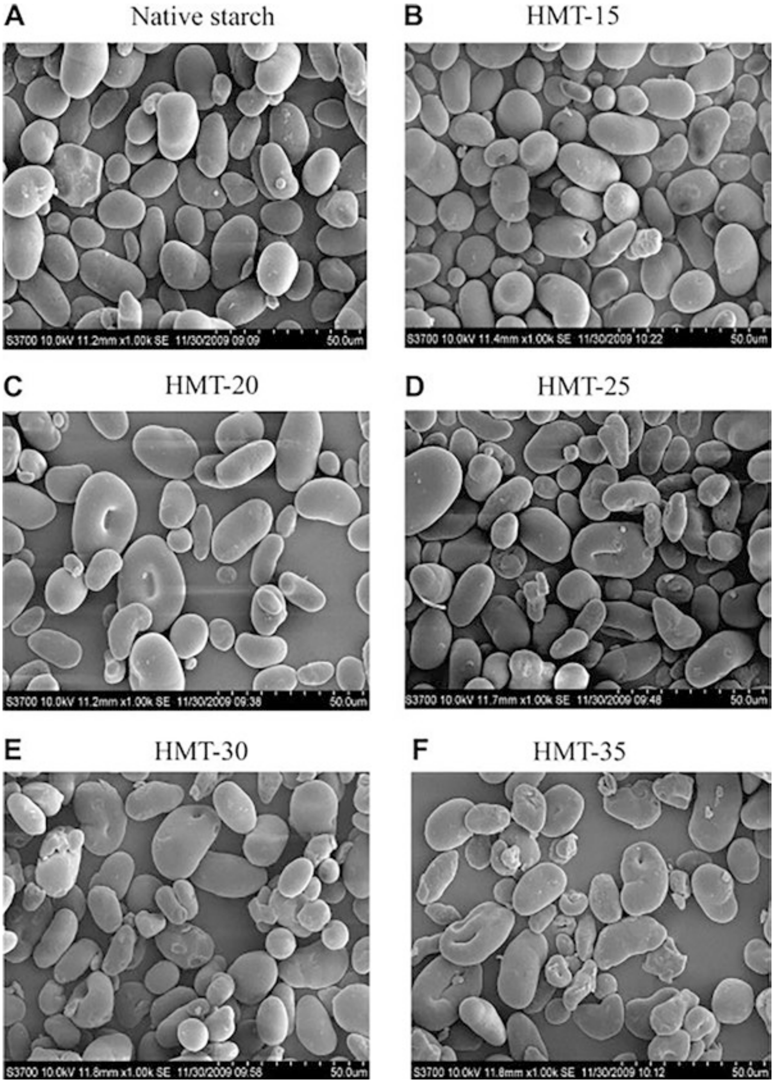

Fig. 8.2 Scanning electron micrographs (at 1000 × magnification) of native and HMT-modified mung bean starches. The moisture level of starch was adjusted to 15%, 20%, 25%, 30%, and 35% before treatment at 120 °C for 12 h. (Reprinted from Li et al. (2011a) with permission from Elsevier)

starches show the absence of spherical structure due to gelatinization, which provides swelling and damages the original starch granules. The crystallinity of potato and brown rice starch decreases after modification (Chakraborty et al., 2021). The decrease in the degree of crystallinity is due to the degradation of amylopectin content in the crystalline matrix and a reduction in the long-chain order of starch granules.

8.3.4 Digestibility

Depending upon chain length, amylopectin chains from the starch structure are three types A, B, or C. The A type is the shortest chain joined by α-1-6 bonds in both amorphous and crystalline phases. The B types are situated in the amorphous regions with high clustering zones. The shorter the chain, and more susceptible to physicochemical modification, acid hydrolysis, and enzymatic digestion. The HMT lowers the content of rapidly digestible starch (RDS) and increases the levels of slowly digestible starch (SDS) and resistant starch (RS) (Luga and Mironeasa, 2019). The structural rearrangement of molecular chains creates a densely packed network less susceptible to enzyme breakdowns, resulting in reduced digestion rates and glycemic index of gelatinized HMT starches. Such a reduction in starch digestibility can be beneficial for managing blood glucose levels. The glycaemic index of starch depends upon the amylose-amylopectin ratio. Higher amylose content lowers the glycaemic index (Fuentes-Zaragoza et al., 2010). Wang et al. (2016) reported HMT modified high amylose maize starch with different moisture levels has high SDS and RS content. The HMT provides structural disorganization that strengthens the starch granules, induces crystallite perfection, and leads to less suspectable enzyme attack. Another possible reason may be that during HMT, interaction between starch, lipids, or protein make it more resistant to enzyme attack because the protein layer is formed on the starch surface, which inhibits the access of amylase (Chen et al., 2015). The digestibility of starch is related to swelling power, thermal properties, and granule size. Low solubility, low swelling capacity, and high gelatinization enthalpy are factors that can increase the association between crystalline regions of HMT starches, which can lead to increased resistance to digestion (Iuga and Mironeasa, 2020). On the other hand, autoclave heating of cassava starch for 20 minutes in boiling water leads to gelatinization, making it more accessible to pancreatin enzymes and decreasing its digestion resistibility (Khurshida & Deka, 2022).

8.4 Applications of HMT-Treated Starches

8.4.1 Applications in Food

HMT of starches has proven highly beneficial in various industrial applications, particularly in the food industry. HMT starches possess essential characteristics such as gelatinization factor, thermal stability, textures, and shear resistance (Kaur & Singh, 2019). In the food industry, HMT-treated starches improve various products' texture, stability, and sensory properties, including bread, noodles, and sauces. HMT-modified starches are also employed as fat replacers in low-fat foods, expanders in biscuits, binders in baked goods, and thickeners in canned goods, creams, and beverages (Schafranski et al., 2021). Subroto et al. (2020) found that mixing HMT potato starch with tomato sauce improves the thermal stability, flavor, color, and overall acceptability on 5 points hedonic scale.

HMT increases the gelatinization temperature of potato starch and increases the viscosity stability of tomato sauce. Gluten-free noodles are getting more popular day by due to the increase in the population with gluten and celiac disease sensitivity. The HMT-modified potato starch is the best alternative with added salt to improve the cooking and textural properties of potato starch noodles (Yang et al., 2022). HMT starches have proven to be desirable for providing good expansion, minimal cooking time, and a firm texture to sauces, confections, canned goods, and pasta (Chandla et al., 2017). HMT has also been utilized to improve the properties of biodegradable films made from rice flour and starch, resulting in enhanced tensile strength, increased stretch resistance, and increased rigidity and extensibility (Majzoobi et al., 2015). However, HMT-modified starch films displayed increased thickness, tensile strength, elongation, and decreased solubility (Indrianti et al., 2018). The starch-based bioplastic film was prepared from HMT-modified green plantain banana starch. The starch showed reduced maximum viscosity, increased pasting temperature, and better paste stability to temperature and shear. The mechanical properties of bioplastic was improved, such as elastic modulus, tensile strength, and elongation (Viana et al., 2022). HMT modifications of starches enhance their thermal stability and lessen their retrogradation, making them highly beneficial for use in the canned and frozen food industries (Jayakody & Hoover, 2008). HMT provides desirable properties, including decreased swelling power, amylose leaching, and increased heat and shear stability, which help make good-quality noodles. As a result of the market for modified starch expansion and development, ingredient industries have been developing starches with distinctive properties to meet the needs of each market segment. Physical modification of starch, specifically HMT, has dominated the global landscape due to its low cost, ease of use, and clean, scalable technology. Modified starches are crucial in processed foods and can be utilized in many industries.

8.4.2 Application in Modifying Dough Parameters

Research has shown that HMT-modified cereals starches (HMCS) can positively impact the texture and stability of wheat-based dough. Specifically, adding HMT-millet flour to wheat-based dough can significantly increase the critical stress and strain values, preventing the dough structure from collapsing (Collar et al., 2020). Similarly, gluten dough made with HMT barley flour has been found to increase storage modulus and decrease loss tangent compared to gluten dough made with untreated barley flour (Lazaridou et al., 2019). However, while oscillatory tests have confirmed these findings, they do not provide insight into the deformation that can be applied during actual processing.

To better understand the effect of HMT, large-deformation mechanical analyses such as the creep recovery test should be used to confirm improvements in dough stability. It should also be noted that the presence of HMCS can reduce dough resistance to shear breakdown and stability in certain situations. Despite rheological data, the architectural characteristics of HMCS-containing dough, such as the internal network's homogeneity and clusters' existence, have not been thoroughly examined. Microscopic measurements could be helpful in this regard. The interactions between starch and gluten fundamentally determine the quality of wheat-based dough. Starch granules act as filling agents, and smaller or irregularly shaped granules can result in a higher filling degree and more harmonious interaction between the dough's components, potentially increasing its stability. The unique distribution of HMCS particles in the gluten reticular skeleton may be responsible for the effect of HMT.

Molecular interactions between HMCS and glucose have not been extensively studied. However, research has shown that dough viscoelasticity is determined by the disulfide bond, which is crucial in maintaining the integrity of the gluten tertiary structure. Exogenous starch has been found to help convert free sulfhydryl into disulfide bonds in the reconstituted dough (Song et al., 2020). The relative crystallinity/short-range order of starch has been found to correlate negatively with dough stability. HMCS has lost its crystallinity, and its exposed hydroxyls may form hydrogen bonds with gluten amino groups, thereby strengthening the inner framework. Infrared spectroscopy can study gluten secondary structure fluctuation and functional groups of HMCS to gain a systematic understanding of the mechanism.

Finally, too many free water molecules can weaken the gluten network, and HMT alters the water hydration capacity of starch. Therefore, it is important to determine whether dough systems experience similar changes and confirm the water status and distribution to support the dough's textural changes caused by HMCS. Understanding the precise function of HMCS in the dough may enhance the quality of finished goods (Wang et al., 2021).

8.4.3 Application in Definitive Food Products

The textural properties of the final products are the most crucial parameters, which affect the consumer's behaviors. According to Chung et al. (2012), noodles made from HMCS-containing flour have a darker appearance and higher yellowness and redness values due to the Maillard reaction between amino compounds and short-chain reducing sugars expelled from starch granules during HMT. Despite this effect, adding HMCS to flour is still considered beneficial for improving the overall quality of noodles, particularly those fortified with bioactive compound-rich cereals.

Another study by Liu et al. (2019) found that using germinated wheat flour in conventional wheat noodles reduced the tensile strength, springiness, and hardness of noodles. However, incorporating HMT-treated germinated flour at the same substituting level significantly mitigated the decrease in these textural parameters, suggesting that improved HMCS-gluten interactions or moderate starch gel rigidity could be beneficial for better HMT wheat noodles.

For gluten-free noodles, Chandla et al. (2017) reported that those made solely from HMCS amaranth were superior in terms of aroma and flavor, as well as hardness and springiness, compared to untreated starch noodles. However, the mouthfeel was found to be poorer, and it is unclear whether the use of HMCS from other cereals would result in similar issues. It may be more appropriate to incorporate HMCS in proportionate amounts.

Furthermore, Fathi et al. (2016) found that baked goods made with HMCS-based flour tend to darken as the HMT intensity increases. It is important to carefully select the appropriate HMT conditions when developing food products to ensure they are visually and sensorially pleasing. However, HMCS has been found to extend the shelf life of baked goods. Chung et al. (2014) reported that HMT-germinated rice flour reduced moisture changes and delayed the hardening of cookies after a week of storage, possibly due to the HMCS's ability to prevent retrogradation. There is no evidence that HMCS interacts with other food ingredients, and studying products supplemented with a single HMCS could provide a clearer understanding of related mechanisms. Overall, the use of HMCS in food production shows promise for improving product quality and shelf life, but careful consideration of its effects on various sensory and textural properties is necessary.

8.5 Summary

Hydrothermal treatments are a sustainable and cost-effective approach for modifying starches, making it a dominant physical modification method globally. This clean technology can be easily scaled and handled. The changes in starch properties and structure depend on factors such as botanical origin, composition, amylose-amylopectin ratio, and treatment conditions like temperature, moisture, and time. These treatments increase the nutritional quality of starch-based systems by boosting the content of slowly digestible starch and resistant starch. HMT-treated starches enhance the texture, stability, and sensory properties of products like bread, noodles, and sauces. Additionally, HMT-modified starches are used as fat replacers in low-fat foods, expanders in biscuits, and binders in baked goods.

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Chapter 9
Annealing of Starch

Pham Van Hung and Trinh Thi My Duyen

9.1 Introduction

Starch has been used as a popular ingredient for producing starch-based foods, which are usually served after being passed a thermal process such as cooking, baking, or frying to enhance its flavor, appearance and palatability. In some cases, these products are dried to prolong their shelf-life and make easier for storage purpose. However, the low moisture starchy foods suffer a change in their molecular level called “staling” caused by “retrogradation” – a phenomenon in which gelled starch exclude water from the polysaccharide matrix. After retrogradation, starch tends to recrystallize and causes a negative impact on the long-term stability, freshness, and quality of the products (Lai et al., 2000). These influences lead to economic losses for both the food industry and the consumer. Although the retrogradation process occurs very quickly, it can be controlled by a range of factors, including storage conditions, especially temperature, starch level, supplement of additives, and physical modification of the starch (Hoover, 1995).

Native starches, which exhibit poor functional properties such as poor thermal, shear and acid stability, and high rates and extent of retrogradation, are not widely applied for food processing. Nowadays, starches utilized in the food industry should be modified such as chemically modified by cross-linking and/or substitution, enzyme modification such as dextrination or debranching or physical modification such as annealing and heat-moisture treatment which modify the physicochemical property of starch without destroying itsgranular structure.

P. Van Hung (✉) · T. Thi My Duyen
Department of Food Technology and Laboratory for Interdisciplinary Research and Analysis on Chemistry, Food, Environment, International University, Vietnam National University (VNU-HCM), Ho Chi Minh City, Vietnam
e-mail: pvhung@hcmiu.edu.vn

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Annealing is a physical modification technique that associates incubation of starch at a specific temperature above the glass transition temperature (T_g) of starch but below the onset temperature (T_o) of gelatinization and an excess water (greater orequal 40%) during a certain time period. This physical treatment modifies the structure and properties of starch to certain extents but does not affect the shape of starch granule (Sharma & Tejinder, 2014). Annealing is considered as one of the most powerful physical modifications of starch to change the undesirable characteristics of usual starch and also keep treated starch in "natural form" without the involvement of any chemical. As the food industry intends to develop more convenient and wholesome processed products, annealed starch is a product which can propel it in that direction.

Starch exists as semi-crystalline structure and annealing affects both its crystalline and amorphous regions. On a molecular level, native starch is plasticized by excess amount of water during annealing which enhances the movement of double-helical chain segments of amylose and amylopectin branches within granules, improves the alignment, as well as lengthens or expands the existing double helices located in the crystalline region, facilitates the expansion and perfection of existing crystallites, forms new double helices through varied associations i.e. amylose-amylose, amylose-amylopectin, amylopectin-amylopectin between starch chains to create more uniform crystallites without breaking native starch chain (Gomand et al., 2012; Tester & Debon, 2000). Annealing also induces hydration, movement as well as structural rearrangement of starch chains within granule amorphous areas, resulting in the increase in gelatinization temperature and rigidness of granule located in amorphous structure (Tester & Debon, 2000). These alterations are the reason for decreased swelling power, solubility and lower rate of retrogradation process, increased stability and resistant starch content of annealed starch compared to its untreated counterparts.

In this chapter, the mechanism, methodology and the change in structure and physicochemical properties of post-annealing starch are reviewed and discussed.

9.2 Modification Mechanism and Methodology

9.2.1 Single, Double and Multi-step Annealing

There are two main methods for annealing treatment. First 'continuous annealing' (single-step annealing) and second 'repeated annealing' that includes double-step or multi-step annealing (Table 9.1). In the continuous annealing, starch granules in plenty (>60% [w/w]) or at medium water content (40% [w/w]) are kept at a temperature between the glass transition temperature (T_g) and the onset (T_o) temperature of gelatinization (Table 9.1) for a set period of time for every 12 h (Hoove & Vasanthan, 1993; Jacobs & Delcour, 1998; Tester & Debon, 2000). During multi-step annealing, starch is initially annealed below T_o of the native

Table 9.1 Annealing conditions and gelatinization parameters of starches from different botanical sources

Starch source	Annealing parameters				Gelatinization parameters						References
	Temp (°C)	Steps (≠)	Time (h)	W:S	S:W	To	Tp	Tc	Tc-To	ΔH (J/g)	
Brown rice flour (native)					1:02	57.2	63.0	69.7	12.5	13.4	Bian and Chung (2016)
Brown rice flour (annealed)	40	Single	24	7:03	1:02	60.0	65.6	72.9	12.9	13.3	Bian and Chung (2016)
Sweet potato (native)					1:03	66.64	69.12	74.65	9.68	8.87	Aijun et al. (2020)
Sweet potato (annealed)	50	Single	24	3:01	1:03	66.58	71.01	75.51	8.97	8.80	Aijun et al. (2020)
	50	Single	72	3:01	1:03	68.29	72.59	76.76	8.47	8.62	Aijun et al. (2020)
	50	Single	120	3:01	1:03	70.20	73.07	76.97	6.77	9.27	Aijun et al. (2020)
Andean oca (*Oxalis tuberosa*) (native)					1:03	51.92	56.50	62.36	10.44	14.53	Puelles-Román et al. (2021)
Andean oca (*Oxalis tuberosa*) (annealed)	42	Single	24	2:01	1:03	55.30	59.20	64.29	9.00	15.13	Puelles-Román et al. (2021)
	42	Single	24	6:01	1:03	55.11	59.1	64.24	9.13	14.61	Puelles-Román et al. (2021)
	50	Single	24	2:01	1:03	59.69	62.77	67.36	7.67	14.14	Puelles-Román et al. (2021)
	50	Single	24	6:01	1:03	59.89	62.75	67.25	7.36	13.74	Puelles-Román et al. (2021)
Oat (native)					1:03	58.18	62.20	67.93	9.75	14.31	Werlang et al. (2021)
	50	Single	24	3:01	1:03	65.28	67.73	70.91	5.63	12.42	Werlang et al. (2021)
Waxy corn (native)					3:11	64.2	70.3	76.3	12.1	14.8	Samarakoon et al. (2019)
Waxy corn (annealed)	54.2	Single	2	3:01	3:11	65.8	71.0	77.3	11.5	14.0	Samarakoon et al. (2019)

(continued)

Table 9.1 (continued)

Starch source	Annealing parameters				Gelatinization parameters						References
	Temp (°C)	Steps (≠)	Time (h)	W:S	S:W	To	Tp	Tc	Tc-To	ΔH (J/g)	
		Single	4	3:01	3:11	66.4	71.3	77.5	11.1	13.8	Samarakoon et al. (2019)
		Single	8	3:01	3:11	67.1	71.9	77.9	10.8	13.6	Samarakoon et al. (2019)
		Single	16	3:01	3:11	67.7	72.0	77.6	9.9	13.7	Samarakoon et al. (2019)
		Single	24	3:01	3:11	67.8	72.0	77.4	9.6	13.2	Samarakoon et al. (2019)
		Single	48	3:01	3:11	69.8	73.7	78.4	8.6	15.0	Samarakoon et al. (2019)
		Single	72	3:01	3:11	69.9	73.7	78.3	8.4	14.6	Samarakoon et al. (2019)
Waxy barley (native)					3:11	56.9	61.4	67.1	10.2	11.9	Samarakoon et al. (2019)
Waxy barley (annealed)	46.9	Single	2	3:01	3:11	61.2	63.9	68.1	6.9	11.4	Samarakoon et al. (2019)
		Single	4	3:01	3:11	62.1	64.5	68.8	6.7	11.7	Samarakoon et al. (2019)
		Single	8	3:01	3:11	62.7	65.1	69.4	6.7	11.3	Samarakoon et al. (2019)
		Single	16	3:01	3:11	63.3	65.6	69.7	6.3	11.9	Samarakoon et al. (2019)
		Single	24	3:01	3:11	63.9	66.2	69.8	5.9	11.8	Samarakoon et al. (2019)
		Single	48	3:01	3:11	64.9	67.1	70.9	6.0	11.9	Samarakoon et al. (2019)
		Single	72	3:01	3:11	64.6	66.7	70.0	5.4	12.0	Samarakoon et al. (2019)
Waxy rice (native)					3:11	59.1	66.5	72.8	13.8	11.3	Samarakoon et al. (2019)
Waxy rice (annealed)	49.1	Single	2	3:01	3:11	60.8	66.9	73.1	12.3	11.0	Samarakoon et al. (2019)
		Single	4	3:01	3:11	61.3	67.2	73.1	11.8	12.3	Samarakoon et al. (2019)
		Single	8	3:01	3:11	62.0	67.6	73.8	11.8	12.0	Samarakoon et al. (2019)
		Single	16	3:01	3:11	62.4	67.7	73.8	11.4	12.0	Samarakoon et al. (2019)
		Single	24	3:01	3:11	62.8	68.0	74.0	11.2	11.8	Samarakoon et al. (2019)

(continued)

Table 9.1 (continued)

Starch source	Annealing parameters				Gelatinization parameters						References
	Temp (°C)	Steps (≠)	Time (h)	W:S	S:W	To	Tp	Tc	Tc-To	ΔH (J/g)	
		Single	48	3:01	3:11	64.9	69.3	74.0	9.1	11.6	Samarakoon et al. (2019)
		Single	72	3:01	3:11	65.5	69.8	74.6	9.1	11.7	Samarakoon et al. (2019)
Waxy potato (native)					3:11	62.9	68.3	73.6	11.1	17.5	Samarakoon et al. (2019)
Waxy potato (annealed)	52.9	Single	2	3:01	3:11	68	71.4	76.3	8.3	17.0	Samarakoon et al. (2019)
		Single	4	3:01	3:11	68.5	71.7	76.4	7.9	16.8	Samarakoon et al. (2019)
		Single	8	3:01	3:11	69.2	72.4	76.7	7.5	16.5	Samarakoon et al. (2019)
		Single	16	3:01	3:11	69.3	72.9	76.7	7.4	16.2	Samarakoon et al. (2019)
		Single	24	3:01	3:11	69.8	72.9	77.3	7.5	16.7	Samarakoon et al. (2019)
		Single	48	3:01	3:11	70.7	73.7	77.6	6.9	17.9	Samarakoon et al. (2019)
		Single	72	3:01	3:11	71.0	73.9	78.3	7.3	17.8	Samarakoon et al. (2019)
Normal wheat (native)					1:03	56.65	61.17	68.77	12.12	8.11	Su et al. (2020)
Normal wheat (annealed)	50	Single	12	3:01	1:03	64.76	66.74	73.05	8.29	9.10	Su et al. (2020)
	50	Two	12	3:01	1:03	–	–	–	–	–	Su et al. (2020)
			12	3:01	1:03	65.81	67.72	74.35	8.54	9.79	Su et al. (2020)
	50	Four	12	3:01	1:03	–	–	–	–	–	Su et al. (2020)
			12	3:01	1:03	–	–	–	–	–	Su et al. (2020)
			12	3:01	1:03	–	–	–	–	–	Su et al. (2020)
			12	3:01	1:03	66.55	68.65	75.32	8.77	9.83	Su et al. (2020)
	50	Six	12	3:01	1:03	–	–	–	–	–	Su et al. (2020)

(continued)

Table 9.1 (continued)

Starch source	Annealing parameters				Gelatinization parameters						References
	Temp (°C)	Steps (≠)	Time (h)	W:S	S:W	To	Tp	Tc	Tc-To	ΔH (J/g)	
			12	3:01	1:03	–	–	–	–	–	Su et al. (2020)
			12	3:01	1:03	–	–	–	–	–	Su et al. (2020)
			12	3:01	1:03	–	–	–	–	–	Su et al. (2020)
			12	3:01	1:03	–	–	–	–	–	Su et al. (2020)
			12	3:01	1:03	66.72	68.74	74.68	7.96	10.63	Su et al. (2020)
	50	Eight	12	3:01	1:03	–	–	–	–	–	Su et al. (2020)
			12	3:01	1:03	–	–	–	–	–	Su et al. (2020)
			12	3:01	1:03	–	–	–	–	–	Su et al. (2020)
			12	3:01	1:03	–	–	–	–	–	Su et al. (2020)
			12	3:01	1:03	–	–	–	–	–	Su et al. (2020)
			12	3:01	1:03	–	–	–	–	–	Su et al. (2020)
			12	3:01	1:03	–	–	–	–	–	Su et al. (2020)
			12	3:01	1:03	67.07	69.19	77.44	10.37	10.40	Su et al. (2020)
	50	Single	24	3:01	1:03	65.69	67.59	73.57	7.88	9.44	Su et al. (2020)
			48	3:01	1:03	66.38	68.46	74.68	8.3	9.78	Su et al. (2020)
			72	3:01	1:03	66.56	68.68	74.58	8.02	10.61	Su et al. (2020)
			96	3:01	1:03	66.67	68.83	76.25	9.58	10.33	Su et al. (2020)
Waxy wheat (native)					1:03	59.29	65.22	74.36	15.07	10.51	Su et al. (2020)
Waxy wheat (annealed)	50	Single	12	3:01	1:03	64.70	68.21	75.54	10.84	10.59	Su et al. (2020)
	50	Two	12	3:01	1:03	–	–	–	–	–	Su et al. (2020)

(continued)

Table 9.1 (continued)

Starch source	Annealing parameters				Gelatinization parameters						References
	Temp (°C)	Steps (≠)	Time (h)	W:S	S:W	To	Tp	Tc	Tc-To	ΔH (J/g)	
			12	3:01	1:03	65.69	68.89	75.57	9.88	10.75	Su et al. (2020)
	50	Four	12	3:01	1:03	–	–	–	–	–	Su et al. (2020)
			12	3:01	1:03	–	–	–	–	–	Su et al. (2020)
			12	3:01	1:03	–	–	–	–	–	Su et al. (2020)
			12	3:01	1:03	66.58	69.57	76.72	10.14	11.74	Su et al. (2020)
	50	Six	12	3:01	1:03	–	–	–	–	–	Su et al. (2020)
			12	3:01	1:03	–	–	–	–	–	Su et al. (2020)
			12	3:01	1:03	–	–	–	–	–	Su et al. (2020)
			12	3:01	1:03	–	–	–	–	–	Su et al. (2020)
			12	3:01	1:03	–	–	–	–	–	Su et al. (2020)
			12	3:01	1:03	66.97	70.03	79.03	12.06	13.00	Su et al. (2020)
	50	Eight	12	3:01	1:03	–	–	–	–	–	Su et al. (2020)
			12	3:01	1:03	–	–	–	–	–	Su et al. (2020)
			12	3:01	1:03	–	–	–	–	–	Su et al. (2020)
			12	3:01	1:03	–	–	–	–	–	Su et al. (2020)
			12	3:01	1:03	–	–	–	–	–	Su et al. (2020)
			12	3:01	1:03	–	–	–	–	–	Su et al. (2020)
			12	3:01	1:03	–	–	–	–	–	Su et al. (2020)
			12	3:01	1:03	67.28	70.42	79.44	12.16	13.2	Su et al. (2020)
	50	Single	24	3:01	1:03	66.27	69.21	77.18	10.91	11.84	Su et al. (2020)
			48	3:01	1:03	67.24	70.06	78.37	11.13	13.14	Su et al. (2020)

(continued)

Table 9.1 (continued)

Starch source	Annealing parameters				Gelatinization parameters						References
	Temp (°C)	Steps (≠)	Time (h)	W:S	S:W	To	Tp	Tc	Tc-To	ΔH (J/g)	
			72	3:01	1:03	67.70	70.82	80.46	12.76	13.25	Su et al. (2020)
			96	3:01	1:03	68.39	71.74	80.16	11.77	13.85	Su et al. (2020)
Wheat (native)					1:03	55.6	60.9	67.3	11.7	10.3	Wang et al. (2017)
Wheat (annealed-freeze dried)	30	Single	24	3:01	1:03	56.2	60.7	66.9	10.7	10.4	Wang et al. (2017)
	40			3:01	1:03	59.5	62.9	67.2	7.7	10.5	Wang et al. (2017)
	50			3:01	1:03	67.7	69.9	73.7	6.0	5.6	Wang et al. (2017)
Yam (native)					1:03	60.0	78.7	89.4	29.4	13.2	Wang et al. (2017)
Yam (annealed-freeze dried)	30	Single	24	3:01	1:03	60.0	78.5	88.5	28.5	13.4	Wang et al. (2017)
	40	Single	24	3:01	1:03	60.1	78.6	89.1	29.0	13.7	Wang et al. (2017)
	50	Single	24	3:01	1:03	62.3	78.5	88.9	26.6	13.1	Wang et al. (2017)
Potato (native)					1:03	55.0	59.5	65.8	10.8	13.0	Wang et al. (2017)
Potato (annealed-freeze dried)	30	Single	24	3:01	1:03	55.1	59.7	66.4	11.3	13.3	Wang et al. (2017)
	40	Single	24	3:01	1:03	56.0	60.2	66.8	10.8	13.5	Wang et al. (2017)
	50	Single	24	3:01	1:03	56.8	61.6	68.2	11.4	13.1	Wang et al. (2017)
Potato (annealed-ethanol dried)					1:03	62.9	67.1	73.2	10.3	15.4	Wang et al. (2017)
	30	Single	24	3:01	1:03	62.9	66.8	72.7	9.8	15.4	Wang et al. (2017)
	40	Single	24	3:01	1:03	64.6	68.1	73.5	8.9	15.9	Wang et al. (2017)

(continued)

Table 9.1 (continued)

Starch source	Annealing parameters				Gelatinization parameters						References
	Temp (°C)	Steps (≠)	Time (h)	W:S	S:W	To	Tp	Tc	Tc-To	ΔH (J/g)	
	50	Single	24	3:01	1:03	68.5	71.4	75.9	7.4	16.2	Wang et al. (2017)
Potato (native)					1:03	61.49	65.53	75.05	13.56	16.7	Xu et al. (2018)
Potato (annealed)	55	Single	12	3:01	1:03	68.02	70.73	79.59	11.57	17.29	Xu et al. (2018)
	55	Two	12	3:01	1:03	–	–	–	–	–	Xu et al. (2018)
			12	3:01	1:03	68.37	70.92	79.67	11.3	17.29	Xu et al. (2018)
	55	Four	12	3:01	1:03	–	–	–		–	Xu et al. (2018)
			12	3:01	1:03	–	–	–		–	Xu et al. (2018)
			12	3:01	1:03	–	–	–		–	Xu et al. (2018)
			12	3:01	1:03	69.79	72.28	80.27	10.48	17.80	Xu et al. (2018)
	55	Six	12	3:01	1:03	–	–	–		–	Xu et al. (2018)
			12	3:01	1:03	–	–	–		–	Xu et al. (2018)
			12	3:01	1:03	–	–	–		–	Xu et al. (2018)
			12	3:01	1:03	–	–	–		–	Xu et al. (2018)
			12	3:01	1:03	–	–	–		–	Xu et al. (2018)
			12	3:01	1:03	70.99	73.43	82.78	11.79	17.95	Xu et al. (2018)
	55	Eight	12	3:01	1:03	–	–	–		–	Xu et al. (2018)
			12	3:01	1:03	–	–	–		–	Xu et al. (2018)
			12	3:01	1:03	–	–	–		–	Xu et al. (2018)
			12	3:01	1:03	–	–	–		–	Xu et al. (2018)
			12	3:01	1:03	–	–	–		–	Xu et al. (2018)
			12	3:01	1:03	–	–	–		–	Xu et al. (2018)

(continued)

Table 9.1 (continued)

Starch source	Annealing parameters				Gelatinization parameters						References
	Temp (°C)	Steps (≠)	Time (h)	W:S	S:W	To	Tp	Tc	Tc-To	ΔH (J/g)	
			12	3:01	1:03	–	–	–		–	Xu et al. (2018)
			12	3:01	1:03	71.38	73.92	84.70	13.32	18.4	Xu et al. (2018)
	55	24		3:01	1:03	68.64	71.36	80.93	12.29	17.77	Xu et al. (2018)
	55	48		3:01	1:03	69.44	71.85	80.19	10.75	18.06	Xu et al. (2018)
	55	72		3:01	1:03	70.42	72.99	82.14	11.72	18.06	Xu et al. (2018)
	55	96		3:01	1:03	71.14	73.48	82.37	11.23	18.45	Xu et al. (2018)
Waxy maize (native)					–	64.8	71.6	81.0	16.2	16.3	Wang et al. (2014)
Waxy maize (annealed)	45	Single	24	2:01	–	66.0	72.0	80.3	14.3	16.1	Wang et al. (2014)
	45	Single	72	2:01	–	66.5	72.0	79.6	13.1	16.8	Wang et al. (2014)
Normal maize (native)					–	66.5	70.7	75.4	8.9	14.0	Wang et al. (2014)
Normal maize (annealed)	45	Single	24	2:01	–	68.0	71.8	75.9	7.9	13.6	Wang et al. (2014)
	45	Single	72	2:01	–	69.0	72.3	76.4	7.4	14.8	Wang et al. (2014)

starch, then, this annealed starch is re-annealed below T_o of the first annealed starch. After annealing, the gelatinization temperature, especially T_o, significantly increased because T_o represents melting of the weakest crystallites (Jayakody & Hoover, 2008), which are more susceptible to crystallite perfection on annealing than crystallites that have higher stability (Jacobs & Delcour, 1998). As the initial annealing increases T_o, the sample could be annealed at a higher temperature i.e., just below the new T_o, without provoking gelatinization. This process could be repeated until no further increase in T_o of the annealed starch (Jayakody & Hoover, 2008). Repeated annealing is more beneficial than its continuous counterpart with the improvement of relative crystallinity and viscosity that further enhances gelatinization temperature and enthalpy value of starches (Su et al., 2020; Zou et al., 2019). Xu et al. (2018) reported that the repeatedly annealed starch exhibited higher thermal stability, degree of relative crystallinity, peak viscosity, and slowly digestible starch content compared to the continuously annealed starch. Therefore, the repeated annealing treatment provide creative insights and novel technology to produce new types of modified starch for food and other industrial applications.

9.2.2 Annealing Mechanism

Figure 9.1 shows the mechanism of annealing of starch. Annealing has been described by Jayakody and Hoover (2008) as: (1) a 'sliding diffusion' that begins with movement of the chains in the crystals that induce the movement of complete molecular sequences within a crystalline lattice and/or (2) a "complete or partial fusion" of crystals and subsequent re-crystallization of the melted components at the annealing temperature. Regarding the mechanism of annealing, there are two important points relating to starch that need to concern. First is the glass transition temperature (T_g), point in which glass transition of the amorphous regions happens. When passing T_g during heating, there is a transformation of the amorphous regions from a rigid glassy to a movable rubbery state. This softening of the amorphous locations is needed before melting of crystallites can occur (Slade & Levine, 1987). On the other hand, gelatinization temperature (T_o) is the point when gelatinization occurs, molecular arrangement of starch is destroyed that lead to changes in shape and size of granules, absorption of water and swelling, crystallite melting, and loss

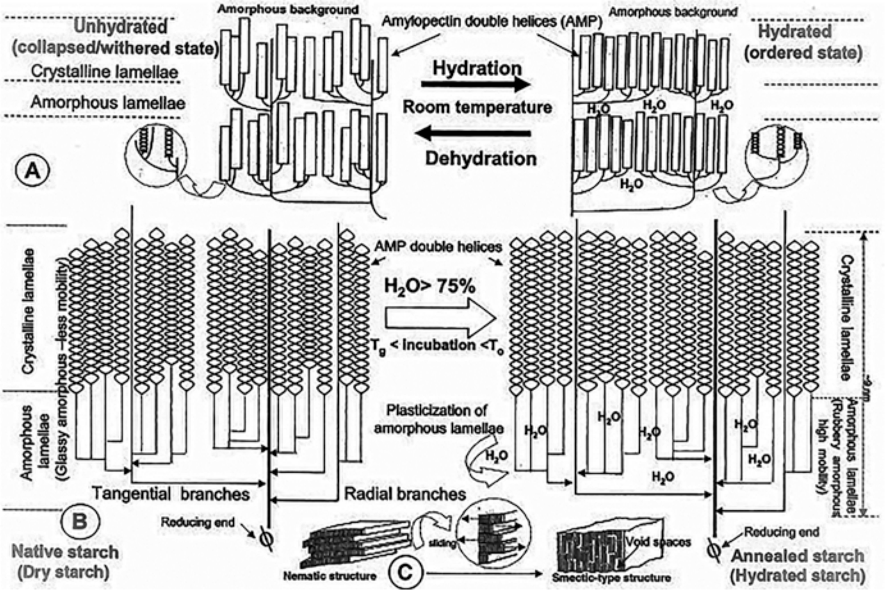

Fig. 9.1 Mechanism of annealing. (Adapted from Jayakody and Hoover (2008) with permission of Elsevier)

of amylose from the granules (Atwell et al., 1988). Annealing is performed between glass transition and gelatinization temperature. Therefore, it affects the semi-crystalline structure of starch in different degrees. The rigid amylopectin double helices are normally linked to an amorphous matrix (Waigh et al., 2000). Because of the variation in lengths of radial and tangential branches, the double helices of the un-hydrated form of starch are originally arranged randomly to form un-defined structure named a nematic, collapsed state or a 'withered state'(Perry & Donald, 2000; Waigh et al., 2000). When applying annealing treatment, the amorphous region of the starch granule is initially susceptible to the initial water absorption and plasticization. This hydration promotes the movement of the amorphous and facilitating vibrational mobility of tangential and radial chains as well as swelling phenomenon in both amorphous and crystalline regions. Finally, it leads to the movement of whole structure of these domains. Although both amorphous and crystalline regions change their location during annealing, the influence on crystalline structure is much lower than its amorphous counterpart. Simultaneously hydration causes limited but reversible granule swelling, allowing mobility of crystalline domains. Owing to the fusion and re-crystallization effect of annealing, more perfect, highly-ordered, well-packed crystalline structure replaces the initially weaker or imperfect crystallites (Lorenz et al., 1984). There are several reasons leading to this homogenous crystalline perfection as suggested by Stute (1992), including:

(i) A change in crystal size from small to large;
(ii) An alteration in structure of crystal;
(iii) A modification in direction of crystal growth;
(iv) Orientation of crystallites;
(v) Crystallites interact together;
(vi) Alterations within the amorphous regions.

Moreover, annealing also increases the amylose chain mobility, the interaction between these movable molecules and between these molecules and its amylopectin counterpart resulting in the formation of new double helices (Tester & Debon, 2000).

9.2.3 Methodology for Evaluation of Starch Change After Annealing

In order to study the effect of annealing on starch, techniques and equipment used for analysis of native starch can be used. This includes study of starch granule morphology using the scanning electron microscope (SEM), thermal properties using the differential scanning calorimetry (DSC), swelling power and solubility by mixing dried starch with water and calculating the weight difference after heating, pasting properties using the rapid viscosity analyzer (RVA) or Brabender visco-amylograph, color values using Hunter Lab Colorimeter. Similarly, other techniques are employed such as starch powder's flowability bulk by calculating sample weight to bulked (V_o) and tapped volume (V_f) (Carr, 1965), amylose leaching by estimating amylose content in hot starch suspension after centrifugation using iodine reactant (Marboh & Mahanta, 2021), molecular weight of amylose and amylopectin using high performance liquid chromatography (Su et al., 2020), freeze thaw stability (% syneresis) using weight of water separated and total sample weight after repeating cycles of freezing and thawing (Yadav et al., 2013). Further techniques are the *in vitro* digestibility using gastrointestinal enzyme, viscoelastic properties by controlled stress rheometer, gel texture employing texture analyzer, crystallinity behavior via X-ray diffraction analysis system (XRD), thermal properties obtained by using DSC, aging process in starch gel after storing samples at 4 °C for one night for DSC measurement and 7 days for XRD analysis (Kun et al., 2016).

9.3 Factors Affect Annealing

Both the annealing treatment parameters (starch/water ratio, heating temperature, heating time) and intrinsic characteristics of original starch (amylose content, botanical source, growth temperature) greatly impact the specific responses to annealing process. These are summarized below in Table 9.1.

9.3.1 Intrinsic Characteristics of Starch

9.3.1.1 Amylose Content

Amylose is more sensitive to annealing than its amylopectin counterparts. It is believed that amylopectin and its disruption are less flexible than amylose. Amylose which is mostly-straight chain molecule is easily able to leach out of granules in hot water and get involved in the formation of double-helical crystallites (Li et al., 2020). Annealing was found to improve the long-range and short-range crystalline domain of the retrograded rice starch amylose, whereas there was no apparent effects on that of the retrograded amylopectin (Guo et al., 2020). The separated X-ray diffractogram results of amylose and amylopectin is another proof to prove this hypothesis. Amylose is more likely to recrystallize than the amylopectin that leads to higher relative crystallinity than that of the amylopectin. The weak steric hindrance of amylose makes this fraction is sensitive to recrystallization (Morris, 1990; Wang et al., 2020).

As mentioned earlier, morphology is not significantly altered by annealing. However, amylose content decides the effect of annealing on the appearance of modified starch. After annealing, pores appear on surface of high-amylose starch granules whereas medium- and low-amylose treated rice starch were more agglomerated compared to native starch (Dias et al., 2010).

9.3.1.2 Botanical Source

The variation in starch source determines the effect of annealing on starch properties. This difference is mainly attributed to the difference in their structure. High amylose starch isolated from two botanical sources, wheat and maize, showed distinct differences in birefringence, leached amount, gelatinization behavior, chain conformation and lamellar structure. The most important point is with a comparable content of amylose high amylose wheat starch (HAWS) has a lower T_o than high amylose maize starch (HAMS) due to its less organized structure. Thus, the effect of annealing is more on HAWS than HAMS, indicated by higher T_o after thermal pre-treatment in HWAS than its maize counterpart. Moreover, the presence of many long chain amylose or "true amylose" ($\gtrsim$2000 DP) in native wheat starch facilitates the development of more number of thermally-stable crystallites. As mentioned earlier, the amylose is susceptible to movement and the greater quantity of very long chains amylose in HAWS improves molecular and lamellar order in developing granules. They are more readily leached and able to support greater structural annealing (Li et al., 2020). The effect of botanical origin on annealing treatment is obviously proved even in the absence of amylose. Higher phosphorous concentration in waxy potato starch could improve its crystalline perfection, thus, the increase in T_o, T_p, T_c, ΔH during annealing. The variation in enhancement of crystallite size and orientation of double helices of different starches has been observed during annealing of waxy starches such as a higher relative crystallinity of waxy corn or more stable crystallites in other waxy starches (Samarakoon et al., 2019).

9.3.1.3 Growth Temperature

Higher temperature of growing condition also causes differences in starch properties partly because annealing also occurs naturally as plant grows and develops. Potato and sweet potatoes were grown at various soil temperatures (15 and 33 °C) and crystallites of starches formed at higher soil temperatures were more perfect than those formed at a lower soil temperature. The effect of this natural annealing is observed through the change in gelatinization characteristics. As the growing temperature increased from 7 to 20 °C, the peak gelatinization temperature of amylopectin structure raised from 42 to 58 °C in normal barley starch and from 44 to 60 °C in waxy barley starch (Kiseleva et al., 2004). The increase in gelatinization temperature of starch during natural annealing is also observed through potato cultivars as growing temperature fluctuated between 10–25 °C (Tester et al., 1999). The gelatinization and swelling characteristics of starches have been found to be modified during biosynthesis by increasing growth temperature even though the background of amylopectin structure and amylose to amylopectin ratio is constant (Kiseleva et al., 2004; Tester et al., 1999). Tester and Debon (2000) have proved that the effects of growth temperature during starch biosynthesis have noticeable industrial consequences such as the energy required for starch gelatinization in food processing (malting and brewing steps in beer production). Storage potato tubers at 55 °C for 7 days exhibited a comparable change in gelatinization temperature and crystallinity of starch when subjected to plenty of water at same time/temperature protocol (Tester et al., 2005). The application of "in situ" annealing to modify starch brings desirable commercial values to develop new products in the food and non-food fields. Hence, modifying the starch structure by "*in vivo*" and/or "*in vitro*" annealing is considered as cost-reducing way in comparison with chemical modification of starches.

9.3.2 Annealing Conditions

9.3.2.1 Moisture Content

Generally, water ensures the flow of the starch molecules, but excess amount of water promotes the leaching of amylose, especially at shorter annealing duration (Guo et al., 2020). Thus, studying the change in starch properties after annealing at varying quantities of water is meaningful. The reduction or enhancement in the retrogradation rate of annealed starch depends on the moisture content. At the incubation temperature of 40 °C, the degree of retrogradation was highest (27.27%) at moisture content of 50%, lowest (17.65%) at 70% of water and increased again (to 25.35%) at moisture content of 90% (Yu et al., 2016). Similarly, as moisture content increased by 10% (from 50% to 60%), the degree of retrogradation values decreased for each incubation time lower than 48 h. Nevertheless, as water content increased from 70% to 90%, retrogradation increased with increasing water content, resulting in a more degraded sample after storage. It is clear that lower moisture content results in more stable starch (Kun et al., 2016). The moisture content also affects the crystallinity pattern after storage (4 °C, 7 days). The samples annealed at ≥80% moisture level had clearer XRD peaks than stored starches treated at that of ≤70%. Above 60% water content, samples showed some additional new peaks accompanied with typical peaks of B-type crystalline. The crystalline fraction varied from 5.92 to 9.14% as moisture content increased from 50 to 80% and dropped to 8.69% when water proportion in annealing was 90%. Regarding the flow properties, higher moisture level resulted in higher loose bulk density (LBD) and packed bulk density (PBD). To be specific, annealed starches at 75% or 80% moisture content exhibited LBD of around 0.7 g/cm^3, which is higher than the LBD (0.6 g/ cm^3) calculated at 70% moisture content. Similarly, the PBD of annealed starches at 75% or 80% moisture content was also higher than that conducted at 70% moisture (Marboh & Mahanta, 2021). The possible reason is the more water during treatment the more interaction between starch granules and water that resulting higher degree of granule disruption and more short chain production.

9.3.2.2 Heating Temperature

An increase in annealing temperature (>T_g) and excess water accelerates the speed of hydration and increases glucan chain movement that permits side by side mobility of the double helices. Consequently, a smectic-type structure is generated and this subsequently improves the order of the amorphous domains as well as the arrangement of double helices of amylopectin molecules (Tester et al., 1999).

At higher annealing temperature, the crystallinity and double helical content decreases significantly because of the change in molecular organization during annealing resulting in the phase transition. To be specific, when the annealing temperature reaches to T_g of amorphous region, water penetrates and expand this region. This expansion consequently affects the alternate crystalline region and high annealing temperature causes a loose connection between these two distinct regions, located in semi-crystalline lamellae. Therefore, there was a reduction of double helix content and crystallinity during annealing (Li et al., 2020). At the annealing temperature lower than 50 °C, the percentage of retrogradation of annealed starch increased by 25% and reduced to just 20% at 50 °C (Kun et al., 2016). The effect of annealing on starch structure is intensified when the annealing temperature is just below T_o to avoid gelatinization. Thus, the high heating temperature significantly affects the structure of the annealed starch. Therefore, annealing temperature should maintain about 5–15 °C below T_o brings the favorable influence (Tester & Debon, 2000).

9.3.2.3 Heating Time

Prolonged treatment duration is also another factor that alters degree of change during annealing. The wheat starch was annealed at 50 °C for 0.5–48 h to study the influence of annealing time. Diffraction intensity of starch determined by a X-ray diffractometry varied and the recrystallization significantly decreased with prolong annealing time from 4–48 h. Annealing time of 4 h exhibited highest crystallinity at 7.82%. Short annealing time such as 30 min is not long enough to permit sufficient intermolecular rearrangement, especially at very low or high moisture content. The most stable starch with lowest degree of retrogradation was obtained at the longest duration in the study of 48 h at moisture condition of 50%. The effect of time on changing direction of crystallinity observed clearly at low moisture level and was insignificant when the temperature was increased to 50 °C (Kun et al., 2016).

9.4 Effect of Modification

Despite starch granules are still unchanged after treatment, annealing treatment could modify structural and physicochemical properties of starch, making the starch more workable and easier to use in some distinctive environments. Table 9.2 gives

Table 9.2 Summary of the effect of annealing on properties of starch

Property	Change	No change	Detail
Granule morphology	☑	☑	Appearance remained (Devi & Sit, 2019; Duyen et al., 2020) Unsmooth surface, more porous (Waduge et al., 2006; Wang et al., 2018)
XRD	☑	☑	Typical peaks remained (Trung et al., 2017; Waduge et al., 2006) Additional peaks of V-amylose–lipid complex (Lorenz et al., 1984; Waduge et al., 2006). Degree of crystallinity ↑ (Waduge et al., 2006)
FT-IR	☑		Peaks of carbohydrate polymer remained (Sit et al., 2013) Ratio of absorbance 1047/1022 cm^{-1} ↑ (Samarakoon et al., 2019)
Color	☑		Lightness remained (Babu et al., 2019; Devi & Sit, 2019) Yellowness, redness ↑ (Babu et al., 2019; Devi & Sit, 2019)
Swelling power	☑		↓ (Zavareze & Dias, 2011; Duyen et al., 2020)
Solubility	☑		↓ (Wang et al., 2018) ↑ (Duyen et al., 2020)
Rheology	☑		
Thermal properties	☑		To, Tp, Tc, ΔH ↑ (Waduge et al., 2006) ΔH remained (Samarakoon et al., 2019)
Leaching amylose	☑		↓ (Zavareze & Dias, 2011)
Pasting properties	☑		Gelatinization temperature ↑ (Alvani et al., 2012) Peak, final, set back, breakdown viscosity ↓ (Wang et al., 2018)
Gel texture	☑		Hardness, springiness, chewiness↑
Molecular weight	☑	☑	Molecular weight of amylopectin remained Molecular weight of amylose ↓ (Bian & Chung, 2016; Su et al., 2020)
Branch chain distribution	☑		Fraction of DP ≤ 5 molecules ↓ (Babu et al., 2019; Chang et al., 2020) Fraction of B2 molecules ↑ (Babu et al., 2019; Chang et al., 2020)
Stability characteristics			Paste clarity & stability ↑ (Devi & Sit, 2019) Acid resistance ↑ (Hoove & Vansanthan 1993; Nazakawa & Wang, 2004; Tester and Debon, 2000) Shear stability ↑ (Sun et al., 2015) Freeze-thaw stability ↑(Devi & Sit, 2019)
Digestibility	☑		↓ (Duyen et al., 2020; Marboh & Mahanta, 2021) ↑ (Samarakoon et al., 2019)
Flow properties	☑		Flow ability ↑ (Marboh & Mahanta, 2021)

(continued)

Table 9.2 (continued)

Property	Change	No change	Detail
Water absorption capacity	☑		↑ (Liu et al., 2016; Marboh & Mahanta, 2021) ↓ (Ali & Hasnain, 2016; Babu et al., 2019)
Oil absorption	☑		↓ (Chen et al., 2021)

a summary of effects of annealing treatment on the morphology, structure and physicochemical properties of starches.

9.4.1 *Starch Morphology*

Annealing does not destroy the overall structure of starch granule. The low temperature applied during annealing maintained the size and shape of treated barley (incubated at 50 °C for 72 h) and mung bean (incubated at 40 °C for 24 h) starch (Devi & Sit, 2019; Duyen et al., 2020). Moreover, the granules of most treated starch remain intact that help in reducing oil absorption during frying (Chen et al., 2021). This aspect is discussed in next section. After annealing, a concave, blurred surface was observed in treated rice starch (Wang et al., 2018). Repeated and prolonged annealing cycle caused dents and cracks on wheat starch due to the recombination of amylose molecules to form a more stable amorphous region, which resulted in a greater internal pressure (Liu et al., 2015; Su et al., 2020). These changes may contribute to the higher enzyme susceptibility observed in annealed waxy corn starch and waxy barley corn starches (Samarakoon et al., 2019). The native starches that possess grooves and pores are more susceptible to the continuous annealing (Su et al., 2020).

9.4.2 *Crystallinity*

The crystalline pattern of starch did not change after being treated by single annealing or combination with other treatments as observed in mung bean, barley, and waxy starches (Duyen et al., 2020; Samarakoon et al., 2019; Waduge et al., 2006). Four typical peaks of type A at 15°, 17°, 18° and 23.5° remained unchanged in modified rice starch, wheat starch, high-amylose barley starch (Trung et al., 2017). However, degree of crystallinity changes due to annealing and the annealed starch is tendency to form stable starch. The change in degree of crystallinity could be attributed to:

- Re-organization of interior starch polymers
- Increase in crystalline perfection
- Generation of new crystallites (from earlier unassociated starch chains)

- Improvement of starch crystal size
- Reorientation of crystalline region (Waduge et al., 2006; Wang et al., 2018; Zavareze & Dias, 2011)
- Better packing of the double helices (Devi & Sit, 2019).

The relative crystallinity of native starch affects the subsequent change of treated starch. Starch having less perfect crystallites in the native structure showed significant changes in comparison with other starch which already had a perfect structure in native form (Samarakoon et al., 2019).

Annealing causes new internal association of starch and the impurities in the isolated starch such as protein or lipid, that exhibits additional peaks of XRD. Among that, fatty acids originated from lipids can associate with amylose to form additional amylose-lipid that raises the intensity of V-amylose-lipid complex (peaks at 20°) via the mobility of surface lipids into the granule interior (through the channels on granule surface) imparted by thermal energy during annealing (Lorenz et al., 1984; Waduge et al., 2006). Enthalpy of the melting of amylose-lipid complex remained stable on annealing. The formation of novel amylose-lipid complexes possibly do not occurred and the enhancement of amylose-lipid peak intensity is attributed to the highly-ordered lipid molecules in amylose-lipid complexes (Waduge et al., 2006).

9.5 FT-IR

As annealing is a physical modification, there is no introduction of any new chemical group and not much change in the position or intensity of the peaks as compared to the native starch. Carbohydrate nature of starch exhibits peaks at wavelength of 570, 930, range 1015–1022, 1048, 1080, 1155, 1365, 1650, 3350 cm^{-1}, and OH-stretching at 2950, 300–3600 cm^{-1} and finally is the CH- stretching at 1420 cm^{-1}(Mano et al., 2003; Sit et al., 2013).

The perfection of crystalline leads to highly ordered structure of the annealed starches as compared to native starch and the ratio of absorbance 1047/1022 cm^{-1} enhanced on single step annealing. The dual annealing further raises this ratio from single step annealing. For modified waxy corn starch, waxy rice starch, waxy potato starch, waxy barley starch, this ratio also increased but not significantly ($P > 0.05$) (Samarakoon et al., 2019).

9.5.1 Molecular Weight (Mw)

In general, there are three categories of molecules separated by high performance liquid chromatogram including amylopectin molecules (large Mw), amylose molecules (small Mw) and medium constituents (Mw between those of amylopectin and amylose). After annealing, Mw of amylose in brown rice starch increased insignificantly from 8.3×10^6 g/mol to 10.8×10^6 g/mol, while the Mw of amylopectin considerably decreased (from 86.3×10^6 g/mol in untreated starch to 79.1×10^6 g/mol in post annealing samples) (Bian & Chung, 2016). Likewise, Mw of amylose in the annealed wheat starch was lesser than that of their native ones (Su et al., 2020). The application of both heat and excess amount of water in hydrothermal treatment favors the formation of short chain glucans (mostly originated from straight chain amylose molecules rather than from complicated branched chain of amylopectin polymers) that further reduced the molecular weight. This also explain for the increase of Mw in amylopectin, as newly formed glucans under the effect of heat and high moisture are easily associated together as well as the preexisting amylopectin to form much larger molecules.

9.5.2 Branch Chain Distribution

During annealing, imperfect and weak crystallites were easily melted and then re-associated, therefore, the chain length distribution of modified starch showed an opposite change in the quantity of intermidiate chains (B_2 chains) and very short chain (A chains) molecules (DP ≤ 5). The replacement of the A chains by the B_2 chains caused by the shearing gradient during annealing induced the re-association of short chain glucans to form more complex, longer chain molecules. The debranched starch with subsequent amount of very short chain molecule or the ultrasound treatment with intense agitation and cavitation activities of starch granule contributed to high proportion of B_2 chains (Babu et al., 2019; Chang et al., 2020).

9.5.3 Color

The white color of the starch is desirable for many food and nonfood sectors and usually expressed by L* value. The lightness or the degree of whiteness of annealed starch was not significantly different from that of the original starch. Besides, there is an increase in yellowness and redness expressed by higher level of a* and b* values as compared to the native starch sample because the participation of residual substance, impurities such as protein that attach to starch under hydrothermal treatment. Scientists have developed dual treatment, annealing and hydroxypropylation or annealing and sonication to improve the white color of starch and reduce the level of non-starch color (Babu et al., 2019; Devi & Sit, 2019). In general, the color of treated product was formed by the following factors: natural color (existing chlorophyll, carotenoid, etc., result of chemical reaction (addition of chemical), enzymatic reaction (addition of enzyme, intrinsic enzyme of material), Maillard reaction (interaction of protein and sugar of short chai glucose under high temperature), etc. Overall annealing treatment is unable to alter the color of starch significantly.

9.5.4 *Leaching Amylose, Solubility and Swelling Power*

9.5.4.1 Leaching Amylose

The amount of leaching amylose is correlated with the solubility of starch because solubility index measures the quantity of soluble molecules such as short chain amylose and simple sugars (glucose, fructose, sucrose, etc.) that are able to leach out in solution. There was a reduction in amylose leaching accompanied with a decrease in solubility. The internal rearrangement of the starch granules (associations between amylose-amylose and amylose-amylopectin chains) intensifies intramolecular bonds and the formation of amylose–lipid complexes within the granules that limit amylose leaching and water solubility of annealed starch (Zavareze & Dias, 2011).

9.5.4.2 Swelling Power

Annealing induces crystallite perfection, amylose-amylose and amylose-lipid association which further inhibit granule swelling and lower the swelling of starch. During annealing, the mobility of amylose chains is promoted, higher ordered-structure of amylopectin and crystalline region are formed, granule become more stable and swelling restricted. In order to overcome this situation, people begin to incorporate hydrophilic hydroxypropyl moieties for loosening the internal structure through applying hydroxypropylation after annealing. This introduction also helps the exposure of hydrophilic OH groups originally present in the starch granule to retain more water molecules and enhance the swelling power of annealed starch at high temperature. Combined modification of annealing and hydroxypropylation improves the solubility of single annealing treatment. The integration of hydroxypropyl groups in the amorphous lamellae of the granules weaken starch granule that further increase amylose leaching and water-associated properties of starch. Highest swelling was observed for one step annealing and dual step annealing when they combined hydroxypropylation with the level of 10%, their figures were surprisingly higher than hydroxypropylation of native starch only (Devi & Sit, 2019).

9.5.4.3 Solubility

Solubility of starches is prevented by following four factors: apparent amylose concentration, amylose-lipid complex, amylose-amylose association, and co-operation of amylose with outer chains of amylopectin.

Except amylose content, which is an intrinsic property, annealing facilitates rest three factors resulting in lower solubility in comparison to native starch sample. Again, annealing triggers the mobility and rearrangement of the amylose and amylopectin molecules in starch granules; thus, it leads to the development of more compact granules with reduced expansion and leakage during re-heating in hot water. Thus, the lower solubility contributed in the smaller cooking loss of rice noodles (Wang et al., 2018).

9.5.5 Water Absorption Capacity

The change of water absorption capacity (WAC) of starch post annealing still remains controversy. Variable observations have been reported by different research. Annealed Foxtail millet starch was reported to absorb less water than untreated form (Babu et al., 2019). The hydroxyl group in polymeric molecules plays an important role in water holding capacity at ambient temperature. More interaction between amylose-amylose chains and amylose-amylopectin chains caused by annealing are due to the hydrogen bonds formed between hydroxyl groups in amylose and amylopectin molecules. Thus, annealing reduces the number of free hydroxyl group involved in the water absorption capacity of starches (Ali & Hasnain, 2016). On the other hand, sohphlang starch showed a higher water absorption capacity after annealing (Marboh & Mahanta, 2021). The reason suggested for increase in WAC was that during hydrothermal treatment, heat and water facilitates the starch hydrolysis into simpler dextrin, molecules with higher affinity for water. Furthermore, annealing also disrupts the hydrogen bonds connecting between amorphous and crystalline structures, releasing more free hydrophilic ends of polymers, thus, increasing the ability of water absorption (Liu et al., 2016).

9.5.6 Oil Absorption Capacity

Oil absorption is one of the most concerned characteristics of starch as this is related to application if starch in various field. Crispiness, attractive appearance, and smell of fried starchy food not only please the consumer but also raises the economic value of the original product. During frying, heat transfer and mass transfer are two primary unit operations. Heat from hot oil is transferred to food that evaporates water and leaves holes in starch structure. Frying destroys granule morphology, crystalline structure, double helices of starch molecules that transform the ordered region (crystalline region) to disordered region (amorphous region). This significant alteration makes oil molecules adsorb onto the surfaces of starch granules and diffuse through them. The condensation mechanism and capillary mechanism make this oil appears as solid fat after frying (Mellema, 2003) and also trigger starch granules to stick to each other though they still easy to spread and smear (Chen et al., 2021). The phenomenon of oil uptake reduces the sensory attribute of final products and also has negative effect on the human health mainly caused by greater consumption of oily, fatty food. Annealing produces a highly compact starch structure and prevent the incorporation of oil molecule in starch structure.

In particular, higher temperatures and moisture levels during annealing promote more internal interaction of starch granule (mobility of polymeric chain, re-organization of double helices formed among the short side chains of amylopectin and the interaction between amylose and amylopectin) to form a denser structure compared with native starch(Zavareze & Dias, 2011). These more compact starch granules are expected to absorb less oil during frying. The highly-ordered structure of starch is also enhanced due to the formation of new amylose-fatty acid complex because triglycerides are degraded to fatty acids during frying, and these acids are absorbed by starch granules and interact with amylose molecules to form more amylose-fatty acids complex (Chen et al., 2021). These alterations reduce the magnitude of the capillary forces and the surface area available for oil to absorb, thereby, decrease in the oil absorption during frying.

9.5.7 Thermal Properties

Thermal properties are regarded as an important physicochemical parameter in terms of starch applications in food products, especially the onset temperature of gelatinization representing the irreversible melting point of the weakest crystallites in a granular starch. The DSC graphs reveal that T_o, T_p, T_c and ΔH of annealed starch are higher than native starches, whereas the range of gelatinization temperature ($T_c - T_o$) is decreased. It is clear that during annealing, new amylose-amylose and amylose-amylopectin interactions as well as double helices are formed. Reduction in imperfection of crystalline region that prevent ability of starch swelling. All these factors delay gelatinization process, resulting in higher gelatinization temperature (Waduge et al., 2006). However, annealing did not alter the gelatinization enthalpy of waxy barley, waxy corn, waxy rice, waxy potato probably because new double helices were not formed on annealing (Samarakoon et al., 2019). There are two more hypothesis related to the maintenance of enthalpy. First is the absence of crystalline melting because of the new complexes of amylose-amylose and amylose-amylopectin. Secondly, the crystalline region is melt but annealing have compensate this loss with similar amount of recrystallization (Gomand et al., 2012).

9.5.8 Pasting Properties

The viscosity of starch in heat processing becomes a crucial factor in the recipe in wide range of food products and in many other industrial processes. Pasting properties refers to the change in viscosity of starch paste at different temperatures when heated in certain quantity of water (Devi & Sit, 2019). After annealing, treated starch has less affinity to water owing to more stable crystalline structure which lowers the peak, final, set back and breakdown viscosity than native sample. The lower setback viscosity of annealed starch with 20% substitution was found advantageous for the qualities of rice noodles by enhancing the smoothness as well as heat and shear stability of the rice noodles (Wang et al., 2018).

However, the reorientation of starch causes an increase in pasting temperature after annealing. The strengthening of intra-granular bonded forces, allows the starch to require more heat before structural disintegration and paste formation occurs (Babu et al., 2019). The degree of the granular disruption depends on the initial pasting temperature of native starch. Highest post-annealing pasting temperature belongs to native starch sample that had possessed the lowest gelatinization temperatures. This is possibly due to the fact that less stable starch structure is more likely to be improved and affected by crystallite perfection during annealing (Alvani et al., 2012). Furthermore, the differences in inter-block chain length (IB-CL) of amylopectin have an impact on the variation of post-annealing changes in gelatinization behavior. Vamadevan et al. (2013) differentiated starches into four categories and treated them to annealing (60 °C below T_o, 80% water (w/w), 3 or 24 h).

Type I starches: Andean yam, oat, rye, barley, and waxy barley
Type II starches: waxy maize, medium-AM rice, waxy rice, and sago
Types III: tapioca, arrowroot, and mung bean
Type IV: potato, waxy potato, canna, and lesser yam

Consequently, they obtained different response based on their type. Type I with extreme short IB-CL is the least responsive to annealing. This type gets readily dissolved in water during annealing and are difficult to be realigned to enhance the crystalline structure. Likely, natural Type IV starches contained a most proper IB-CL and external chain lengths that possessed very few defects for stable crystallization, thus, undergoing little further enhancement or transformation upon annealing. Nevertheless, types III and IV were considered to be most amenable to annealing, showing greater flexibility and mobility of double-helical chain segments. This allows the segments to align and improve molecular order (Vamadevan et al., 2013).

Products containing annealed starch are required more energy to completely gelatinize because the post-annealed starch has higher gelatinization temperature. The incomplete gelatinized starch affects the quality of products, e.g., reduced loaf volume of bread and undesirable food texture (Li et al., 2020). Combining hydroxypropylation with annealing can lower the energy consumption to create starch paste or cook starchy products. The hydrophilic nature of added hydroxypropyl groups helps water penetrate into the starch granules and loosens the granular structure that lowers the pasting temperature and increases the viscosity. The substitution of hydroxypropyl groups at 10% combined with either single step or dual step annealing exhibited a more improvement in the final viscosity than only applying hydroxypropylation treatment for native starch.

Annealing increases the crystallinity and lowers the affinity between starch and water, resulting in the decrease of the final viscosity. The final viscosity decreases because of the starch structure weakening. Hydroxypropyl groups is easily attached to starch matrix during the early gelatinization period and increase final viscosity, especially high level of hydroxypropylation (Devi & Sit, 2019).

9.5.9 Stability Characteristics

9.5.9.1 Paste Clarity and Stability

Storage of starch paste leads to decrease in the paste clarity. Annealing which reduces amylose leaching and slow down retrogradation process owing to its stable crystalline structure will improve the paste clarity during cooling (Devi & Sit, 2019).

9.5.9.2 Acid Resistance

Mechanism of acid hydrolysis is described as follows: acid first attacks the amorphous region of starch, then, the amorphous area is separated from the rest of crystalline region, finally, the unlocked amorphous is subjected to acid hydrolysis. The influence of annealing on acid resistance ability still remains controversy. Annealed starch from same origin behave differently when associated with acid (Hoove & Vasanthan, 1993; Nakazawa & Wang, 2004; Tester & Debon, 2000). Variation in starch source, type and concentration of acid used, condition of treatment (single, double, multi-step annealing, annealing temperature) are main factors that cause variation in the end product of acid hydrolyzed starch sample (Jayakody & Hoover, 2008). There are several reasons attributed to a decrease in level of acid hydrolysis including (1) perfection of starch crystallites (Waduge et al., 2006), (2) generation of double helical structures between amylose molecules (Jacobs & Delcour, 1998), and (3) formation of V-amylose-lipid complexes (Waduge et al., 2006). However, annealing treatment also increases quantity of α–glucan in amorphous area and void space in crystalline region through its perfection, resulting in the resistance of acid hydrolysis (Nakazawa & Wang, 2004).

9.5.9.3 Shear Stability

Annealing contributes to the reticulation within the starch granule leading to an amylose network offering resistance to shearing and acidic treatment. The re-organization within the granule and generation of resistant starch in the annealed starches makes them to be more resistant to shear (Sun et al., 2015).

9.5.9.4 Freeze-Thaw Stability

Syneresis refers to exuding of large quantity of water as frozen or refrigerated foods are thawed. Freeze-thaw stability implies the resistance of food from syneresis. Thus, freeze-thaw stability is an important property of starch for indicating the structural alteration that further negatively affect texture and other sensorial attributes of food. Starch suffered more syneresis after being annealed and had a lower freeze-thaw stability as compared to native starch. After annealing, starch formed compact, high ordered structure and reduced quantity of free hydroxyl groups resulting in lower ability of holding water during thawing (Devi & Sit, 2019). Therefore, annealed starch has much lower amount of water oozed out during defrosting as compared to native starch. The stable post-annealed starch with lower rate of retrogradation that permits the water molecules to be bound and trapped in the amylose-amylopectin crystalline matrix making it difficult for water to get out of the structure. High freeze thaw stability allow starch to resist physical changes during freeze-thaw treatment.

9.5.10 Flow Properties

The flow properties of powder are expressed by the bulk density that indicates the ability of packing together of the powder particles. Annealed starch had lesser bulk density and took prolonged time to sediment, resulting in unpacked powder. The Carr index below 25% and Hausner ration values less than 1.30 of treated starch means the starch powders exhibit passable flowability trend (Marboh & Mahanta, 2021). High flow ability behavior reduces the loss of powder that stick on the packaging, time of pouring materials, as well as prevent uncontrollable changes (interparticulate interactions, microbial contamination).

9.5.11 Digestibility

Annealing is believed to rearrange the internal structure of starch granule, especially facilitate the associations between amylose-amylose and amylose-amylopectin chains which further enhance the perfection of both crystalline and amorphous lamellae. This compact structure resists the attack of digestive enzyme and hydrolysis, resulting in the improvement of resistant starch fraction. The formation of slowly digestible starch (SDS) and resistant starch (RS) after annealing is attributed to the presence of additional interactions that leads to the rearrangement of ordered structure. Annealing significantly improved the RS content of foxtail millet starch from 18.20 to 42.32% (Babu et al., 2019). The SDS and RS fractions of treated samples were higher than the native sohphang starch, especially the RS content. The RS increased from 11.30% in untreated samples to 21.58% after annealing at 80% moisture content (Marboh & Mahanta, 2021). Cooking or gelatinization of starch, phenomenon make inter- and intra-molecular hydrogen bonds between starch chains weaker, that leads to a continuous amorphous structure generation, which further enhances the susceptibility of starch granules to digestion. Then, cooking reduces RS fraction in both native and modified starch. However, annealing improves the RS fraction with nearly tripling RS content (4.67% RS in cooked sohphang, 11.54% RS in cooked annealed starch) (Marboh & Mahanta, 2021). In addition, the enzyme susceptibility depends on the starch granule size. Relatively greater surface area per unit mass of small granules makes them more susceptible to enzyme hydrolysis than large granules. Moreover, crystalline type also affects hydrolysis rate, especially, waxy cereal starches, A-type crystalline starches have a higher fraction of short chains with DP 6–12 and amylopectin branch points which are distributed in crystalline lamellae, forming weak points, and making more vulnerable to enzymatic attack than the B-type starches. Waxy starches with B-type crystalline pattern and large granule such as waxy corn and waxy barley starches were more accessible to enzymes and annealing treatment continuously increased their enzyme susceptibility while decreased in waxy rice and waxy barley starches (B-type, large granule) (Samarakoon et al., 2019).

9.5.12 Degree of Retrogradation (DR)

Staling through retrogradation of wheat starch cause a transformation from dry, crisp, pleasant texture of new-baked bread to soft, leathery, unpleasant texture of stale crust. Therefore, understanding the mechanism of retrogradation and controlling it helps lower economic losses for both the industry and the consumer. Experiment of aging process in starch gel to stimulate the staling of bread as well as test influence of annealing conditions on starch retrogradation.

Retrogradation is triggered by a fast recrystallization of amylose chains followed by a slow recrystallization of amylopectin polymers after starch is heated. Annealing hinders the amylose leaching and thereby reduces retrogradation rate. Kun et al. (2016) found that wheat starch at 70% moisture and annealed at 40 °C for 48 h had the lowest degree of retrogradation (17.65%). Although this considerable high water/starch ratio was found to promote the amylose leaching and subsequently the fast retrogradation, annealing facilitated the rigidity of the swollen granules, thereby reducing DR (Lii et al., 1996; Tsai et al., 1997).The reduction of retrogradation degree in annealed starch was confirmed by XRD result with less crystallinity, and resulting in less retrogradation compared with native wheat starches stored for the comparable length of time (Kun et al., 2016).

9.6 Application of Annealed Starch

Annealing starch with notable thermal stability and lower degree of set-back viscosity has been widely applied in canned, frozen food industries, and noodle processing. Specifically, annealing hinders starch swelling, amylose leaching at cooking temperature that improves the gel quality of rice paste (Zhou et al., 2003). The substitution of 50% of native rice flour by annealed rice starch resulted in obtaining a comparable textural quality (adhesiveness, chewiness, tensile strength) of rice starch noodle as compared with commercial products (Hormdok & Noomhorm, 2007). The greater amount of resistant starch in annealed starch suggest it can become an ingredient in processed foods without affecting any appearance, textural properties, owing to its bland taste, white color and micro-structure. The annealed starch can be used as a fat replacer or to increase the dietary fiber content of food because of its characteristics described above. Spray drying of annealed alpha-amylase hydrolysates starch at 160 °C caused formation of spherical agglomerates. These agglomerates exhibited several promising effects of compressible excipient such as high compressibility, increased disintegration time, high crystallinity and low friability in comparison with other commercial fillers available in the market (Tukomane et al., 2007). Despite of many alterations in properties of starch during annealing, the morphology of starch remains the same as the native form. Therefore, the annealed starch is applied to produce starch-based foods for people with kidney disorder such as powders, granules, cookies, noodles. Reduced swelling power, solubility, paste viscosity, and high gel hardness of annealed starch are desirable properties for making high quality noodles. Moreover, the treated starch can interplay the role of emulsifier or thickener to improve the overall qualities of the rice noodles. Noodles made from annealed rice starch with sufficient low cooking loss were also developed (Wang et al., 2018). When the level of substitution was 40% (w/w), the final noodles had the best overall quality. The improvement of starch's functional properties such as mouthfeel, firmness in cooked rice noodle after annealing prove the power of this physical modification without using chemical additives. Annealing (annealed condition: moisture content of 60% at room temperature for 7 days) was also applied to pretreat the blends of poly (lactic acid)/corn starch in order to enhance thermal stability and mechanical properties (Lv et al., 2014). Nanocrystals produced from annealed waxy corn starch had higher crystallinity and higher melting temperature (Ji et al., 2019). Recently, annealing has been used for modifying the structure of V-type starch to improve the ethylene concentration encapsulated in starch structure as well as stability during storage (Shi et al., 2019).

9.7 Case Studies

Case study 1 Annealing treatment of sweet potato starch (Fig. 9.1)

9.7.1 Step 1: Cleaned, Peeled Sweet Potato Tubers

The fresh harvested sweet potato tubers were washed with clean water several time and peeled using peeler for removing dirt and other physical contamination as well as to ensure the high purity of obtained starch through excluding any possible contaminants.

9.7.2 Step 2: Make Blend with Water

The peeled sweet potato tubers were sliced into thin layer or cut into small piece for easier blending process. They were blended with water with the ratio of 1:1 (w/v) to make the blender using a Waring blender (7015 N, Waring®Commercial, USA).

9.7.3 Step 3: Starch Separation

Based on the difference in size between starch and other components of this tuber (protein, fat, fibers…), starch was separated by passing the pulp through a sieve of 0.232 mm in aperture size. The experiments were repeated three times to separate all starch from the pulp and a final time through a sieve with 0.105 mm in aperture size to obtain the highest purity degree of isolated starch.

9.7.4 Step 4: Starch Purification and Recovery

The slurry after sieving was centrifuged with the speed of 1500 × *g* for 20 minutes. The supernatant was discarded, and the residue was continually wash thoroughly in distilled water. This technique was repeated for three times to remove any non-starch component that can be dissolved in water. Starch was recovered by oven-drying at 40 °C for 24 h to 10–11% moisture and grinded to powder form for further analysis. This starch should be stored in desiccator for preventing the re-absorption of water in outside environment to starch and lead the spoilage of starch.

9.7.5 Step 5: Annealing Treatments of Sweet Potato Starch

9.7.5.1 Step 5.1: Add Distilled Water to Starch Powder at a Ratio of 1:2 (W/W)

- Step 5.1.1: Measure the moisture content of starch powder that was isolated in previous steps. The moisture content of starch powder was determined using Rapid Infrared Analysis or drying oven by putting starch sample at 10 °C and weigh it every hour until its weight stable (the moisture content was calculated based on the difference between initial weight and final weight of dried starch, multiply by 100). Record the result

- Step 5.1.2: Take 100 g of isolated starch (dry basis) by using the below formula:
- $m_{wb} = m_{db}/(1\text{-}MC)$
- m_{wb}: weight of actual starch powder that you take for annealing (g)
- m_{db}: weight of required starch mentioned in method (g)
- MC: moisture content of starch powder
- In this case study, the m_{wb} is unknown, m_{db} is 100 g, MC is supposed to be 10% equal 0.01, so $m_{wb} = 100/(1–0.01) \approx 101$ g
- Step 5.1.3: 200 g or 200 mL distilled water was mixed with 100 g starch powder (dry basis) in 500 mL Erlenmeyer flask. The water was added to create a suitable environment for annealing because water can facilitate starch granule disruption during heating operation that favors the re-organization of short chain molecules and form a more stable starch (Fig. 9.2).

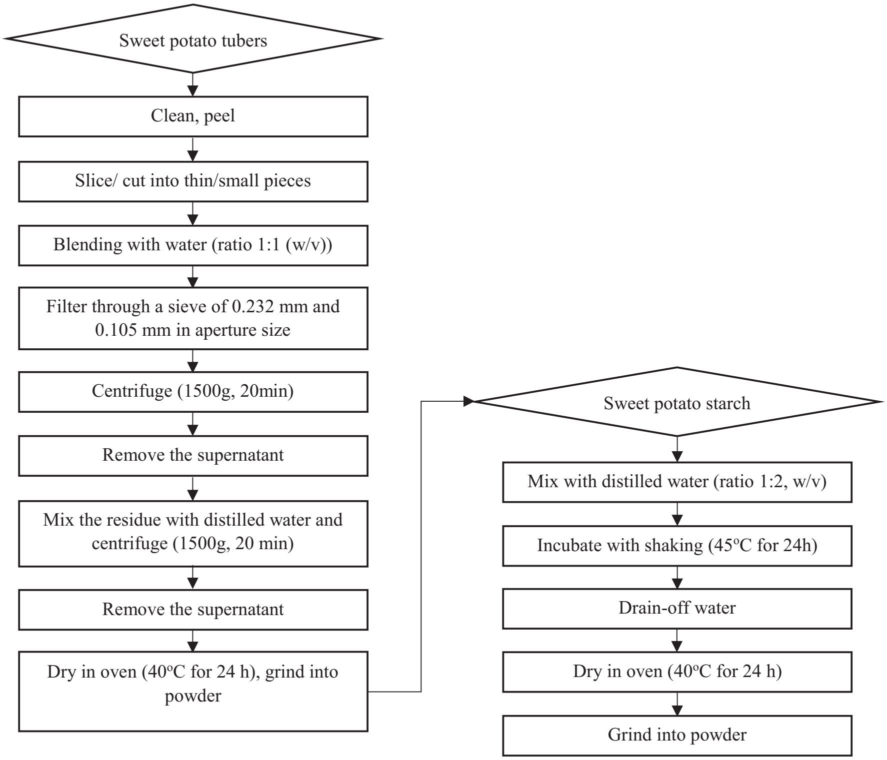

Fig. 9.2 An annealing process of sweet potato starches (Trung et al., 2017)

9.7.5.2 Step 5.2: Incubation and Recovery of Annealed Starch

The flask contained mixture of starch and water was covered to prevent water evaporation and incubated in a water bath shaking at 45 °C for 24 h. After incubation, the starch sample was pour into tray and put in drying oven that operated at 40 °C for 24 h to a moisture content of 9–10%.

9.7.5.3 Step 5.3: Grinding Annealed Starch

The annealed starch was grinded using usual blender and sieving through 0.232 mm-diameter sieve, the large particles remained on the sieve will be re-grind to obtain uniform small size starch particles (Table 9.3).

Case study 2 Annealing combined citric acid hydrolysis process of Mung bean starch (Fig. 9.3)

9.7.6 Step 1: Seed Coat Separation

The raw Mung beans were soaked in distilled water supplemented with 0.3% sodium bisulfate at room temperature for 8 h. The addition of low concentration of sodium bisulfate was enough to bleach the beans without altering the properties of beans and contaminating bean sample. Then, the water was poured out and seed coats were separated using hands.

Table 9.3 Summary of major findings on applications of annealed starch

Food industry	Characteristics	References
Breadmaking	Modify the dough viscosity and texture.	Miyazaki et al. (2006)
	Improved adhesiveness and chewiness of bread.	Hormdok and Noomhorm (2007)
	Unique bread texture and economical cost of final product.	Yao et al. (2018)
Frozen and canned foods	Improved thermal stability and shear resistance.	Yao et al. (2018)
	Lower setback temperature and retrogradation.	Stute (1992)
Rice noodles	Improved overall noodle textural attributes such as chewiness and adhesiveness	Yao et al. (2018)
	Decrease in granular swelling and amylose leaching, and the increase in heat and shear stability.	Jayakody and Hoover (2008)
	Acceptable textural quality (adhesiveness, chewiness, tensile strength) of the rice noodle Enhance resistant starch	Hormdok and Noomhorm (2007)

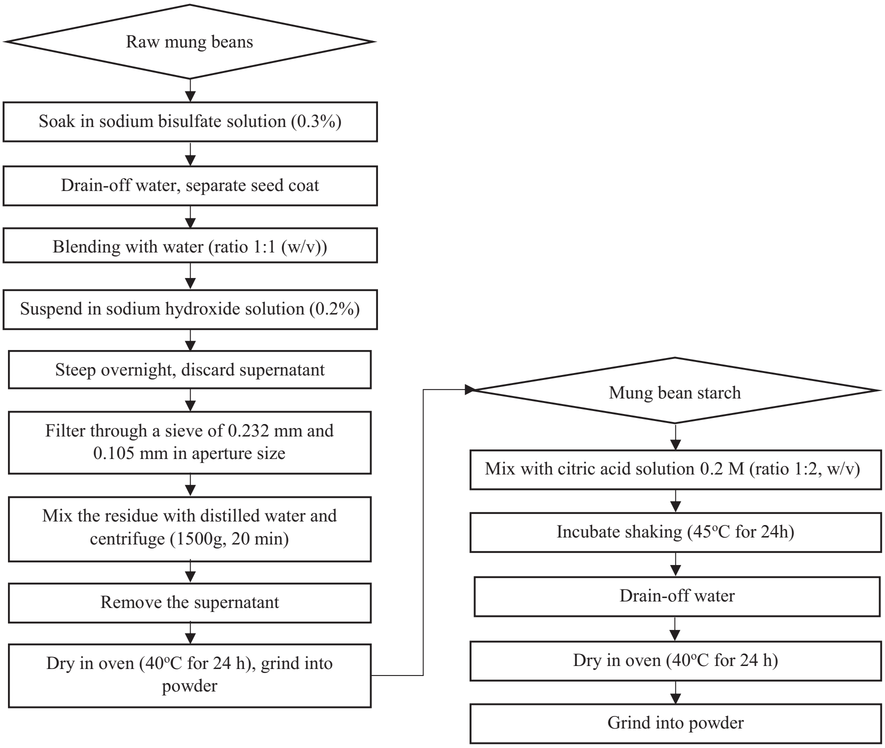

Fig. 9.3 An annealing combined citric acid hydrolysis process of mung bean starch (Duyen et al., 2020)

9.7.7 Step 2: Alkaline Extraction

After seed coat removal, Mung bean cotyledons were blended and suspend in water that supplemented with 0.2% sodium hydroxide at a ratio 1:1 (w/w). The mixture was kept overnight, and the supernatant was discarded to eliminate high protein fraction contained in Mung bean variety.

9.7.8 Step 3: Starch Separation

Similar to sweet potato starch, starch was separated by passing the pulp through a sieve of 0.232 mm in aperture size based on the difference in size between starch and other components of this tuber (protein, fat, fibers…). The experiments were repeated three times to separate all starch from the pulp and a final time through a sieve with 0.105 mm in aperture size to obtain the highest purity degree of isolated starch.

9.7.9 Step 4: Starch Purification and Recovery

Similar to sweet potato starch, starch was purified using centrifugation and drying operation. The dried starch was grinded into powder.

9.7.10 Step 5: Annealing-Citric Acid Hydrolysis Treatments of Mung Bean Starch

– *Step 5.1: Add citric acid solution to starch powder at a ratio of 1:2 (w/w)*

Instead of using pure water to create the environment for sample annealing, **200** g or **200** mL of citric acid solution (**0**.**2** M) was mixed with **100** g starch powder (dry basis) in **500** mL Erlenmeyer flask. The supplement of citric acid induces the reorientation of starch granule and improve its stability.

– *Step 5.2: Incubation & recovery of annealed starch*

The mixture of starch and water in flask was sealed and incubated in a water bath shaking at **45** °C for **24** h. After incubation, the starch sample was pour into tray and put in drying oven that operated at **40** °C for **24** h to a moisture content of **9**–**10**%.

– *Step 5.3: Grinding annealed starch*

The annealed starch was grinded using usual blender and sieving through **0**.**232** mm-diameter sieve, the large particles remained on the sieve will be re-grind to obtain uniform small size starch particles.

9.8 Conclusion

Annealing is a typical physical modification method of starch. It involves the intentional incubation of starch at a plenty amount of water (usually more than 40%, w/w), and at a certain temperature (usually lower than 60 °C) between glass transition and gelatinization points for a period (usually more than 16 h) or happen naturally during plant growth and starch isolation. Annealing modifies structural and physicochemical properties of starch in a predictable trend, which improved the starch function for broader field of starch utilization. Besides, annealing is referred as a “clean-label” starch modification technique, because it only involve water and heat. Therefore, annealing treatment has received extensive attention from science field and food manufacturer. Annealed starches have been widely used in food industries such as canned and frozen food industries, noodle and bread making industries. In addition, the increase in the RS content indicates that the annealed starch can be incorporated in producing low-carb foods. Even though lots of studies on annealing of starch with various starch sources, heating temperature and time and water level, further studies are still needed to clarify the change in micro structure inside starch granules while the morphology of granules keeps unchanged. The studies on application of annealed starch in food and pharmaceutical industries also get more attention.

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Chapter 10
High Hydrostatic Pressure Treatment of Starch

Lorena Deladino, Aline Schneider-Teixeira,
and Antonio Diego Molina-García

10.1 Introduction

High pressure technology has experienced a distinct increase in the last decades, whether the number of industrial installations dedicated to food processing, or the annual production are considered. Many high pressure-treated food products of all types are currently in the market and the associated fresher, healthier, and chemicals-free labels make them widely appreciated by consumers.

High hydrostatic pressure (HHP) is an expanding technology that is quickly developing in worldwide food technology. However, the term “novel technology” is not really adequate, as the application of HHP to food is now over a century old, after the first food-related pressure studies (Bridgman, 1964). Pressure is a key property determining the states of matter, in a similar way to temperature. But, while thermal treatments’ effects include chemical reactions acceleration, pressure does not have this effect. Pressure in term of MPa (10^6 Pa) are usually employed as hydrostatic pressure units (1 Pascal, the SI derived pressure unit, equals 1 kg m^{-1} s^{-2}, 1 MPa equals to 10 bar or 9.86923 atmospheres).

HHP treatments are currently applied to food because they are non-thermal and chemical-free processes, and so, they have a smaller energy consumption associated, while the undesired effect of added chemicals is avoided. Nevertheless, the instrumental investment required is significant. Figure 10.1 shows a scheme of a laboratory/pilot plant HHP processing equipment, while Fig. 10.2 depicts an industrial semi-continuous device. In both cases, samples/products are introduced

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L. Deladino (✉) · A. Schneider-Teixeira
Centro de Investigación y Desarrollo en Criotecnología de los Alimentos (CIDCA), CONICET, Facultad de Ciencias Exactas, Universidad Nacional de la Plata, La Plata, Argentina

A. D. Molina-García
Instituto de Ciencia y Tecnología de Alimentos y Nutrición (ICTAN-CSIC), Madrid, Spain

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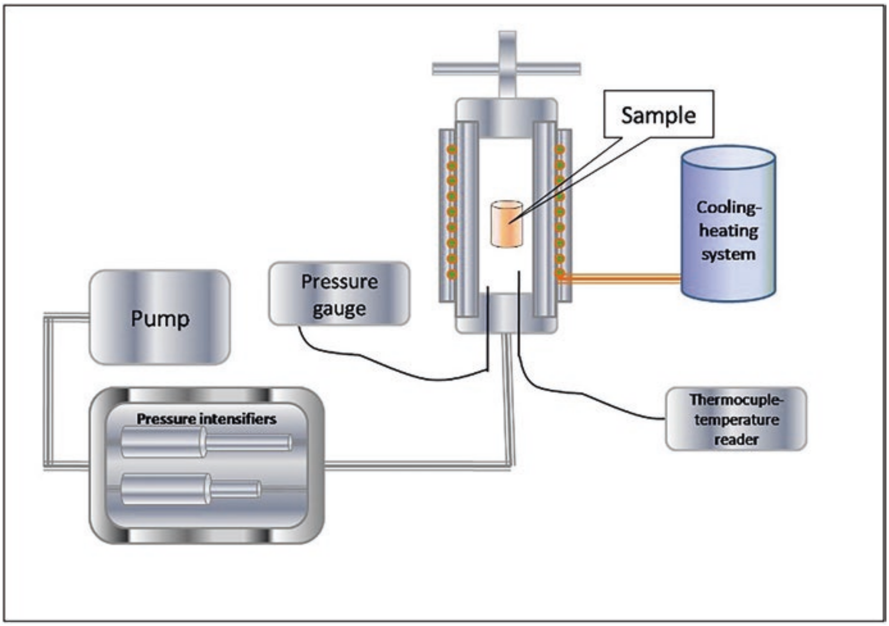


Fig. 10.1 Cartoon showing a research food high hydrostatic pressure processing unit. HHP processing equipment, whether at a laboratory, pilot plant, or full industrial scale, is heavy, bulky and expensive. However, the total cost per food unit (weight or volume) is not too high, as there are considerably less energetic expenses. Additionally, water consumption is scarce and there are no wastes associated with the process

Laboratory equipment can be obtained at many different sizes and designs, but always the principles of Figs. 10.1 and 10.2 are followed: water (or a suitable food-compatible fluid) is injected by means of pumps and hydraulic pressure intensifiers into a metal cylindrical chamber, where the sample is pressurized. Steel is usually employed, although other alloys, such as copper-beryllium can be used in smaller vessels. Additives common for avoiding freezing problems are sometimes used (often the food-compatible propylene glycol). Freezing (which can be deleterious for the equipment) can take place upon decompression if work is carried out at temperatures a few degrees over the freezing point, as the adiabatic cooling can cause over 10 degrees temperature reduction. However, this fluid is not in contact with food. A flexible wall container, allowing pressure transmission, but still being a barrier for the external fluid, to avoid contamination, must be employed. This means plastic, although there is active research going on in developing biodegradable packaging materials fit for HHP use (e.g., Marcos et al., 2008). Even starch has been proposed as a source of biodegradable plastic packaging: starch-made packages could be used to pressurize starchy foods (e.g., Khan et al., 2017)

into a steel chamber (of considerable thickness and weight, to withstand the pressures applied, several times those of the deepest abyssal marine trench). Pressure is supplied by different pumping systems and pressure intensifiers, which basically inject pressurizing fluid into the chamber (a pressure-transmitting fluid is required, as well as a suitable elastic container to separate external fluid from actual food, see below and Figs. 10.1 and 10.2 legend). Despite a large number of industrial applications of high pressure technology being directed to extend food self-life

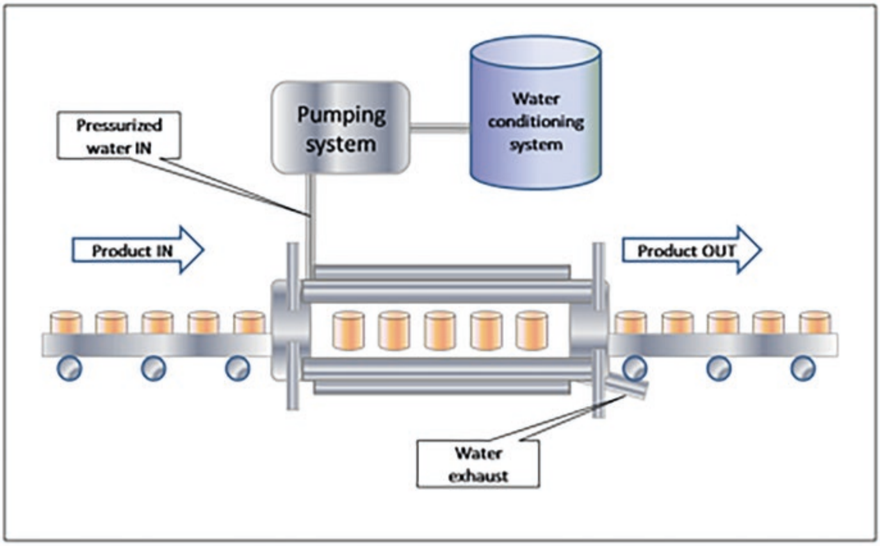


Fig. 10.2 Cartoon showing an industrial semi-continuous food high hydrostatic pressure processing unit

HHP processing is basically a batch process: although equipment has been designed for industrially processing liquid foods, its working principle is essentially semi-continuous. Industrial equipment, as that shown in Fig. 10.2, loads a train of pre-packed food through one end of the pressure chamber. Then it is closed, pressure is built and held for a given time, and afterward, quickly released, for the load exiting through the other end. For industrial use, the processing time is short. The shorter the treatment duration, the cheaper it results. And it has been found that often a few minutes are enough to get the desired effect.

HHP laboratory equipment often allows thermal control, to choose the processing temperature. It can go from as high as 110 °C to as low as −20 °C. Measuring systems (often thermocouples) give information on temperature, even in different locations within the vessel. However, industrial scale equipment does not have these thermal control systems, and influx fixed temperature water is employed.

Starch gelatinization is a process in which hydrogen bonds among starch polysaccharide chains are interchanged with those of water. This water required for the gelatinization process acts as an internal pressure-transmitting medium

through microorganisms elimination, HHP offers many other effects of technological interest, such as enzyme activity modulation or inactivation, freezing-under-pressure transformations, or induction of structural and textural changes.

It must be noted that (actually, as well as with thermal processes) pressure treatments by themselves induce only reversible changes in matter: i.e., once the pressure is released and atmospheric conditions recovered, the changes that may have occurred are reverted. Even the adiabatic heating, a phenomenon by which temperature rises upon pressure increments (and that, depending on the physicochemical nature of each material, can increment temperature over 10 °C or more), are reverted, with a similar temperature reduction upon decompression (further details on adiabatic heating can be obtained in, for example, Otero et al., 2000).

Biological polymers are especially sensitive to high hydrostatic pressure. Meanwhile, small molecules, such as those mainly responsible for food taste, color, aroma or nutritional properties, are scarcely affected, at the pressure levels currently employed in food industry (up to 600 MPa) (Tauscher, 1995). This is due among other factors, to the existence of alternative conformations for biopolymers, energetically similar to the native ones. These conformations are often involved in the biomolecule physiological function. On the other hand, other biopolymers, such as cellulose, are scarcely affected by pressure.

The complex behavior of corn starch under pressure has been extensively studied (Douzalset al., 1998; Buckow et al., 2007, 2009; Oh et al., 2008; Kim et al., 2012; Okur et al., 2019, Rahman et al., 2020, Castro et al., 2020, Heydari et al., 2021). Starches from many biological origins have been investigated, since their pressure-behavior is depending on its origin (Belmiro et al., 2020; Conde et al., 2022; Leite et al., 2017). However, this chapter will refer primarily to corn (maize) starch, the most studied starch type, as a model for other sources.

In practice, and especially in such products as food, with a large number of highly concentrated ingredients, reversibility is impaired by many unspecific changes. Thermal processes add the disturbing effect of increased Brownian clashes among molecules in suspensions/solutions, and this gives rise to molecular drifts that alter structures, taking them often far from the original one, which impairs the recovery of the original conformation upon return to atmospheric pressure. Pressure treatments are freer from drift but (as well as thermal ones) cause easily the unspecific aggregation of structures altered by pressure.

All effects induced by changes of hydrostatic pressure are driven by the consequent volume changes associated: pressure increase occasions a volume reduction to minimize the free energy of the system, as described by Le Chatelier's principle. Van't Hoff equation describes the dependence of the equilibrium constant with pressure, in relation with the volume change in the process, within thermodynamic, equilibrium conditions. A similar relation can be found for non-equilibrium, kinetic processes, in which the reaction activation volume (volume difference with the activated intermediate state) relates pressure with the rate constant. For details of these equations see, for example: Heremans and Smeller (1998), Molina-García (2002) or Knorr et al. (2006).

The volume increments associated to a number of processes driven by pressure are known, but it must be noted that the volume to be considered is that of the global system, including hydration or solvation layers, which makes difficult to obtain accurate data and to extrapolate them for different treatment conditions. Moreover, unavoidable thermal fluctuations, as well as pressure (and temperature) dependent second order thermodynamic derivative properties, and molecular mobility related factors (viscosity, diffusivity), impair the practical use of volume changes to predict the effect of HHP on food systems. Additionally, pH and ionic dissociation, in general, are affected by HHP (Molina-García, 2002).

Moreover, while protein pressure unfolding can be, under certain circumstances, related with relative ease, to the volume changes induced by pressure, the behavior of starch under HHP is even more difficult to predict, as starch gelatinization is a multi-step process, including irreversible stages.

Proteins undertake somehow similar phenomena when submitted to thermal treatments or to HHP: partial or total (reversible or irreversible) unfolding, dissociation of monomers, denaturation. However, the process and the resulting modified state are not quite the same, for the two perturbations (Heremans & Smeller, 1998). Starch behaves likewise, giving rise to similar but not identical modified products.

10.2 Modification Mechanism and Methodology

The result of HHP treatment of starch aqueous suspensions is its gelatinization, i.e., the well-known phenomenon in which water hydrogen bonds substitutes the intermolecular ones that keep the tight starch granular structure in its place (Muhr et al., 1982). Several observations follow: (1) Water is required for starch to gelatinize under pressure, in the same way that it is needed for thermal gelatinization. Pressure treatment at the levels usually employed for food processing, i.e., up to 600 MPa, in the absence of water (for example, suspending starch in ethanol), have no significant consequences on the granules (Molina-García, unpublished data). (2) No rupture of the starch amylose or amylopectin chains can be expected: the energy for breaking a covalent bond being much higher than that provided to the system by the previously mentioned pressure levels (Heremans & Smeller, 1998; Katopo et al., 2002). (3) Hydrostatic, isostatic pressure is always considered, i.e., a fluid is required to transmit pressure quasi-instantaneously and uniformly for all points in the vessel. For food uses, water is employed (with food-compatible additives in case the treatment involves temperatures close to freezing, to avoid mechanical damages in the pressure equipment). Pressure treatments in dry state cannot be considered hydrostatic: in them, pressure depends of directional vectors, scissor forces are generated and pressure has not a single and constant value over the whole pressure vessel (making possible, in this case, covalent bonds breaking).

Starch gelatinization is a well-known and exhaustively studied process, in which intermolecular hydrogen bonds are replaced by those with water. Gelatinization is essential for allowing accessibility of starch chains to digestive enzymes, as well as for most industrial uses of this product. It carries an associated crystallinity reduction, irreversible granule swelling and partial or total amylose chains release (Waigh et al., 1997). The complex and condensed starch structure is disentangled in a number of steps, some of them irreversible, which endows the whole process of irreversibility.

Figure 10.3 shows a cartoon focusing on two of the phenomena associated to starch pressure gelatinization: granule swelling and reorganization of polysaccharide chains. During gelatinization, starch basic polymeric chains gradually become released from the granules (causing them to swell), interacting with chains from other granules, which gives rise to gels, even when the whole granules are not completely dissolved, still keeping their individual character.

Granule swelling and the interaction of amylose protruding chains from different granules causes large viscosity increases and gelification. However, pressure-treated

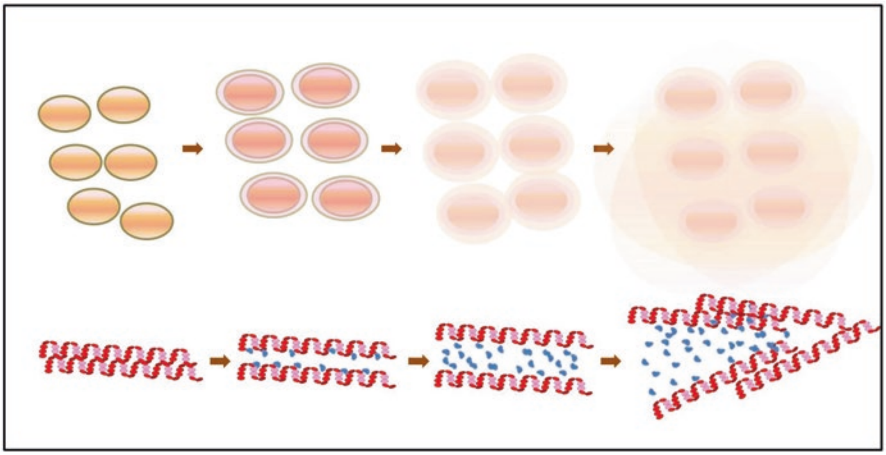


Fig. 10.3 Cartoon showing two phenomena associated to starch pressure gelatinization: granule swelling and reorganization of polysaccharide chains. Starch granules incorporate water and polysaccharide chains become increasingly separated as gelatinization proceeds. Water-amylopectin hydrogen bonds substitutes previous bonds between amylopectin chains within the granule. Interaction among granules, mediated by the protruding amylopectin chains, increases viscosity, creates strong gels and, eventually, causes the complete granule disorganization. While in thermally gelatinized starch the exit of amylopectin from granules and its intergranular interaction is the main cause of viscosity increases, in HHP gelatinized starch viscosity is more dependent on granular swelling

starch granules preserve their individuality (Figure 10.4b, c) (as a difference with thermally gelatinized ones), with a scarce degree of amylose complete solubilization (Figure 10.4d). Pressure-treated granules can preserve a good part of their crystalline character, even after intense treatments (Katopo et al., 2002). A-crystalline pattern starch (proper of cereals starches) has been observed to change to B (more common in tuber starches), as a result of pressure-induced reorganizations (Yang et al., 2013). A consequence of the interactions among chains from different granules being less extended and part of the crystalline structure still retained in pressure-generated starches, is the weaker character of pressure gels (as compared to thermally induced ones).

Pressure has a synergic effect with temperature (Fig. 10.5) and the pressure level required for starch gelatinization is lower at higher temperatures. As well and in the same order as for thermal treatments, starch gelatinization pressures are depending on the biological origin of the starch (Knorr et al., 2006). Treatment time is also a parameter to be considered (Stolt et al., 2000). It has special relevance in industrial contexts where very short pressure processing times are favored. Although some of the effects of hydrostatic pressure are basically instantaneous, the kinetically controlled phases of starch gelatinization are reflected in a progress of the process in time. Figure 10.6 shows a comparison of DSC thermograms for starch treated at the same temperature and pressure but differing in duration. It can be appreciated

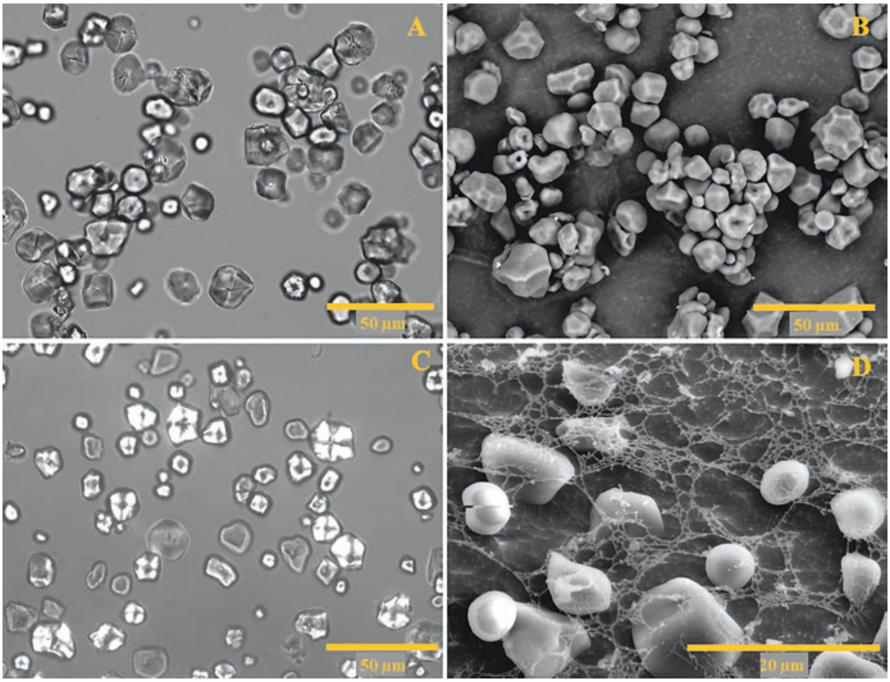


Fig. 10.4 Microscopic observations of corn starch granules: (**A**) Optical micrograph of native granules (untreated); (**B**) SEM micrograph of samples treated at 400 MPa, 40% w/v starch concentration, 35 min at 40 °C; (**C**) Optical micrograph of the same treatment as **B, (D**) Cryo-SEM micrograph of the same treatment as **B** with higher magnification. A small water content remaining in the starch cryo-SEM preparation allowed visualization of the amylopectin network protruding from granules, normally not visible in SEM micrographs. The bars correspond to 50 μm (**A**–**C**) or 20 μm (**D**)

that, while gelatinization temperature peak and onset are scarcely affected, the area of the gelatinization peak (proportional to the energy of thermally completing the gelatinization of the still ungelatinized starch fraction) is further reduced as treatment time increases.

Starch pressure gelatinization can be studied by different techniques, which can give information on the progress of the different steps of this process. Transient information (during pressure processing itself) is difficult to obtain in HHP treatments. But treated starch suspensions can be studied just after treatment (and before other processes -thermal, drying, retrogradation- have a chance to alter the HHP outcome) by techniques focusing on chemical groups interactions (RMN or FTIR), energetic balance of the thermal gelatinization still to be completed (DSC), crystallinity related ones, such as loss in birefringence, or molecular mobility and viscosity (Stolt et al., 2000).

Especially useful is DSC, as it has been shown that it can be employed to study starch in different gelatinization states, after drying to powder the resulting

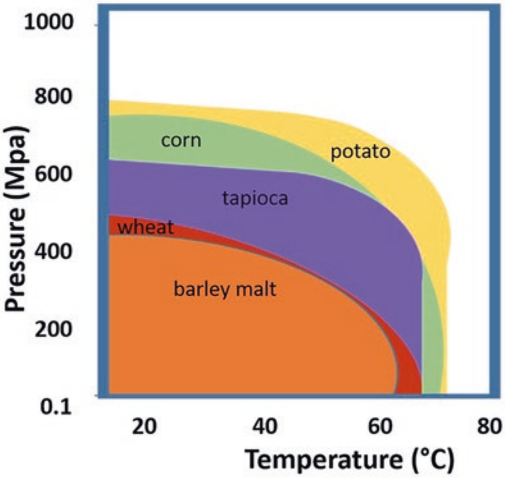


Fig. 10.5 Starch gelatinization pressure-temperature phase diagram: cartoon after several authors (Douzals et al., 1998; Bauer & Knorr, 2005; Knorr et al., 2006; Buckow et al., 2008). A synergy exists between temperature and pressure, so that starch gelatinization at a given pressure is facilitated by higher temperatures. Other factors of relevance are not considered here, such as time under pressure, starch/water ratio or water incubation previous to pressure treatment

pressure-treated suspensions and later reconstituting with water (Teixeira et al., 2018). X-ray diffraction can also be performed in the dry state to obtain important information on crystallinity.

10.3 Starch Modifications by Pressure

10.3.1 Molecular Weight

Based on the gel permeation chromatography results, ultrahigh hydrostatic pressure treatments (690 MPa) did not change the molecular weight distribution of starch samples (Katopo et al., 2002). As already mentioned, hydrostatic pressure treatments average compression energy by means of the pressure transmitting medium. Without these media, pressure effects would be uneven. The average compression energy is not sufficient to break starch covalent bonds. However, in real foods, starch may coexist with amylolytic enzymes. These enzymes could degrade starch polysaccharide chains, once pressure gelatinization has facilitated its digestion.

Another high-pressure food technological process, not to be mistaken with HHP, is high pressure homogenization. In it, sample solutions or suspensions are forced through small perforations at high pressure. This gives rise to extreme scissor forces and can degrade starch granule reducing the size of polysaccharide chains (Apostolides & Mandala, 2020).

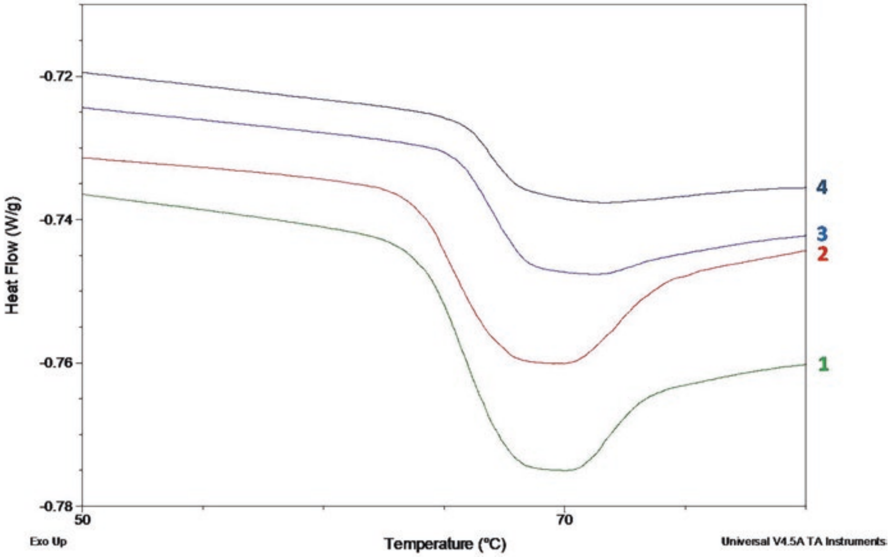


Fig. 10.6 Comparison of Differential Scanning Calorimetry thermograms for starch treated at the same temperature and pressure but differing in treatment duration. Thermal starch gelatinization is globally a two-step process, although its irreversible stages make the global process also irreversible (as shown by the curve wide from). The initial and final states can be seen to be clearly different as having a very distinct heat capacity (position of the baselines before and after gelatinization). In this experiment, corn starch suspensions were partially pressure gelatinized (400 MPa, 25 °C), for different times: **1**–0 min, **2**–15 min, **3**–30 min, **4**–60-min. The position of the gelatinization event in the temperature axis (onset and peak temperatures), related to the gelatinization mechanism, are not changing with time. But as time proceeds, the process enthalpy (proportional to the area over the curve), which is related to the number of intramolecular bonds replaced for those with water, is smaller, as a result of pressure gelatinization having advanced, and a smaller fraction of starch being still not gelatinized

10.3.2 Structure and Morphology

Gelatinization implies starch structural alterations, such as a progressive degradation of the granule crystalline regions, based on the double helix arrangement of amylopectin, from initial distortions caused by incipient hydration to a later water intrusion in the crystalline core, for its subsequent complete hydration (Lund, 1984; Oh et al., 2008). Starch crystallinity and micro- and macro-structural properties alterations are closely related to thermal properties (Lemos et al., 2018; Rahman et al., 2020).

The progress of gelatinization is visibly evidenced by changes in the morphology of starch granule. Corn starch granules have polyhedral shape, irregular sizes, and a relatively smooth surface. Granules contain specific channels and cavities penetrating its interior, connected to open surface pores (Fannon et al., 1993; van de Velde et al., 2002). These granule morphological parameters show only slight changes, as observed by scanning electron microscopy (SEM), after moderate HHP treatments (300 MPa during 15–30 min, Rahman et al., 2020, or 400 MPa at 38 °C for 35 min, Deladino et al., 2015), when size is slightly increased, and surface gets faceted and rougher. At the latter conditions, the alteration of pores connecting surface and granule inner core is observed by confocal laser scanning microscopy, which also allowed observing the increased granule size, due to a limited swelling. A higher level of penetration of FITC dye was attributed to these pores and to the presence of voids and fractures due to incomplete HHP-induced gelatinization (Teixeira et al., 2015).

At these intermediate pressure levels, a narrowing in the profile of native starch pore size (from 8 to 60 nm, with a maximum number of pores between 8 and 12 nm, to pore diameters ranging from 4 to 10 nm), accompanied of an increased of the total pore volume, is reported, as determined by mercury intrusion. Moreover, when using the nitrogen adsorption/desorption technique, HHP treated starch showed a decrease in pore size distribution and changes in the distribution curve shape, with an increase in the number of smallest pores. The BET surface area increased from 0.277 m^2/g for native starch to 0.407 $m^{2/}g$ for the HHP treated starch, showing an increase of 47% due to HHP treatment (Deladino et al., 2015). These results were attributed to granule reorganization induced by high pressure, which may enhance the connection between external pores with interior channels that were not accessible in untreated starch.

Higher pressures can alter granular surface, creating cracks, flattening granules, which collapse at its center, and completely disintegrating granules (Błaszczak et al., 2005; Douzals et al., 1998; Heydari et al., 2021; Rahman et al., 2020). After Okur et al. (2019), morphological changes develop slowly, in lengthy treatments, at pressures over 500 MPa.

The observation of the gelatinization process by light microscopy photographs of I^2 solution-dyed starch treated at 400 MPa (at 40 °C for 15 min), showed a high level of disorder at the center of the granule while the classic non-gelatinized granular ordering of native starch was intact at the external layers. However, at more drastic conditions (700 MPa, at 35 °C, for 25 min) the erosion of the surface and the granule integrity loss and the formation of a gel network, are the features appreciated (Teixeira et al., 2018) and might be associated to the hydration of the amorphous phase and/or melting of crystallinity (Wang et al., 2008). Rahman et al. (2020) also observed by SEM that maize starch granules were completely disintegrated and gelatinized, showing large starch granules and lamellar structure when treated at 500 MPa for 15 and 30 min.

Starch crystallinity is based on the double helix arrangement of amylopectin. X-ray diffraction provides information on these crystalline domains. It allows the classification of native different biological origin starches as A-type (cereals: rice, corn), B-type (tubers: potato) and C-type (tapioca, pea) starches (Cheetham & Tao, 1998; Hibi et al., 1993; Le Bail et al., 1999; Zobel, 1988). Starch crystalline helices are more compact in A than in B-type, which has a more hydrated core. C-type is often considered as a mixture of both A and B-types (Teixeira et al., 2018).

Li et al. (2011) reported a weakening of diffraction peaks with increasing pressure, related to the destruction of crystalline structure in gelatinization. B-type starches resist pressure more than A- or C-type starches (Katopo et al., 2002; Oh et al., 2008; Rahman et al., 2020; Rubens et al., 1999; Stute et al., 1996). A-type could have more scattered and flexible branching amylopectin structures than B-type, and can be rearranged under pressure, generating channels letting water molecules in, which triggers the pressure-induced transformation from A to B-type (Katopo et al., 2002; Yang et al., 2016; Teixeira et al., 2018). Actually, Teixeira et al., (2018) found that peaks characteristics from A-type and B-type patterns coexist after HHP treatment, evidencing the partial gelatinization transformation. Similarly, Rahman et al. (2020) detected the decrease of intensity of A-type peaks and the appearance of peaks typical of B-type pattern in treatments at 500 MPa and attributed the transformation of the diffraction pattern from A-type to B-type to the disruption of the crystalline regions of maize starch granules.

The crystallinity fraction (CF) is closely related to the molecular structure and contents of both of amylopectin and amylose (including chain length, branching and polydispersity). It is also related to starch interesting properties, such as pasting and starch digestibility (Irani et al., 2017). CF is reduced after HHP treatments, as follows from crystalline structure weakening and destruction (Heydari et al., 2021). Native corn starch CF, 24%, as derived from X-ray diffraction data (Teixeira et al., 2015), is reduced by nearly a 50% by HHP treatments at moderate pressures (400 MPa, 30 °C, 15 or 35 min), while higher pressures (700 MPa) leave a residual CF of 2.8%, only a 11.7% of the native crystallinity (Teixeira et al., 2018). Other authors also found a similar loss of native crystalline structure and molecular order (Katopo et al., 2002). At the same type, the B-type crystallite increased, more drastically at the more elevated pressures (in good agreement with the GD obtained from DSC measurements) (Teixeira et al., 2018). This A and B-type patterns mixture resulting from HHP treatment was suggested to be induced by recrystallization (Choi et al., 2009), just after pressure-gelatinization. Different starch/water ratio (SWR) are not correlated to significant modifications in peak positions and intensities, or CF (Teixeira et al., 2018).

In samples submitted to very high pressure (700 MPa) or long treatment times at 400 MPa, an additional X-ray diffraction pattern, V, was detected. V-diffraction pattern reflects the presence of complexes of amylose with lipids and similar molecules (Cheetham & Tao, 1998; Shi et al., 2017). The preservation of starch structure after harsher pressure treatments (9 min at 650 MPa), although its crystallinity decreased, has been justified resorting to V-pattern, in high amylose samples also containing lipids, whose complexes would impair swelling stabilizing the granular structure (Katopo et al., 2002; Teixeira et al., 2018; Wang et al., 2008). Peaks characteristic of V-pattern (Shamai et al., 2003) have intensities increasing with treatment time and SWR at moderate pressures (Teixeira et al., 2018). More extreme conditions (700 MPa, 25 min, 30 °C), gave rise to a high intensity for this peak. This implies also a reduced amylose leach, as it is complexed in the granule (Teixeira et al., 2018). Similar results were obtained by Le Bail et al. (1999).

Addition of hydrocolloids before pressure treatments does not alter starch crystalline fraction. However, V-type polymorph X-ray peak intensity increases, in correlation with lower amylose concentration in solution. The formation of the

V-crystalline complex would be favored, as hydrocolloids, linear polysaccharides, could contribute to amylose stabilization, leading to lower amylose release of HHP treated starch (Katopo et al., 2002; Teixeira et al., 2018).

HHP affects starch component chemical bonding, and this can reflect on FTIR spectra (Cui & Zhu, 2019). HHP treatment respects corn starch FTIR spectra major peaks but there is a clear effect on peak intensity. Broadbands (3100–3700 cm^{-1}) are related to starch OH− stretching (Jiang et al., 2011). Corn starch OH − stretching band width and intensity was reportedly reduced after HHP treatments at 500 MPa, while unaffected at low (100 MPa) or medium (300 MPa) pressures, although there are significant changes in the fingerprint area. Moreover, the asymmetric C–H stretching band also shows peak lowering and narrowing, reflecting changes in conformation and crystallinity of amylose-amylopectin (Kizil et al., 2002; Rahman et al., 2020). Other peaks, related to firmly bound water and characteristic of the anhydrous glucose ring C-O stretch, had also their intensity reduced after pressure treatment (Fang et al., 2002).

RMN T2 transverse relaxation time studies suggest that longer HHP treatments at elevated temperatures have a complex effect, in terms of hydration and starch structural changes. T2 increases after HHP treatments, meanwhile heat-induced gelatinization is associated to T2 decreases (Okur et al., 2019; Ozel et al., 2017). Other heat and HHP-induced starch gelatinization differences can also contribute to the reverse effect on T2, such as the shear forces associated to stirring, usually absent in HHP treatments (BeMiller & Huber, 2015). The apparition of B-type crystals in pressure-induced gelatinization implies reduced double helix dissociation (while heat gelatinization causes intense double helix dissociation) (Pei-Ling et al., 2010), which produced less swollen starch granules due to reduced amylose leaching (Yang et al., 2016).

10.3.3 Swelling and Solubility

Gelatinization gives rise to hydration of starch amorphous regions, causing granular swelling. A second step of this process implies further granule weakening and swelling, release of amylose chains and interaction among these from different granules, with loss of granule identity, in the way to gel formation: a dispersion of free amylose and amylopectin including granule remnants (BeMiller & Whistler, 1996; Lund, 1984; Oh et al., 2008). HHP gelatinized starch shows differences from the heat-induced product, including less amylose release, and reduced starch granule swelling and disintegration (Knorr et al., 2006; Stute et al., 1996).

Granule birefringence under polarized light is closely associated to swelling and loss of internal structure. Its characteristic Maltese cross is due to amylopectin double helix radial orientation in starch crystalline regions (Castro et al., 2020; Deng et al., 2014). While potato starch retains birefringence after a strong pressure treatment (600 MPa, 30 min), corn and tapioca starches loss this property totally or partially at these pressures. At lower pressures (150–300 MPa), birefringence was still shown. However, at intermedium values (400–450 MPa), birefringence reduction was noted, while it was partially maintained (Castro et al., 2020; Oh et al., 2008; Teixeira et al., 2015). In a similar way to other starch properties related to structure, or to its thermal parameters, swelling behavior after HHP depends on starch biological origin and treatment parameters (Oh et al., 2008). Some authors consider it dependent on granule size and its distribution, and on the amylose content (Heydari et al., 2021; Oh et al., 2008).

The swelling power of HHP treated starches has been reported as higher than in native starch (Heydari et al., 2021) and, contrariwise, lower than in untreated starch (Okur et al., 2019). Oh et al. (2008) found an 18% swelling degree after pressurization at 400 MPa for corn starch. In a similar way, Deladino et al. (2017) found that HHP almost doubled the swelling power of corn starch treated at 400 MPa (at 38 °C, for 30 min), over a thermal gelatinization value around 8%. However, Rahman et al. (2020) found that both solubility and swelling power of maize starch were significantly decreased with increasing pressure (0.1 to 500 MPa) in comparison with native starch. Corn starch swelling at 600 MPa was under 50% (Oh et al., 2008; Stute et al., 1996). Some starches including corn, have a reduced swelling associated to HHP treatment, perhaps related to a lower thermal drift effect, as compared to thermal gelatinization. On its hand, waxy corn starch was not found to swell considerably at intermediate pressures (400 MPa), while achieving 100% swelling at 600 MPa. Potato starch was much less affected by swelling (Oh et al., 2008). Some authors (Li et al., 2012), consider swelling as caused by amylopectin, as amylose would be scarcely solubilized, as most granules would be still intact. A limited granular swelling is usually taking place after HHP treatments (Stolt et al., 1999; Heydari et al., 2021).

Some authors (Katopo et al., 2002; Teixeira et al., 2018), observed (by scanning electron microscopy) a retention of granular identity in corn starch, even after HHP treatment at 690 MPa. Lower amylose release and less water binding could justify this granular preservation (Douzals et al., 1998), while amylose–lipid complexes could have a role in restricting swelling (Katopo et al., 2002; Liu et al., 2016).

Amylose solubilization, taking place at the more advanced gelatinization phases, can also be affected by HHP treatments, either increasing or limiting it, through the amylose-amylopectin interaction (Liu et al., 2016). Different balances of inter-associative forces within starch amorphous and crystalline domains, amylose/amylopectin ratio and their branching characteristics, together with the role of other minor starch components may be the cause of these solubilization differences (Kumoro et al., 2012; Liu et al., 2016). Corn starch gelatinized under HHP conserve most of its granule independence and the solubilization of amylose is limited (Douzals et al., 1998; Knorr et al., 2006; Stolt et al., 2000; Teixeira et al., 2018). Birefringence reveals only some granules gelatinized and swollen at moderate pressures and temperatures (400 MPa, 30 °C), while harsher treatments (700 MPa) affect most granules, whose integrity is lost, while a gel network can be detected (Oh et al., 2008; Teixeira et al., 2018).

The main differences of HHP gelatinized starches with thermally treated ones can be summarized in a smaller amount of amylose released from the granules, a limited granule swelling and starch granules appearing intact or just partially disintegrated (Knorr et al., 2006; Teixeira et al., 2018).

10.3.4 Thermal Properties

Thermal parameters are a suitable measurement of the gelatinization progress. A DSC experiment with native starch produces a single endotherm, providing the SWR under 30%. At higher SWR values, gelatinization, basically a reaction with water, is incomplete, and additional thermal transitions are obtained. Meanwhile, higher water contents ensures a complete gelatinization, practically independent of the actual ratio (Wang et al., 2014, 2016). Some concerns about the availability of water for starch gelatinization in HHP treatments have been addressed elsewhere (Teixeira et al., 2018).

The endotherm is roughly symmetrical, though not completely, reflecting the nonreversible character of gelatinization. The temperature at which it takes place: onset temperature (T_o) (the endotherm starts, usually considered the gelatinization temperature) is a measurement of the stability of the initial starch state. T_{peak} and T_{endset}, reflect starch heterogeneity, which widens the transition. The area under the thermogram, the endotherm enthalpy (ΔH), corresponds to the energy exchange in the process. Both enthalpy and temperatures are characteristic of each starch type.

A DSC thermogram of previously treated starch informs of the extent of the gelatinization undergone (the gelatinization degree, GD) and the remaining intramolecular bonds, still not replaced by hydration (Oh et al., 2008). After a given HHP (or thermal) treatment, the ΔH is reduced, indicating partial gelatinization. If the process is complete, no endotherm will be observed. For corn, depending on authors, varieties and other process parameters, this takes place between 500 and 700 MPa (Teixeira et al., 2018). This enthalpy decrease reflects the energy employed in breaking the intramolecular hydrogen bonds in helical chains of the crystalline regions and, therefore, the progress in the crystallinity and internal granule order destruction by the different treatments leading ultimately to gelatinization (Castro et al., 2020).

While the decrease of enthalpy upon treatments inducing gelatinization is widely accepted, in dependence of treatment pressure, duration and starch biological origin (Stolt et al., 2000; Bauer & Knorr, 2005; Kawai et al., 2007a, b; Teixeira et al., 2018; Cui & Zhu, 2019), this is not the same with gelatinization temperature. Many factors related to starch origin, amylose/amylopectin composition, crystalline degree, etc., are involved, and results differ for different cases and authors. The actual extent of gelatinization may be also a crucial factor. Justification of this temperature drift can be based in the destabilization of crystalline structure by partial gelatinization (which would contribute to T_o decrease), or in the preferential gelatinization of the smaller (less resistant) granules, leaving intact the larger and more stable ones (which would cause T_o increase) (Castro et al., 2020; Teixeira et al., 2018). According to some authors, starches with higher crystallinity degrees would exhibit higher ΔH and T_o (Heydari et al., 2021).

HHP treated (600 MPa) corn starch T_o was reported to increase over that of native starch (Katopo et al., 2002; Oh et al., 2008). However, Rahman et al. (2020) found a significant decrease at treatments at 300 MPa (with a widened T_o range), while at 500 MPa, no endotherm was reported. Similar results were observed in other biological origin starches (Guo et al., 2015; Heydari et al., 2021; Li & Zhu, 2018). The decrease in T_o has been ascribed to granule structure disruption and crystallinity reductions (Błaszczak et al., 2005). Other authors report virtually no change (or only a slight reduction) in T_o for potato varieties starch (Cui & Zhu, 2019) and corn at moderate pressures (300–400 MPa), while at 700 MPa, T_o could be seen to decrease (Teixeira et al., 2018; Heydari et al., 2021).

The degree of starch gelatinization measured by DSC increased accordingly with the intensity (pressure level and exposure time) of the HHP treatment. The data from several studies is summarized in Table 10.1, which shows that HHP treatment may have a range of effects on starch, including inducing gelatinization, altering crystalline structure, rheology and improving the thermal stability.

Achieving only a partial gelatinization degree through HHP treatment is not always a failure: partially gelatinized starch shows mixed properties: granular identity and independence, but incipient interactions among the partially degraded granules and with other molecules. HHP provides more homogeneous and better-defined partially gelatinized products, with a specific crystallinity degree, in a process easier to monitorize (Heydari et al., 2021; Knorr et al., 2006; Teixeira et al., 2018).

The presence of hydrocolloids did not introduce significant differences in these parameters (Teixeira et al., 2018; Tester & Sommerville, 2003). However, for thermal gelatinization, a possible stabilization of the granular structure by hydrocolloid has been reported (Biliaderis et al., 1997). On the other hand, enthalpy may be reduced by bacterial cellulose fibrils (Díaz-Calderón et al., 2018).

10.3.5 Pasting Properties

Several studies support the fact that the application of pressure imparts structural changes on starch, restricting the leaching of amylose and amylopectin, increasing pasting temperature and reducing viscosity (Ahmed & Thomas, 2017; Heydari et al., 2021; Hoover et al., 2010; Liu et al., 2016). Pasting temperature agrees with the temperature at which gelatinization of starch begins. Li and Zhu (2018) reported that the pasting temperature decreased at 600 MPa for corn starch, whereas the peak pasting temperature was increased, agreeing with the result previously informed by Oh et al. (2008) for normal and waxy corn starch.

Regarding viscosity, Oh et al. (2008) found that the initial viscosity for waxy corn starch increased from 5.7 MPa s^1 before pressure treatment to 3530 MPa s^1 after pressure treatment at 600 MPa. BeMiller (2019) also reported that the viscosity increased in association to the increase in swollen starch granules and its maximum values corresponded to the formation of complexes among different granules.

Table 10.1 Some studies in the literature on the impact of high pressure on granule of corn starch and its properties

Reference	Treatment	Impact on granule properties			Achievements
		Gelatinizationdegree	*Rheology*	*Crystallizationdegree*	
Cappa et al., 2016	1:0.5, w/v, 600 MPa, 40 °C, 5 min	–	At low strain levels, G’ always exceeded G’‘	–	HHP treatment applied to mixtures with high sample-to-water ratios, had beneficial effects on gluten-free bread features.
Heydari et al., 2021	10, 15 and 20% (w/w), 600 MPa, 25 °C, 20 min	Starch granules depicted a higher gelatinization than the HHP-treated starches	–	Increasing starch concentration from 10% to 20% caused an increase in the relative crystallinity	HHP treatment and high concentration provoked a considerable increase in water absorption capacity, oil absorption capacity, zeta potential values, and a pronounced decrease in peak, trough, breakdown, setback viscosities; gelatinization, peak and conclusion temperatures, and gelatinization enthalpy.
Larrea-Wachtendorff et al., 2020	5 and 10% (w/w), 0–700 MPa, −40 to +120 °C 5, 10, 15 and 20 min	–	The critical strain was equal to 3%. Below this threshold value, the structure of the hydrogels remained intact and behaved like a solid.	–	Viscosity and G’ values of corn starch HHP hydrogels decreased with increasing glycerol concentration. 5% glycerol concentration was identified as the critical value to obtain stable HPP hydrogels with good rheological and texture properties.

Reference	Treatment	Impact on granule properties			Achievements
		Gelatinizationdegree	*Rheology*	*Crystallizationdegree*	
Larrea-Wachtendorff et al., 2021	20% (w/w), 600 MPa, 25 °C 5–15 min	Corn starches were completely gelatinized	The hydrogels obtained displayed a shear-thinning and gel-like behavior ($G' > G''$)	–	HHP hydrogels showed a cream-like structure. The mechanical properties of hydrogels produced were improved by utilizing processing times of 15 min.
Li et al., 2016	30% (w/w), 300, 450, and 600 MPa, 15 min	Samples treated at 600 MPa for 15 min were completely gelatinized. The starch was not fully gelatinized when treated at lower pressures.	–	HHP treatment at 600 MPa resulted a substantial loss of A-type crystallinity. Retrograded corn starch exhibited a weak A-type structure, and the V-structure becomes more visible.	Comparing corn starch and their HHP-gelatinized starch gels retrograded at different storage time, the differences in amylose content and crystallinity characteristics provided an explanation for their different properties.
Liu et al., 2022	20% (w/w), 400–600 MPa, 25–300 °C, 10–20 min	Five degrees of gelatinization (DG) were achieved. DG1: approx. 20%,(400 MPa/10 min); DG2: approx. 50%, (500 MPa/10 min); DG3: approx. 70%,(550 MPa/10 min); DG4: approx. 85%,(600 MPa/10 min); DG5: approx. 100%, (600 MPa/20 min).	–	The relative proportion of double-helix and single-helix components gradually decreased, and the amorphous content correspondingly increased with increasing degree of gelatinization.	The HHP reduces the binding of immobilized water and raises freedom degree of water molecules. Molecular dynamics simulation was employed to provide supporting evidence on the intra- and inter-hydrogen bonding interactions in pressure-treated amylose-water systems. HHP tends to make water serve as a plasticizer of the amorphous region.

(continued)

Table 10.1 (continued)

Reference	Treatment	Impact on granule properties			Achievements
		Gelatinizationdegree	*Rheology*	*Crystallizationdegree*	
Papathanasiou et al., 2015	5% (w/w), 300–800 MPa, max 29 °C, 15 min. and same HPP, 80 °C, 15 min.	Gelatinization degree rises with the increase of the pressure applied.	–	–	The pressurized starch suspensions, in comparison to the equivalent thermally treated samples, release less amount of glucose after 120 min of enzymatic digestion.
Rahman et al., 2020	20%, (w/w), 100, 300, 500 MPa, 25 °C, 15 and 30 min	The onset temperature, peak temperature and gelatinization enthalpy of HHP treated starch significantly decreased at the pressure up to 300 MPa. Starch treated at 500 MPa was completely gelatinized.	–	At 500 MPa, starch granules were fully disrupted, indicating the transformation of the diffraction pattern from A-type to B-type.	500 MPa for 30 min had a significant impact on the modification of corn starches in terms of amylose and damaged starch contents, particle size, microstructure, thermal and pasting properties.
Shen et al., 2018	30, 50, 70% (w/v), 200, 400, 600, 800 or 1000 MPa, 30 °C, 15 min	–	–	Crystallinity significantly increased with pressure level, but almost no changes were observed due to concentration.	Authors propose that a lower treatment pressure leads to the rearrangement of crystalline structure or molecular chains to make the starch internal structure of the starch granules more organized than its native status.
Yang et al., 2016	10 w/w%, 600 MPa, 25 °C, 15 min	–	Increase of G* as a function of storage time compared with non-treated control sample	–	The rheological and FTIR analysis of HHP treated starches demonstrate that retrogradation does not depend on the pressure releasing rate.

10.3.6 In Vitro Digestibility

Starch can be divided into three groups according to its digestion rate: resistant starch (RS), rapidly digestible starch (RDS), and slowly digestible starch (SDS). Digestion of SDS occurs at a lower rate than RDS, so SDS raises blood-glucose levels more slowly than RDS, being helpful in preventing hyperglycemia-related diseases (Okur et al., 2019). Accordingly, SDS would be the more appropriate type of dietary starch, since its digestion in the small intestine is retarded and it would reduce postprandial blood glucose levels (Huang et al., 2020). Shen et al. (2018) studied the effect of high-pressure treatment on the internal structure of high amylose corn starch and its influence on digestion. They found that HHP treatment at 200 and 400 MPa led to a lower digestion rate compared to native starch. But HHP treatment at 600, 800 and 1000 MPa resulted in a significantly enhanced digestion rate. In the same way, HHP caused a significant decrease at 400 and 500 MPa for RS content of corn starch, while SDS content increased significantly (Okur et al., 2019). Papathanasiou et al. (2015) measured the available glucose content after the enzymatic digestion of starch type A, B, C and resistant starch was examined. The pressurized starch suspensions were treated, achieving different gelatinization degrees (from 25% to 100%) and they released less amount of glucose after enzymatic digestion in comparison to the equivalent thermally treated samples. Among the studies samples, resistant and waxy corn starch exhibited the lowest levels of released glucose after enzymatic digestion. So, they could be used to produce starch-based low-glucose foods. Besides, the effect of freeze-drying on their available glucose content after enzymatic digestion was not significant, meaning that treated starches tolerated one of the typical industrial procedures. Meanwhile, pressure-gelatinized (600 MPa, 15 min) samples of normal and waxy corn starches showed a rise in resistant starch content and relative crystallinity degree with the increase in storage time. These retrograded starches also showed restricted starch swelling power and lower pasting viscosities.

Moreover, different moisture contents (30–70%) digestion rates of high amylose corn starch did not change significantly at pressures in the range 200–800 MPa but did at 1000 MPa, suggesting that applied pressure plays an important role rather than moisture content in altering starch digestion (Shen et al., 2018).

According to these studies, treatments at low pressure would lead to the reordering of crystalline structure or molecular chains, to make the internal structure of starch granules more organized than its native state. However, a high-pressure application can destroy the internal arrangement, giving rise to a less-organized structure, which enhances the susceptibility of starch molecules to enzymatic hydrolysis.

10.4 Applications

Many of the structural changes that HHP provokes on starch granules positively impact its functional and nutritional properties, diversifying its uses in food, medical or pharmaceutical applications. Figure 10.7 reviews some potential applications of HHP treated corn starches.

10.5 Previous and Recent Trends in Feasibility and Utilization of Modified Starch

HHP induces modifications on biopolymers and represents a workable opportunity to avoid chemical or genetically modified ingredients in health and food-related industries, attending to consumers' claims on chemical-free, clean, and/or green technologies.

High hydrostatic pressure commercialized products are mainly sterilized or pasteurized stuff, such as processed meat, fish, juices, fruit-based preparations and ready-to-eat products in general, products where the microbial safety is important (restricts microbial spoilage in fruit juices and milk). Also, it has been adopted in emulsion processing (reduction in droplet size contributes to emulsion increased stability) and pharmaceuticals (O/W emulsions employed in drug delivery to increase bioavailability) (Aganovic et al., 2021; Dumay et al., 2013; Raghunathan et al., 2021). Figure 10.8 summarizes the regulation institutions and the current legal status of HHP worldwide.

10.6 Case Studies

Several combinations of pressure and temperature have been performed while studying the effect of HHP on corn carrying properties (Deladino et al., 2015, 2017; Teixeira et al., 2015, 2018). The different treatments were applied on 10 g/100 mL corn starch aqueous suspensions. The crystalline fraction (CF) was calculated from X-ray diffraction (XRD) patterns as the ratio between the absorption peaks and the total diffractogram area, expressed as a percentage (%) (Teixeira et al., 2015). The degree of gelatinization of starches was determined by DSC (Tp: peak temperature (starch gelatinization temperature); ΔG: enthalpy of gelatinization on dry basis). Table 10.2 summarizes the most important effects of HHP treatments on carrying ability of corn starches and/or their potential for the design of new ingredients for food formulations with nutritional and functional added value.

High pressure caused partial granule gelatinization and increased the granule specific surface area (Table 10.2). This increase in surface was due to the generation of pores, which favored the adsorption of the yerba mate hydrosoluble polyphenols

Carrier systems.

- Metal carriers: High pressure gelatinized starch (400 MPa, 35 min, 40°C) was used as a vehicle for the inclusion of metals (zinc and magnesium) for food products applications (Fernández et al. 2008); when HHP treatment was performed simultaneously with alkali, the high pore volume created more binding sites to load high amounts of minerals, such as zinc and magnesium (Deladino et al., 2017).
- Natural antioxidants: the increase of the specific surface area of granules by HHP (400 MPa, 35 min, 40°C) favored the adsorption of the yerba mate hydrosoluble polyphenols (Deladino et al., 2014).
- Combined systems: zinc and magnesium combined with natural antioxidants were successfully vehiculized by HHP treated corn starch (400 MPa, 35 min, 40°C) (Teixeira et al., 2015).
- Theophylline: starch-based matrices prepared via high hydrostatic pressure (650 MPa, 9 min, 30°C) exhibited controlled drug release (Błaszczak et al., 2015).

Resistant starch production.

- RS type 3 (RS3): it can be obtained by HHP leading to a product with high thermal stability and preserved nutritional functionality (Ma et al., 2018).
- RS type 5 (RS5): HPP treatment (500 to 600 Mpa, 30 min, 25°C) of lotus seed starch resulted in the formation of amylose long-chain fatty acid complexes, defined as type-5 of resistant starch (Guo et al. (2019).

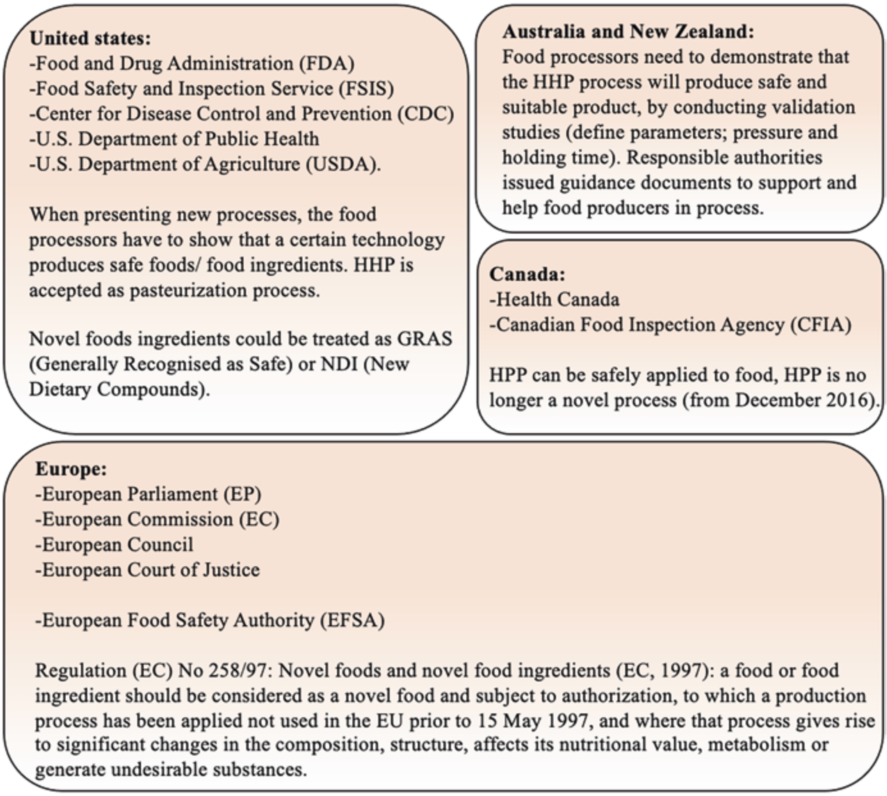
Food ingredients.

- Improved flours: High pressure treatment (600 MPa, 5 min, 40 ºC) applied to raw materials (corn starch and rice flour) was used to better gluten-free bread shelf-life, the presence of HHP treated corn starch or rice flour was effective in slowing down the staling rate of bread crumb (Cappa et al., 2016).
- Fat replacer: HHP-treated starches (600 MPa, 20 min, concentration> 10%) presented a creamy texture with high stability during storage, and appropriate viscosity, so they can replace fat in many food systems, especially, in food emulsions (Heydari et al., 2021).
- On-demand special-purpose starches.
  HHP treated starches (600 MPa, 20 min, concentration> 10%) showed high swelling power and oil absorbance levels, so consumer claims for chemical-free, clean, and/or green technologies could be attended by adding these modified starches in the formulation of food emulsions for producing low-calorie foodstuff emulsions (Heydari et al., 2021).

Hydrogels.

- The incorporation of glycerol as humectant was explored, starch-based hydrogels have potential use in pharma and cosmetic sectors to produce creams, gels and ointments. Also useful in the food industry as functional foods and products for personalized nutrition. The concentration of 5% glycerol was the critical value to obtain stable HPP hydrogels (600 MPa) with good rheological and texture properties (Larrea-Wachtendorff et al., 2020).
- Obtention of natural products for use in applications where either creamy or gummy structures are needed. Both, the processing time and starch source, had an effect on the formation of gels from starch suspensions affecting the physical characteristics of HPP hydrogels (600 MPa, applied at a compression rate of 8.4 MPa/s for 5 and 15 min, 25°C). Rice, wheat, and corn starch HPP hydrogels showed a cream-like structure (Larrea-Wachtendorff et. al., 2021).

Fig. 10.7 Potential applications of high hydrostatic pressure treated starch



United states:
-Food and Drug Administration (FDA)
-Food Safety and Inspection Service (FSIS)
-Center for Disease Control and Prevention (CDC)
-U.S. Department of Public Health
-U.S. Department of Agriculture (USDA).

When presenting new processes, the food processors have to show that a certain technology produces safe foods/ food ingredients. HHP is accepted as pasteurization process.

Novel foods ingredients could be treated as GRAS (Generally Recognised as Safe) or NDI (New Dietary Compounds).

Australia and New Zealand:
Food processors need to demonstrate that the HHP process will produce safe and suitable product, by conducting validation studies (define parameters; pressure and holding time). Responsible authorities issued guidance documents to support and help food producers in process.

Canada:
-Health Canada
-Canadian Food Inspection Agency (CFIA)

HPP can be safely applied to food, HPP is no longer a novel process (from December 2016).

Europe:
-European Parliament (EP)
-European Commission (EC)
-European Council
-European Court of Justice

-European Food Safety Authority (EFSA)

Regulation (EC) No 258/97: Novel foods and novel food ingredients (EC, 1997): a food or food ingredient should be considered as a novel food and subject to authorization, to which a production process has been applied not used in the EU prior to 15 May 1997, and where that process gives rise to significant changes in the composition, structure, affects its nutritional value, metabolism or generate undesirable substances.

Fig. 10.8 Regulation institutions and legal aspects of HHP

(Deladino et al., 2015). Alkali treatment also increased the number of pores in the granules whereas the combined procedures (HHP + alkali) led to a central disruption and an enlarged deep pore number (Fig. 10.9). Accordingly, the high pore volume found in alkali-treated starches created more binding sites to load high amounts of zinc and magnesium (Teixeira et al., 2018). Also, the decrease in temperature and enthalpy of gelatinization, associated to high pressure granular starch suspensions, was moderated in alkali-treated samples by the creation of new charged interactions.

The X-ray diffractometry A pattern is associated mainly with cereal starches in the native granular forms, the C pattern is a mixture of both A and B types, the last one being more resistant to amylolytic digestion (Liu et al., 2016).

The fact that all samples with hydrocolloids showed higher intensities of V-type polymorph peak was correlated with a lower amylose concentration in the supernatants, compared to the HHP treated sample without gum, indicating that the formation of the V-crystalline complex would be favored by hydrocolloid presence under pressure (Teixeira et al., 2018). Hydrocolloids, being linear polysaccharides, would contribute to the lixiviated amylose stabilization, leading to lower amylose contents in the supernatants of HHP treated samples.

Table 10.2 Effect of HHP treatment on corn starch properties. Case studies

HHP Treatment	CF (%)/crystal type	Tp (°C)/ ΔG (J/g)	Main improved characteristic/pretended use
400 MPa, 35 min, 40 °C	24%/A-type	68.6/9.9	Porosity increased 47% with respect to native granules, BET surface area = 0.407 m^2/g, Bimodal narrow profile, pore volume decreased, and the number of small size pores was lower in comparison to native starch, ranging from 3 to 14 nm with maxima at 4 and 8 nm/ Vehiculization of active compounds.
400 MPa, 35 min, 40 °C + NaOH	8%/A-type	72.6/9	Bimodal distribution, lower size interval at 3–17 nm (max. at 6 and 10 nm) and a wider larger size interval at 26–87 nm (max. at 52 nm)/ higher extent of gelatinization, both the small and large size pores increased in number compared to the native granules and the larger size pores reached diameters that qualifies as macropores with a pore volume four-fold higher/ Vehiculization of minerals.
400 MPa, 35 min, 40 °C + Carragenan gum	13.6%/C + V-type	68.5/8.5	The presence of hydrocolloids in the suspension during HHP processing would guarantee water availability. Higher intensities of a peak corresponding to the V-type polymorph, in comparison to the control without hydrocolloid addition. Lower concentrations of amylose released. The V-crystalline complex formation would be favored by hydrocolloid presence under pressure / So, hydrocolloids could contribute to the lixiviated amylose stabilization, leading to lower amylose contents in the supernatants of HHP treated samples.
400 MPa, 35 min, 40 °C + Guar gum	14.2%/ C + V-type	67.7/8.8	
400 MPa, 35 min, 40 °C + Xanthan gum	15.8%/ C + V-type	67.9/9.2	Gelatinization degree was lower in starch with added xanthan gum in comparison to the other hydrocolloids ($p < 0.05$) / Stabilization effect of this gum on the starch granular structure during gelatinization.
400 MPa, 120 min, 30 °C	8.3%/V-type	67.7/6.2	Increase in gelatinization degree and V-type polymorph peaks, maintaining integrity.
700 MPa, 25 min, 35 °C	2.8%/B + V-type	60.3/4.2	Highly gelatinized samples (>60%), loss of integrity/extremely drastic treatment.

10.7 Research Gaps and Scope of Future Studies

Research on HHP-modified starch future trends would be surely bond to both the developments taking place in HHP-treated food products, as well as the market and consumer requirements for starch as a main food component, not forgetting the newest consumer acceptance trends.

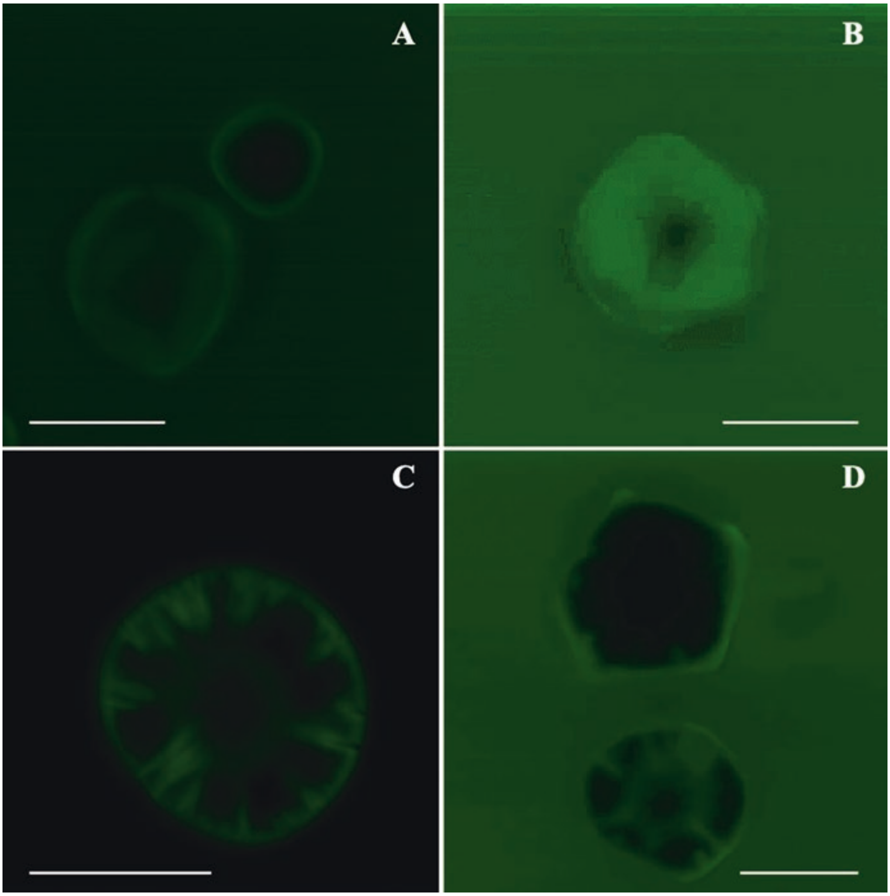


Fig. 10.9 Confocal laser scanning micrographs of corn starch granules: (**a**) Native granules (untreated), (**b**) Alkali-treated starch, (**c**) Samples treated at 400 MPa, 10% w/v starch concentration and 35 min at 40 °C, and (**d**) HHP treatment as in C + alkali. The bars correspond to 10 μm. Large and deep pores can be seen developing in pressure-treated samples (**c**, **d**), which can be of interest for incorporating binding agents (e.g., minerals, nutrients)

An always rich source of new ideas and developments lies on the study of starch biopolymer within foods, either as a natural component or as an added ingredient (with nutritional, textural or other purposes). The complexity of HHP effects on starch gets multiplied when research is focused in complex mixtures, with a limited water availability and, moreover, when thermal or other simultaneous processes (may be homogenization, blending, ultrasounds, etc.) are to be considered.

Other source for new products to be studied come from the introduction of different plants in diet, bringing associated different starch types that will have to be characterized and whose potential applications should be tuned with the more interesting technological approaches. Starch can be used also as a binder, texture creator, encapsulating agent and for many other structural derived purposes.

Combinations of the already known products and their properties with novel ones, would multiply the richness that this product can endow to food production.

A present trend in consumers is the search for less energy-rich food ingredients that still preserve the textural and flavor properties of traditional foods. All type of research studies on HHP starch dwelling on the generation of resistant starch, its characterization, and the progress in the knowledge of how it behaves in real nutrition, will find an immediate echo, both in industry and consumers.

Lastly, food properties that are currently carried out by proteins, especially animal origin ones, are growingly being replaced by different molecules, that could mimic meat properties in its interactions or, in a very particular case, substitute the remarkable gluten properties that give rise to the wide bread products family. To modify starch in ways in which the sought binding, elasticity, gas retaining properties of meat proteins and gluten can be substituted, is very surely to be one of the trendier food processing lines in the near future, and HHP-treated starch can have a very promising role in the achievement of this goal.

10.8 Conclusions

HHP is a chemical free tool to achieve modifications on starch. The broadened uses of this technology in food industry would counteract the drawback of the high cost of the equipment, since nowadays companies can rent their pressure equipment, increasing the accessibility of this technology for obtaining new starch-based materials. The main advantage of this treatment is the possibility of obtaining tailor-made ingredients, by modifying the operating conditions (time, pressure) and the incorporation of other ingredients during the process. Many factors have a strong influence on starch response to HPP, principally the biological origin, but also a range of treatment parameters, such as pressure levels, process temperature, treatment duration and starch to water ratio.

The differences between HHP and thermally gelatinized corn starches can be summarized in a smaller amount of amylose released from granules, a limited granule swelling and the partial preservation of granular integrity. The presence of hydrocolloids does not have a significant influence on HPP induced corn starch gelatinization, though may give rise to crystallinity changes implying a limited release of amylose.

The possibilities of achieving only a partial gelatinization degree though HHP treatment may be of practical interest, as partially gelatinized corn starch shows a mix of properties: granular identity and independence are preserved, but incipient interactions among granules and with other molecules are formed. HHP would yield more homogeneous, better-defined partially gelatinized products with a specific crystallinity degree than the thermal treatment, in an easily controllable process.

HHP treatments are a valuable resource for increasing the range of starch derived formulations for applications so different as transport of molecules of nutritional interest, controllable behavior within complex food products and gelatinization versatility.

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Lorena Deladino obtained a PhD in Exact Sciences, from the National University of La Plata (UNLP) (La Plata, Argentina, 2010). She is currently an Independent Researcher from the National Scientific and Technical Research Council Argentina (CONICET) in the Center for Research and Development in Food Cryotechnology (CIDCA) and she is Head of practical work in the course of Design of Experiments of the Exact Sciences Faculty, from UNLP.

Her research has been focused on the encapsulation of natural antioxidants extracted from yerba mate (*Ilex paraguariensis*) in calcium alginate and chitosan beads (2007–2012). Then she worked on the use of corn starch as a carrier system itself or as a component of complex encapsulation systems with a particular application in food systems. In this way, she explored the High Hydrostatic Pressure technology as a green modification of starches to improve its carrier ability as future food ingredients (2012–2017). Since 2016 she has worked on obtaining green fertilizers from yerba mate by-products. His actual research is related to the application of the mentioned industrial waste, rich in minerals and vitamins and mainly constituted from cellulose, in food systems as an integrated approach to valorization.

Aline Schneider -Teixeira obtained a PhD in Biotechnology and Genetic Resources of Plants and Associated Microorganisms, from the Polytechnic University of Madrid (UPM) (Madrid, Spain, 2013). She is currently an Adjunct Researcher from the National Scientific and Technical Research Council Argentina (CONICET) in the YPF – Technology (Y-TEC) and she is a graduate assistant in the course of Physicochemical of the Engineering Faculty, from the National University of La Plata (UNLP).

His career has always been focused on the area of science, she accumulated experience and skills with stays in various international research centers (Brazil, Portugal, Spain, the United States, the Czech Republic, South Africa and Argentina) dedicated to biophysics, multipurpose encapsulation, biopolymers, fertilizers and germplasm conservation and cryopreservation. Her main line of research aims to reveal the fundamental bases of desiccation and tolerance to stress at low temperatures in plant genetic resources. She also investigates the application of plant natural products such as fertilizers systems and in food. Her studies are multidisciplinary, and commonly involve making structural, physical and chemistry determinations.

Antonio Diego Molina-García (B.Sc. -Chemical Sciences, Complutense University of Madrid, Spain-, PhD (Physical Biochemistry, University of Nottingham, UK) is based in Madrid, Spain, at the ICTAN-CSIC (Institute of Food Science and Technology and Nutrition, within the Spanish "Consejo Superior de Investigaciones Científicas" network).

He carries out research in high hydrostatic pressure-low temperature effects on food systems, including starch and other polysaccharides, and over enzymatic reactions enhanced by pressure, within food and related contexts. He also studies ice dynamics and glassy state in cryopreservation, by DSC and cryo-SEM.

Chapter 11
Ultrasonication of Starch

Rohan Jitendra Patil, Anand Kishore, Anupama Singh, Khushbu Kumari, and Pradeep Kumar

11.1 Introduction

Starch has significant applications as a functional ingredient in product development and for making biodegradable packaging material (Sid et al., 2021). In product development, starches are used in snacks, bakery items, cereals, pasta, bars, confectionaries, dairy products, etc. Furthermore, Starch is non-plastic in its natural condition due to hydrogen bond-induced crystallinity and can be transformed into a thermoplastic material with thermal-mechanical treatment. Recently, starch from bio-sourced material has been proven appropriate for bio-based polymers and nanocomposites (BeMiller & Whistler, 2009, Kishore et al. 2023). However, most starches have drawbacks in their natural states, such as low tolerance for various operating conditions, poor functional characteristics, poor solubility, limited shear stress resistance, and heat degradation and hence, several strategies have been adopted for modifying starch to overcome its limitations for a wider range of industrial application.

Physical, chemical, enzymatic, and genetic approaches are used for starch modification to achieve the desired functionality and physicochemical properties for further application. Among all treatments, physical modifications are widely used due to low cost and are recognized as safe compared to other methods. Ultrasonication is one of the emerging physical modifications for starch which minimizes the use of chemicals, reduces waste, and is eco-friendly. Ultrasonication has been used in various starch-related food and non-food applications for shorter processing time,

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R. J. Patil · A. Kishore (✉) · A. Singh · P. Kumar
National Institute of Food Technology Entrepreneurship and Management,
Kundli Sonepat, India

K. Kumari
National Dairy Research Institute, Karnal, India

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environmental friendliness, lower energy usage, and lower production costs. It modifies starch's physicochemical and functional properties due to the cavitation process (formation, growth, and rapid collapse of bubbles) under different sonication process conditions. The applied frequency, sound wave amplitude, and treatment time majorly affect starch's physical, chemical, and biochemical effects (depolymerization of starch). This technique can also improve starch solubility, viscosity, and stability, and may have the potential for producing new and innovative starch-based products.

Different ultrasonication treatment conditions produce different effects with different operating conditions on the same starch. Vela et al. (2021) reported that when rice flour is exposed to ultrasounds at 24 kHz, functional, thermal, pasting, and rheological properties indicated substantial differences. Surface microstructure and particle size of treated samples showed physical variations, which define their interaction with water, and, as a result, changes in the pasting profile and rheological behavior were observed. The findings revealed that the flour content does not strongly influence the impact of ultrasonication during treatment, and only up to 10 min of time change could be detected; after that, there were no changes in the rice flour.

The ultrasonication process can be applied either on native starch granules suspended in solution or on gelatinized starch. Abral et al. (2018) developed the film from starch granules after ultrasonication, and another film was made by sonicating the starch gel. The data shows that the film made after treating the native starch has the highest transparency, lower water vapor permeability, highest thermal stability, and tensile strength compared to a film made from sonicated starch gel. Several studies have been conducted on the effect of ultrasonication treatment on the composition, physicochemical characteristics, and structure of starch, which is shown in Table 11.1.

Recently, starch-based biopolymer has been given attention as an eco-friendly biodegradable film alternative to synthetic plastics. Several studies reported that ultrasonication is effective in improving film quality. Abral et al. (2019) reported that ultrasonication-treated sago starch-based sample (2.5, 5, and 10 min) has a more compact structure and better moisture barrier properties than those without treated film. The duration of treatment also affects the physical properties of the film. They also found that the film of starch treated for 10 min has the highest transparency and thermal resistance. However, the five-minute duration increased the tensile strength of the film by 227%, and its moisture absorption decreased by 29.83% compared to non-treated film.

This chapter summarizes the mechanism of ultrasonication of starch and its effect on the physical, and functional properties of starch and their application in the food industry.

Table 11.1 Effect of ultrasonication treatment conditions on various properties of starch

Sr. No.	Starch	Ultrasonication treatment condition	Rheological properties	Thermal properties	Structural properties	Functional properties	References
1.	Tapioca	Ultrasound power (400 W, 24 kHz- 50% and 100% amplitude), treatment time (10,15 and 30 min).	–	–	Crystalline structure of starch granules destructed and transformed to its amorphous form at 100% amplitude and 30 min).	Solubility and swelling power values increases as comparing to native and heat-treated starch.	Manchun et al. (2012)
2.	Cereal (wheat, barley, rice and maize)	Ultrasound power (100 W, 24 kHz- (100% amplitude), treatment time (15 and 30 min)	Storage and loss modulus (G′ and G″) of cooked starch pastes increased and decreased for 15 and 30 min, respectively.	–	Pores and depressions generation on the surface of wheat and maize starch granules after ultrasonication treatment.	Increased solubility and swelling power of cereal starches. Increased rapidly digestible starch (RDS), and resistant starch (RS) of ultrasonicated starches with increased treatment time.	Kaur and Gill (2019)
3.	Elephant foot yam	Citric acid (CA) + ultrasound (US) modification Ultrasound treatment for 10 min at 25 °C.	Reduced the pasting properties of starch except pasting temperature and pasting viscosity.	Decreased gelatinization temperature for modified starch as compared to native.	CA + US treatment broke the granules and caused pores and cracks.	Increased oil absorption and water absorption capacity for ultrasound along with CA-treated starches.	Singh and Sharanagat (2020)

(continued)

Table 11.1 (continued)

Sr. No.	Starch	Ultrasonication treatment condition	Rheological properties	Thermal properties	Structural properties	Functional properties	References
4.	Oat	Horn sonicator Ultrasound power (150, 250, and 350 W, 20 kHz- at intensities 39, 48, 63 W/cm^2), (10 and 20 min).	High ultrasound intensity decreased the paste viscosity of the starch.	Increased gelatinization, onset and peak temperatures.	At high sonication intensity size of starch granules is reduced. Generation of pores and cracks on the surface of horn sonicator-treated starch granules. Decreased degree of crystallinity.	Enhanced solubility, swelling power, water and lipid holding capacity, Increased amylose content, and transmittance, whereas reduced gel hardness.	Falsafi et al. (2019)
5.	Sweet potato	Ultrasound power (300 W, 20 kHz), treatment time (15, 20, 25, and 30 min),	Decreased peak viscosity, final and breakdown viscosity of gelatinized starch.	Decreased pasting temperature.	Pores and cracks formed on the surface of starch granules with increasing treatment time. Decreased relative crystallinity values of ultrasound-modified starches compared to native starches.	Increased solubility, swelling power, and amylose content of modified starch.	Wang et al. (2020a, b, c)
6.	Corn	(a) Treatment-U1 (420W, 40 min and 50 °C) (b) Treatment-U2 (480 W, 30 min, 40 °C) (c) Treatment-U3 (540 W, 20 min, 60 °C)	Pasting parameters decreased with ultrasound treatment, especially for U2 treatment.	The gelatinization temperature and enthalpy of modified starch decreased as compared to native starches.	Reduced relative crystallinity of modified starch Pores and a notch formed on the surface of starch granules.	Increased starch hydrolysis due to increased rate constant of enzymolysis reaction.	Li et al. (2018)

7.	Rice	Frequency (22 kHz), ultrasound power (150, 300, 450, 600 W) for 20 min.	Peak and breakdown viscosity increased, Peak time decreased.	Gelatinization enthalpy and the pasting temperature were decreased.	Amorphous areas of starch granules were slightly get destructed by ultrasound treatment. Cracks and pores formed on the starch granule surface with less effect on granular size.	–	Yang et al. (2019a)
8.	Cassava	Amplitude (60% and 80%), treatment time (10, 20, 30 and 40 min), temperature (20 ± 1 °C).	Higher paste clarity compared to native starch. Decreased paste viscosity.	–	–	Increased amylose content, solubility and swelling power of modified starches.	Satmalawati et al. (2020)
9.	Potato	Ultrasound power (60, 105, 155 W), frequency (20 kHz), treatment time (30 min).	–	–	Groove and notch formed on surface of starch granule.	In an excess water, amylose region absorbed more water than amylopectin lamella.	Zhu et al. (2012)
10.	Wheat	Frequency (30 kHz), treatment time (15 and 30 min).	–	–	Increased relative crystallinity, showing a higher percentage increase for 15 min than 30 min ultrasonication. A-type starch granules increased with 15 and 30 min treatment, but B-type starch granules reduced after 30 min treatment.	Increased oil absorption capacity, swelling power, and amylose content of ultrasonication-treated starch.	Karwasra et al. (2020)

11.2 Mechanism of Ultrasonication of Starch

Ultrasonic treatment of starch is generally done using an ultrasonic bath and probe system. In most reported procedures, an ultrasonic probe system is used for the treatment of starch, which is equipped with a booster horn (probe) in varying lengths and diameters. The probe tip diameter dictates the amount of sample that can be effectively processed. The probe receives energy from the transducer and resonates at a specified frequency, transmitting ultrasonic energy to the sample. During the treatment, the starch can be placed in a beaker with distilled or deionized water in specific ratios and immersed in the ultrasonic bath with enough water to stabilize the beaker. During sonication, the temperature increases, which can be controlled by adding ice to the bath system.

Cavitation and radical attack are the two primary mechanisms governing the changes in the characteristics of starch during the ultrasonication process. The cavitation process comprised the formation, growth, and rapid collapse of bubbles. The generation of gas bubbles starts first, followed by producing intense microjets with velocities higher than 100 m/s and a shockwave that causes the particles to collide. The rapid collapse of each microbubble generates energy to increase high pressure (1000 atm) and temperature (4000 K). Moreover, the shear forces arise due to rapidly collapsing bubbles and disrupt the polymer chains that cause the depolymerization of starch (breakage of bonds and formation of long chain radicals). Second, a radical attack occurs when water is partially decomposed into hydroxide (–OH) and hydrogen (H^+) radicals, causing starch polymer breakdown.

Ultrasonication of starch involves the application of a mechanical wave with a frequency greater than 16 kHz. Low-intensity and high-intensity ultrasounds are the two types applied to food materials. At a frequency of 100 kHz-1 MHz, the power used with low-intensity ultrasound is generally less than 1 W cm^{-2}, which does not affect the material structure. On the other hand, high-intensity ultrasound has a power level of 10–1000 W cm^{-2} and operates at 20–100 kHz frequency. Low-frequency and high-intensity ultrasound with sufficient energy can cause physical damage or encourage chemical reactions like emulsion formation, crystallization control, and oxidation reactions (Mi et al., 2021).

Ultrasonication is also used to produce starch nanoparticles (SNPs) for various applications in the food industry and has recently become a topic of increasing interest in academic research and industry. The mechanism to produce SNPs from starch granules by the high-intensity ultrasonication by cavitation process with a sufficient amount of water. Energy is transferred to the starch particles via acoustic cavitation during the ultrasound treatment. As a result, the starch granules progressively disintegrated into their most minor building materials and released the globular blocklet structure. These blocklets were broken again under the combined effects of ultrasonication and water and released amylopectin chains that reorganized into 2D nanoparticles. A necessary amount of water in the solvent is required to form SNPs from starch granules because water molecules assist disintegration by weakening internal cohesion through hydrogen bonding. The ultrasonic energy reduces the blocks' cohesiveness and enables easy separation from the starch grains.

The amylopectin chains are then released from the block structure as small NPs. Due to the increasing collapse of the hierarchical structure caused by strong ultrasound treatment, amylose was also leached out and distributed randomly throughout the radial amylopectin chains. Boufi et al. (2018) reported the production of starch nanoparticles using ultrasonication treatment. The results showed that after 75 min of ultrasonication at 25 °C, starch granules were completely converted from micron to nanoscale. Also, with the increasing ultrasonication time, there is a decrease in particle size; the particle size was obtained to be 40 nm after giving ultrasonication treatment for 57 min.

The ultrasonication process is influenced by sonication conditions (frequency, power), treatment time, temperature, and starch properties (viscosity, surface tension). The ambient conditions like temperature and pressure also affect the modification in properties of starch. Kunyanee et al. (2020) reported that after ultrasound treatment, low temperature contributed to the rearrangement of starch molecules and caused a decrease in the glycemic index of rice.

11.3 Effect of Ultrasonication on Starch Properties

Ultrasonication is a non-thermal treatment that enhances the physicochemical characteristics of starches derived from various botanical sources. The ultrasonication treatment is mainly used for starch modification because it does not involve potentially dangerous chemicals or biological agents. This section will describe the effect of ultrasonication in improving starch during modification and the changes in the amylose content, granule morphology, particle size distribution, structure, swelling, and pasting properties of the starches. The effect on ultrasonication on the various properties of starch is shown in Table 11.1.

11.3.1 Amylose and Amylopectin Content

Starch is a heterogeneous material that has both linear and branching microstructures. Amylose is a linear molecule, whereas amylopectin is a highly branched molecule. Amylopectin makes up 75 to 80% of starch, while amylose makes up 20 to 25%. Amylose may be essential in determining starch's physicochemical characteristics and use. Higher amylose content is required to make starch-based films because amylose content contributes to film strength. At the same time, amylopectin's branching structure often results in a film with poor mechanical qualities.

Amylose and amylopectin are fundamentally incompatible molecules, with amylose having a lower molecular weight and a longer form than amylopectin, which contains large yet compact molecules. The presence of amylose reduces the crystallinity of amylopectin and affects the ease with which water can penetrate the granules (Dabhi and Hirpara, 2021).

Ultrasonication treatment enhances the amylose content of potato, maize, sago, mung bean, and pea starches may be due to partial depolymerization of amylopectin chains by the ultrasound treatment, which enlarged the number of linear fragments, increasing the amylose content of starch granules (Han et al., 2021). Wang et al. (2020c) studied the effect of ultrasonic treatment on sweet potato starch, where ultrasonication was performed at a power of 300 W and frequency of 20 kHz for varied times (15, 20, 25, and 30 min). The amylose content (AC) of treated sweet potato starch with ultrasonication was determined using the iodine-binding colorimetric method. The research found that ultrasonicated starches had a greater AC than native starches, which became more evident as the sonication time was increased from 15 to 30 min. The results showed that the amylose content of starch increased after ultrasonication, indicating that the ultrasound treatment contributed to the stronger linear fractions through molecular scission of chains and depolymerization of amylopectin. Ultrasound may help boost the solubility and swelling capacity of starch granules. The amylose content of native and ultrasound-treated starch enhanced from 25.1 to 28.75%.

Similarly, Satmalawati et al. (2020) studied cassava starch modification using ultrasonication treatment. Variations in amplitude (60 and 80%) were used in the ultrasonication process on the 5% (w/v) of starch suspension at four different time intervals (10, 20, 30, and 40 min) and a constant temperature of 20 ± 1 °C. The starch suspension was precipitated and dried for 24 hours after the ultrasonication. The observations showed that the hydrolysis of the glycosidic bond caused depolymerization during the starch oxidation process. The increased level of amylose was caused by the cleavage of the branches of amylopectin into a linear structure and read as amylose. The ultrasonic technique had a considerable impact on the amounts of starch amylose. The hydrolysis of starch led to the depolymerization of amylopectin and amylose molecules, resulting in the production of smaller-size chains. According to the findings, the duration of the ultrasonication treatment had a substantial impact on the amylose content. At 60% amplitude and after 40 min of ultrasonication treatment on the cassava starch, the amylose content observed was 44.13 ± 0.28. Similarly, at 80% amplitude and after 40 min of ultrasonication treatment on the cassava starch, the amylose content observed was 41.23 ± 0.25. The ultrasonic procedure would induce microbubbles and cavities in the solution. The spreading of microbubbles releases much energy, turning into high temperatures and pressures, causing polymer breakdown. Cavitation occurs when sound waves move through a solution and cause the disintegration of micro-sized bubbles (microbubbles). The cavitation process generates enough energy to disrupt covalent bonds in polymeric materials. Several studies concluded that the duration of exposure to ultrasonication on the starch significantly affects the increased amylose content.

11.3.2 Particle Size Distribution

Ultrasonication treatment time significantly increases the particle size of the starch granules. (Wang et al., 2020c) reported an increase in the starch granule size after the ultrasonic treatment on the sweet potato starch, which indicated that the starch granule tended to move towards a larger particle size than the native sample with the increasing sonication time. The significant increase in the particle size of starch granules is due to the superficial adhesiveness between granules or, the re-aggregation of starch granules. Similar results were reported by Kaur and Gill (2019) on the ultrasonication treatment of the starches obtained from different cereals. After the ultrasound treatment on wheat, corn, rice, and barley starch, the increased size of starch granules was observed due to ultrasonic cavitation. Mechanical activity increased the starch's granular structure by generating changes in its physical geometry. The barley starch granules (28 μm) were found to be greater in size, followed by corn (21.7 μm), wheat (19.27 μm), and rice (15.4 μm) starch granules, respectively. Results indicated that surface and microstructural alterations in the physical geometry of granules were generated by ultrasonication, with no effect on the overall integrity of starch granules. The increase in the size of starch granules reported was because the large starch granules were broken into tiny particles by ultrasonication, resulting in small starch fragments re-aggregating into new granules or adhering to the surface of starch granules.

A decrease in the particle size has been observed in the study reported by (Karwasra et al., 2020) on the ultrasonication of Indian wheat cultivar starches. The size distribution of starch granules was categorized into three categories: A-(>15.5 μm), B - (5.5–15.5 μm), and C -type granules (5.5 μm). A-type granules contributed the most to the total percent volume among all starches (ultrasonicated and native), followed by B-granules and C-granules. After ultrasonication treatment for 15 and 30 minutes, the percent volume of A-type granules increased. However, after 30 minutes of ultrasonication treatment, the percent volume of B-type and C-type granules decreased. The intensity and frequency of the ultrasound treatment affected the particle size distribution. SEM results revealed that larger-size granules were affected by the ultrasonic treatment, increasing the percent volume because ultrasonic waves loosen the compactly bound crystalline and amorphous lamellar structures, and some granules that were previously in the B-type size pattern transferred to A-type sizes. The differences in particle size percentage volumes of various cultivar starches were caused by this. Ultrasound changes the physicochemical properties of starch by altering the size of starch granules (decreases their size).

11.3.3 Granule Morphology

In nature, starch granules have different sizes and shapes. Spherical, oval, polygonal, lenticular (disc), kidney, and elongated are all common shapes of starch granules (BeMiller & Whistler, 2009). Starch's structural changes can be caused by various physical, chemical, and enzymatic processes discovered through microscopic analysis. Scanning Electron Microscopy (SEM) examines the microstructure of untreated and ultrasonically treated starch samples. Various studies have shown that ultrasonication affects the size, shape, and birefringence of starch granules. Raghunathan et al. (2021) investigate the application of ultrasonication for the modification of cereal starches and their effect on their properties. It was reported that cereal native starch granules had an irregular form with smooth surfaces and without any cracks. The ultrasound treatment caused low-intensity cracks and holes in the granule surface. This impact was particularly noticeable in big grains. The level of mechanical damage increased as ultrasonic power raised due to rapid bubble formation and collapse during cavitation, resulting in the scrapping of the outer layer from the periphery.

Similarly, Yang et al. (2019b) provided a more detailed explanation of the morphological response of waxy corn starch to ultrasound treatment. The researchers found that the power of ultrasonication treatment affects the degree of alteration in the morphological structure of waxy corn starch. The morphologies of native and ultrasonicated waxy corn starches are examined using SEM to understand the better effect of different ultrasound powers on waxy corn starch. Waxy corn starch in its native form (wcs-ns) was a mixture of rounded and angular granules with small irregular fragments. In waxy corn starch treated with ultrasonication at 100 W (wcs-u100) and waxy corn starch treated with ultrasonication at 400 W (wcs-u400) granule surfaces, some notches, and grooves are observed. Some starch granules were significantly damaged after ultrasound irradiation, and cracks and grooves developed on the surface of wcs-u100 and wcs-u400. The stronger shear stresses from bubble collision enhanced the OH radical concentration of the waxy corn starch solution as the ultrasonic power raised. The explanation for this was that the changes caused waxy corn starch granules to be damaged due to the rapid bubble creation and collapse. The energy acquired from ultrasonic waves and the transitions between bubble production and collapse could also be responsible for the disturbance of the outer granule layers.

Along with the power of ultrasonication, treatment time also affects the morphology of the starch granules. Rahaman et al. (2021) reported ultrasound-based modification of cassava and corn starch. Untreated cassava granules had a spherical, oval, or oval-truncated shape, but untreated corn granules had a slick, compact surface with a solid ball and polygon morphology. SEM analysis showed no cracks; a smooth granule surface was observed for both the untreated starch samples. The ultrasonic treatment did not affect the size of starch granules, but roughness on the surface of the granules was observed, which increased with treatment time (Fig. 11.1). After 10 and 20 min of ultrasonic treatment, the groove and notch occurred on the surface of starch granules. The observed structural changes can be

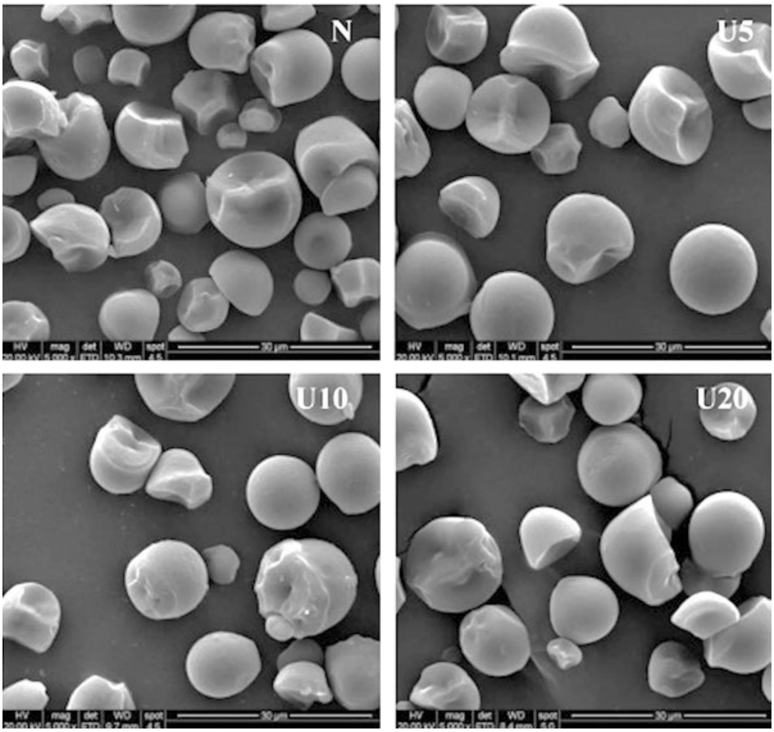

Fig. 11.1 Effect of ultrasound treatment time on the microscopic structure of native cassava starch granules (N), and samples treated with ultrasound for 5, 10, and 20 min (U5, U10, U20). (Adopted from Monroy et al. (2018) with permission from Elsevier)

produced due to shock waves that generate frictional/shear force. The development of localized hot spots caused by bubble collapsing and cavitation leads to high-pressure gradients in the surrounding area. Frictional/shear force is sufficient to destroy the polymers, resulting in depressions and small cracks on the granules' surface. Based on the results, the researcher concluded that with the increasing ultrasonication treatment time, the structural changes in the surface of the cassava and corn starch were observed based on SEM results. However, in their granule size, there was no significant change.

11.3.4 Functional Properties

11.3.4.1 Swelling Power and Solubility

Swelling power is related to the water holding capacity of starch and at a given temperature and the amount of starch molecule that solubilizes is known as solubility (Alcázar-Alay & Meireles, 2015). Swelling power and solubility indicate the degree of starch chain interaction in amorphous and crystalline areas. The gelatinized temperature, amylose/amylopectin ratio, intermolecular interactions between their chains, the aggregation structure of starch granules, protein, lipid, and phosphorus content have a significant role in the variation of swelling power (SP) and solubility (S) (Oyeyinka et al., 2021).

Satmalawati et al. (2020) reported that ultrasonicated treated cassava starch has a higher solubility and swelling power than native starches and concluded that the sonication process changes the starch granule structure and physical geometry of pores, which allows water to penetrate the large volume and hence solubility increases. The data shows that the solubility increased as the amplitude (60–80% and treatment time (10, 20, 30, 40 min) increased as it increased the mobility of amylose. However, the amplitude and treatment time do not significantly affect swelling power. Ultrasonication significantly increased the swelling power of wheat, barley, rice, maize, and sweet potato starch as compared to native starch (Kaur et al., 2018; Wang et al., 2020c). However, microwave heating of starch generally reduced the starch swelling due to the rearrangement of crystalline regions within the starch granules (Oyeyinka et al., 2021). In the ultrasonication process, observations revealed that the hydrogen bonds were destroyed when the starch slurry was heated above the gelatinization temperature. This allowed water molecules to hydrate with free hydroxyl groups, resulting in a swollen volume of starch granules. Moreover, Physical damage to starch granules, disruption of crystalline structure, and disassociation of short-range ordered structure and ordered molecular structure allowed for easy hydrogen bridge bonding between water molecules and free hydroxyl groups of starch polymer chains, responsible for high swelling power and enhancing water ingress into the amorphous and crystalline areas of starch granules. And the results of the solubility of starches showed that ultrasonic treatment at various treatment times increased amylose chain mobility and improved their hydration performance with water molecules, facilitating amylose dissolution. It has been reported that the higher solubility caused by ultrasonication has been attributed to an increase in amylose content, improved fragmentation of shorter branched chains, and the formation of pores and cracks on the surface (Wang et al., 2020c).

11.3.4.2 Oil Absorption Capacity (OAC)

The amount of oil absorbed or entrapment within the material is the oil absorption capacity (OAC). Oil absorption capacity is the essential functional property of food (Wang et al., 2020a). There is also a need to know about the oil absorption capacity for starch because various products can be manufactured using those starches. The ultrasonication treatment time and power significantly affect the oil absorption capacity of the starches. Karwasra et al., 2020 reported that after the ultrasonication treatment for 15 and 30 min, the oil absorption capacity significantly increased, and values ranged from 1.13–1.55 g/g and 1.36–1.73 g/g, respectively. One of the wheat grains samples (PBW-343) showed greater values of oil absorption capacity after ultrasonication treatment for 30 min. Similarly, after ultrasonication treatment, oil absorption capacity values were increased in corn, rice, and potato starches (Sujka et al., 2013). Physical entrapment of oil inside the starch structure due to the lack of non-polar sites in starch, compared to proteins, is the leading cause of oil absorption capacity in starch (Oneh Abu et al., 2006).

11.3.4.3 Hydration Properties

The starch's hydration properties are the starch's ability to absorb water. High hydration enhances the viscosity, gelatinization ability, and swelling of starch granules (Cornejo-Ramírez et al., 2018). Vela et al. (2021) reported a slight enhancement in the rice flour's water absorption capacity (WAC) when ultrasound treatment was given for 2 and 5 min, and significantly decreased at a longer exposure time. The hydrophilic parts primarily determine water absorption capacity in proteins and carbohydrates. Ultrasound treatment can cause proteins to dissociate and change their secondary structure, affecting their water-binding sites. Water absorption index (WAI) and swelling power (SP) showed a strong enhancement in the treated samples after 40 and 60 min of treatment with ultrasonication, 133% higher than the native rice flour sample. The lowest concentration of rice flour sonication was adequate, and the SP and WAI were 138% greater than the native samples. It was figured that the enhanced surface area of starch granules in treated flours (due to the accumulation of smaller particles and increased exposed area), as well as the dissociation of proteins, which increases their water binding sites, facilitates water interaction, resulting in greater water uptake and retention. The water solubility index (WSI) of every treated sample decreased due to the modification of the amylose/amylopectin ratio after sonication. Amylose content has a negative relationship with solubility. The gel structure becomes more compact with a higher amylose content, making it more difficult for soluble substances to overflow outside the granules, resulting in low solubility values (Vela et al., 2021).

11.3.5 Pasting Properties

After gelatinization, changes in the starch structure are referred to as the pasting of starch. It is one of the most important indicators of structural and functional properties of starch, which includes peak, setback, trough, final, breakdown viscosities, and pasting temperature. In general, peak, breakdown, and setback viscosities and pasting temperature were significantly reduced by ultrasound treatment (Vela et al., 2021). However, the sonication power, processing time, and starch types affect the pasting properties of starch. When ultrasound treatment was given to the waxy corn starches, the peak viscosity obtained for waxy corn starch treated by ultrasonication at 100 W and waxy corn starch treated by ultrasonication at 400 W reduced by 98.67 BU and 178.00 BU, respectively from native starch (Yang et al., 2019b). The explanation was that during ultrasonic irradiation, the long chains in waxy corn starch were scissored into multiple short chains caused by strong physical forces (shear pressures, shock waves, etc.) and OH free radicals. The peak viscosity (PV) was caused by starch granule swelling, which weakened the internal core network of starch granules. Lower PV values are usually related to an increased ratio of amylose to amylopectin in starch and smaller granule sizes (Vela et al., 2021). The setback viscosity indicates the retrogradation capacity of starch and the lower SV indicates a lower amylose retrogradation (Wang et al., 2020b). The setback viscosity (SV) values decreased when the rice starch was exposed to low-intensity ultrasound treatment, which was caused due to depolymerization and degradation of leached long-chained amylopectin and amylose (Yang et al., 2019a). Thus, the intensity has shown a significant effect on the viscosity properties of the starch materials. Moreover, the setback values of waxy corn starch treated by ultrasonication at 100 W, and waxy corn starch treated by ultrasonication at 400 W were smaller than those of native waxy corn starch (109.33 BU) and were 100.00 BU and 88.67 BU, respectively. So, it concluded that ultrasound irradiation might slow short-term retrogradation, as evidenced by a decrease in setback viscosity in ultrasonicated waxy maize starch. Furthermore, Li et al. (2018) also reported that the ultrasonication treatment of the corn starch for 30 min at 480 W power resulted in a decrease in peak, trough, final, setback, and breakdown viscosities, which were decreased by 12.7%, 10.5%, 14.2%, 20.5%, and 20.8% respectively compared with that of the native starch. The loss of granule integrity and stiffness caused by glycosidic link cleavage resulted in a drop in peak viscosity after irradiation. Ultrasound can break starch's macromolecular chains and disrupt its crystalline structure, lowering the viscosity resistance of the paste. The lower breakdown viscosity of treated starches resulted from a lower peak viscosity, indicating that they are more resistant to shear-thinning during heating. The polymerization of leached-out amylose and long linear amylopectin causes the final and setback viscosities. Final viscosity and setback viscosity reduced insignificantly after sonication compared to the native starch. The drop was attributed to a reduction in paste viscosity caused by a decrease in the degree of polymerization of treated starch due to starch chain breakage. However, Yang et al. (2019a) reported an increase in peak viscosity value in ultrasound-treated rice starch than the native sample of rice starch. The conclusion is that ultrasound treatment influences viscosity properties differently, which varies for the type of starch material. Also, ultrasound treatment made the starch granules more susceptible to disintegration, indicating higher resistance to high shearing force and temperature, and also showed the increased value of the setback viscosity. In the report of Yang et al. (2019b), pasting properties between ultrasonicated and native waxy corn starches were observed. The observations showed that the native waxy corn starch had the lowest pasting temperature (67.43 °C). In contrast, waxy corn starch treated with ultrasonication at 100 W had 67.86 °C, and waxy corn starch treated at 400 W had 68.00 °C pasting temperatures, respectively. Ultrasonicated waxy corn starch showed excellent resistance to swelling, as seen by its higher pasting temperatures. However, Wang et al. (2019) reported that ultrasonication slightly reduced the pasting temperature (PT) of sweet potato starch. The low pasting temperature indicates low resistance to swelling and rupture. The drop in pasting temperature could be due to starch breakdown, particularly amylopectin, leading to lower swelling resistance.

11.3.6 X-Ray Diffraction and Relative Crystallinity

The X-ray diffraction (XRD) technique is a helpful technique for determining changes in starch structure. Using XRD has proven that starch has four types of crystalline patterns (A, B, C, or V polymorphs). The packing arrangement of double helices within amylopectin and the hydration level distinguishes between the A and B crystalline forms. Because the double helices are closely packed in the A-type, it is less hydrated than the helical core of the B-type. Most studies on the ultrasonication of starch revealed that the crystalline pattern remained unchanged after the ultrasound treatment (Yang et al., 2019a). Vela et al. (2021) studied ultrasonication of rice flour and showed a typical A-type X-ray pattern for all samples of cereal starches with distinct peaks at 2-theta of 15°, 17°, 18°, 23°, and 26°; the reflection at 20° is also observed, which is usually associated with V-crystallinity. Although ultrasonication had no significant effect on rice flour crystallinity, samples treated ultrasonically for 2 min had a lower value than the control sample. The crystallinity remained unchanged when rice starch was sonicated at 24 kHz for 15 and 30 min. When sonicated at 150 W, the results showed a reduction in the crystallinity, which indicated it was because of damage to the amorphous region, not the crystalline region. However, amorphous regions are more susceptible to destruction by ultrasonication treatment. The ultrasonication effect on the crystallinity of starches to promote decrease, increase, or no change mainly relies on the type of starch, experimental conditions, and the specific arrangement of amorphous and crystalline parts in the starch granule which are responsible for the attack by the ultrasounds.

Most studies on the ultrasonication of starch have shown no significant changes in the crystallinity pattern of the starch samples; however, ultrasonication combined with the other treatment technique has shown changes in the crystallinity patterns. The study reported by Han et al. (2021) has shown the effect on the crystallinity pattern of the pea starch samples when heat moisture treatment is assisted by ultrasonication. High moisture treatment for 4 h or more and two kinds of dual ultrasonication and heat moisture modification changed the pea starch's crystalline structure from type C to type A. The relative crystallinity pattern of pea starch granules decreased more when pretreated by ultrasonication than the starch samples, which were singly treated by the heat moisture treatment and post-treatment of ultrasonication showed an increase in the crystallinity. This indicates that the ultrasonication treatment and any other treatment significantly affect the crystalline properties of the starch samples.

11.3.7 Thermal Properties

When heat is applied to a material, thermal properties are related to a material-dependent response. Examples of this response are a temperature rise, a phase transition, a change in length or volume, the starting of a chemical reaction, or a change in some other chemical or physical quantity. The transfer of energy to or from a sample undergoing a chemical or physical change is commonly measured by differential scanning calorimetry (DSC). In this process, the mixture of starch and water mixture is heated in a tightly sealed aluminum pan and measures, onset (T_o), peak (T_p), conclusion gelatinization temperature (T_c), and enthalpy change (ΔH) (Zhu, 2015). The gelatinization temperature of starch is affected by several factors like sonication power, treatment time, the chain length distribution of amylose and amylopectin, degree of crystallinity, and starch structure. Rahaman et al. (2021) studied the thermal properties of native and sonicated cassava and corn starches. Native corn starch had higher values of T_o, T_p, and T_c than native cassava starch. It could be because corn starch has a high degree of crystallinity than cassava starch, which gives the granules more thermal stability against gelatinization. Corn starch has a higher degree of crystallinity, which results in a higher gelatinization temperature. Results showed that sonication treatment did not affect the cassava and corn starch's gelatinization temperatures compared to native samples. Moreover, after 10 minutes of sonication, the enthalpy value of cassava and corn starch was less than that of their native starches, indicating that the treatment generates a structural disparity in the crystalline area of the granules, which is caused by hydration of the amorphous regions. However, after 20 min of treatment, cassava and corn starch's enthalpy value increased because breaking intermolecular connections in starch molecules required more energy. The enthalpy values show that starch constituent organization is essential in gelatinizing starches modified by ultrasound treatment.

Ultrasound irradiation treatment on the waxy corn starch reported by Yang et al. (2019b), showed that the application of high ultrasound power of 400 W on the waxy corn starch showed a significant increase in the values of T_o, T_p, and end temperature (T_e) as compared to the native waxy corn starch sample. The T_o, T_p, and T_e increase because ultrasound irradiation disrupted weak crystalline structures inside waxy corn starch granules. Hence, ultrasonicated waxy corn starch needed a higher gelatinization temperature to dissociate the strong crystalline structure. The gelatinization enthalpy (ΔH) of waxy corn starch was less than that of the native waxy corn starch sample. The ΔH value of waxy corn starch is positively associated with amylopectin branch-chain length, with longer chain lengths displaying higher gelatinization enthalpy. The decreased value of ΔH showed that, after ultrasound irradiation, double helices of waxy corn starch were wounded, and some of the external chains were destroyed. Furthermore, studies of ultrasonication treatment on corn starch were studied by Li et al. (2018), who reported a decrease in the enthalpy and gelatinization temperatures (T_o, T_p, and T_c). Usually, gelatinization temperature and enthalpy are higher in starches with greater crystallinity and longer amylopectin branch chains; in this case, gelatinization temperatures and enthalpy decrease due to the absence of long-range crystallites and less amylopectin content. Lower gelatinization temperatures imply that these starches need less heat to break the bonds formed between crystalline amylopectin chains and freely moving amylose molecules. Furthermore, it reported that the drop in enthalpy was mostly due to the breakage of the double helices instead of the longer-range disruption of crystallinity. The ultrasonic treatment causes some starch to lose its double helix structure, which aids in gelatinization and liquefaction.

11.3.8 Digestibility

In humans, starch digestion occurs in the intestinal tract, within which salivary and pancreatic α-amylases break down starch molecules such as maltotriose, maltose, and other branched oligosaccharides known as α-limit dextrins, which are then transformed into glucose in the small intestine. To analyze the nutritional quality, the digestibility of starch has been interconnected to the glycemic index (GI) (Magallanes-Cruz et al., 2017). Strong shear forces can depolymerize starch chains and break granule crystallinity during ultrasonication. Furthermore, ultrasonication caused pores to develop in granules of starch and the double-helix structure to be disrupted, resulting in increased enzyme access at the digestive enzyme-sensitive locations. Changes in starch molecular architecture impact starch digestibility (Bonto et al., 2021). According to the rate of glucose release and absorption in the gastrointestinal tracts, starch can be classified as rapidly digestible starch (RDS), resistant starch (RS), and slowly digestible starch (SDS). RDS is the portion of starch that induces a rapid increase in blood sugar (glucose) after ingestion, whereas SDS is the fraction that is completely digested in the intestinal tract at a slower rate than RDS. RS cannot be digested in the small intestine but is fermented in the large intestine by microbes to form short-chain fatty acids. Starch botanical sources, the molecular structure of amylopectin, amylose chain length, degree of crystallinity, and amylose content are the various factors that affect the digestibility of starch (Oyeyinka et al., 2021).

Starch digestibility can also be significantly affected by the type of modification treatment. Ultrasonication treatment time reportedly affected the contents of rapidly digestible starch, resistant starch, and slowly digestible starch in native and gelatinized corn starch. After 16 min of sonication treatment (24 kHz), the resistant starch content of native starch rose from 4.7 to 6.2%, while rapidly digestible starch and slowly digestible starch contents remained unchanged with sonication time. The increase in resistant starch content is because XRD analysis revealed an increase in long-range crystallinity, supported by the rise in gelatinization enthalpy. The hydrolysis rate of amylolytic enzymes is limited because of the compressed rearrangement of starch's-double-helix structure, leading to slower disruption of amylopectin chains. The increased crystallinity content also reflects improved rearrangement of the intrinsic granule structure caused by ultrasound waves.

Crystalline regions are more tightly ordered than amorphous regions, making them less vulnerable to amylolytic enzyme attacks. In the scenario of gelatinized starch sonicated for the same time (16 min), resistant starch increased from 2.1 to 3.0%, a percentage increase similar to that of sonicated native starch. In contrast, rapidly digestible starch content increased from 44.2 to 60.0% and slowly digestible starch content decreased by approximately 68%. Increased ultrasound energy treatment on starch dispersions resulted in many short-chained molecules more susceptible to enzymatic degradation. Moreover, the short-range crystallinity index suggests the breakage of double-helix structures dispersed in the gelatinized starch dispersion, contributing to enzyme accessibility to amylolytic attack sites (Flores-Silva et al., 2017). The ultrasonication treatment reportedly increased the rate of digestibility in the gelatinized starch as compared to native at the same treatment time. A similar effect has been reported by Kaur & Gill, (2019) on cereal starches. The increase in ultrasonication time from 15 to 30 min along with 24 kHz frequency and 100 W power, caused the increase in rapidly digestible starch and resistant starch, whereas the content of slowly digestible starch was obtained to be decreased. Furthermore, Ultrasonicated rice showed a higher glycemic index than control rice, which is associated with increased enzymatic activity in the degraded starch crystalline region. Increased digestion rate was observed in ultrasonicated rice. These findings imply that ultrasonicated rice crystallinity and digestibility may vary depending on ultrasonic parameters and rice variety (Bonto et al., 2021).

11.4 Applications of Ultrasonicated Starch

Many studies have revealed that the starches modified with ultrasonication have enormous potential for various food products and starch-based biodegradable packaging applications. The application of ultrasonicated modified starch is highlighted below-

1. After modification, a reduction in setback viscosity may be used in reducing staling tendencies in bread and other food products.
2. Ultrasonication modification improves the thermal properties of starches, so they could be helpful in high-temperature applications-based processed products.
3. The ultrasonic treatment enhances the film's strength due to the uniformly distributed materials in the matrix (Mi et al., 2021). Ultrasonication effectively lowers the size of biopolymer particles (starch molecules) and enables their dispersion in the matrix.
4. Edible films made of starch have some drawbacks, such as high-water penetration and poor mechanical characteristics. After starch treatment with ultrasound, the starch films produced using ultrasonicated starch showed a more cohesive structure, improving the mechanical and barrier properties (Cheng et al., 2021).
5. Coconut shell liquid smoke (CSLS)-Ginger starch films with ultrasound treatment showed better mechanical, thermal, barrier, and antibacterial properties than control films (Rahmasari & Yemiş, 2022).

11.5 Conclusions

The starch modification industry is constantly evolving, as modification processes can considerably improve the physicochemical properties and structural attributes of native starch and increase its industrial worth. Cold water solubility, viscosity, and swelling capacity upon cooking, retrogradation tendency, and the disintegration of starch structural order and consequent syneresis of systems are affected by the modification process. There are many ways of modification to alter its structure to create a functional product with the right qualities for specific industrial applications, enhancing its added value. The ultrasonication treatment is a promising starch modification technique because it does not involve potentially dangerous chemicals or biological agents. The power, frequency, temperature, and treatment time influence the ultrasonic modification process. The amorphous region of the granule is affected mainly by ultrasound, whereas the form and size of the granule are preserved. The starch surface becomes porous, changing attributes including the paste's swelling capacity and solubility. Ultrasonication treatment increased the amylose content and digestibility of the starches, mainly dependent on the ultrasonication duration time. The intensity and type of starch significantly affect the pasting properties of the starches, and the ultrasonication treatment also affected the crystalline properties of the starch samples. A similar effect was observed in the thermal properties and the particle size distribution of ultrasonicated starch. So, ultrasonication pretreated can be an effective way to get the desired characteristics in the starch to improve the quality of food products and the development of bio-based packaging material with improved properties. Future studies would be required to investigate their application in product development and starch-based packaging system.

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Chapter 12
Deep Freezing and Thawing Modification and Its Impact on Starch Properties

Xu Lu, Xiaoqing Ma, and Yi Lei

12.1 Introduction

Starch is a polysaccharide stored in plant cells and distributed in different parts of plants in different forms. Crops, beans, seeds, and other plants contain large amounts of starch. The raw starch is a semicrystalline granule consisting of amylose and amylopectin, wherein the amylose and the amylopectin are linear and branched structural molecules (Manners, 1989), respectively, and their branches are located toward the non-reducing end (Zhou, 2017). On the basis of the proportion of amylose, raw starch is classified into wax, ordinary, and high amylose types. Due to the hydrogen bond, amylose in plants usually assumes a left-handed helical conformation (every six glucose forms a single helical cycle) and the degree of polymerization is 300–5000 (Mua et al., 1997). Plant lipids can be embedded into the hydrophobic interior of the spiral to form a complex. The degree of molecular polymerization of amylopectin is approximately $1 \times 10^5 \sim 1 \times 10^7$, and the main side chain exhibits a cluster structure.

Xu Lu (✉)
College of Food Science, Fujian Agriculture and Forestry University, Fuzhou, China

Teagasc Food Research Centre, Food Chemistry and Technology Department, Moorepark, Fermoy, Co.Cork, Ireland

Institute of Food Science and Technology, Fujian Agriculture and Forestry University, Fuzhou, China

China-Ireland International Cooperation Center for Food Material Science and Structure Design, Fujian Agriculture and Forestry University, Fuzhou, China

X. Ma · Yi Lei
College of Food Science, Fujian Agriculture and Forestry University, Fuzhou, China

China-Ireland International Cooperation Center for Food Material Science and Structure Design, Fujian Agriculture and Forestry University, Fuzhou, China

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The starch resources in China are abundant and varied and have the characteristics of increasing food viscosity, good stability, easy to improve texture, low cost, and easy degradation (Carr, 2010). Given the current fast pace of life, frozen food has become an important product for families to purchase due to its characteristics of easy processing and good flavor. Many frozen foods, such as fruit fillings, soups, sauces, frozen batter and cream-based products, use starch as a base ingredient or additive, further improving the texture of the food (Eliasson & Gudmundsson, 1996). These products generally experience a series of temperature fluctuations after long-term storage or freezing and thawing before consumption (Wang et al., 2015), which leads to deterioration of quality through melting of ice crystal, loss of water and softening of the texture. This loss of quality is not acceptable to consumers. Which could be due to the amylose and amylopectin reassemble to form a double helix during the cooling and storage of starch paste, accelerating retrogradation. For example, the water absorption of starch granules after freezing and thawing is an important factor in determining the rheological quality of dough food. The water content is inversely proportional to the hardness of the bread. Multiple freeze-thaw cycles accelerate the formation of ice crystals in frozen starch and cause granular damage. With increasing storage time, the formation of microcrystalline amylopectin in the dough becomes more obvious, and the hardness increases (Tao et al., 2016). Therefore, starch properties have an important influence on the freeze–thaw stability and quality characteristics of starch food, among which the water retention, freeze–thaw stability, and viscoelastic properties of starch gel represent key issues affecting the quality of starch-based food (especially frozen food). Furthermore, this problem also serves as a bottleneck, restricting the development of the starch-based food processing industry. Starch-based food is prone to freeze–thaw phenomena (cryogenic freezing and thawing) due to temperature fluctuations in the process of storage, transportation, and sales, which easily cause starch granules to exhibit water loss, skin cracking, hardening, brittleness, and other deterioration phenomena, which are directly related to freeze–thaw stability. In this chapter, the relevant factors affecting the stability of starch freezing-thawing in existing research are described, providing a theoretical basis for the improvement of the quality of starch-containing foods during storage and transportation.

12.2 Freeze–Thaw Modification and Freeze–Thaw Stability of Starch

The freeze-thaw cycle has a negative effect on the structure of starch granule's, for example, changes in moisture and temperature change the starch granule crystal shape and texture characteristics (Wang et al., 2016), the specific action mechanism is shown in Fig. 12.1 (Wang et al., 2022b). First, starch granules are prone to retrogradation due to the molecular rearrangement of amylose and amylopectin

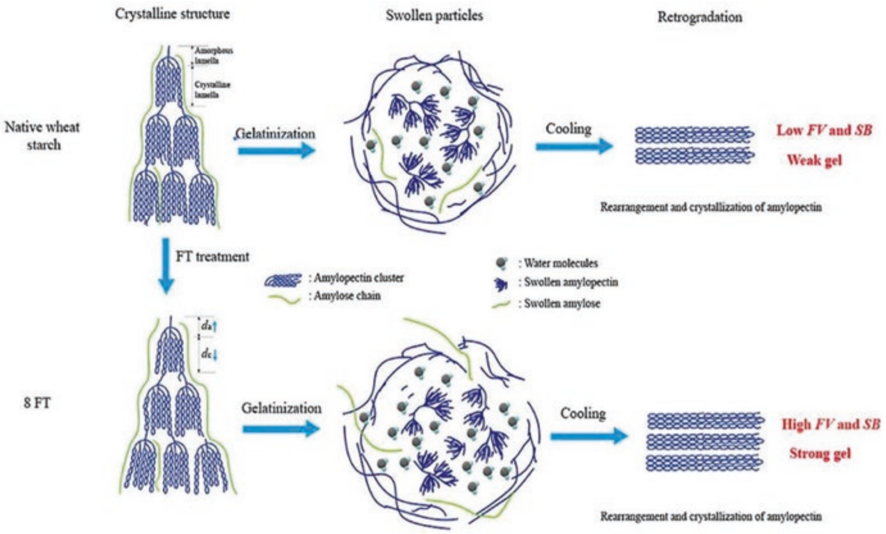

Fig. 12.1 Action mechanism of freeze-thaw treatment on starch (Wang et al., 2016)

during cooling and storage, impacting food texture. The reaggregation of amylose to form a double helix structure also accelerates retrogradation and increases the elasticity of starch glue. Amylopectin side chains also slowly form partially ordered structures (Tao, 2017). Second, repeated freeze–thaw cycles accelerate the formation of ice crystals in starch, and the mechanical force generated by ice crystals embedding into the spongy network structure exerts physical pressure on food tissues, causing damage to starch granules. The physical damage can promote the hydration and expansion of starch granules, so starch granules can spontaneously gelate. The quality of the extract increased with increasing number of damaged starch granules, resulting in branched chains of starch enriched and swollen in proportion (Tao et al., 2015b), as well as loss of water and softening of the texture, which affects the quality, sensory quality and shelf life of frozen foods.

12.3 Effect of the Freeze–Thaw Process on Starch Structure and Properties

The freeze-preservation technique is commonly used in food preservation because the storage time of the food, facilitates transportation and long-time preservation are greatly prolonged, but wich has different effects on different types of starch (Table 12.1).

Table 12.1 Effect of freeze-thaw treatment on different starch types

Starch type	Treatment conditions	Main findings					References
		XRD	Infrared	SAXS	viscosity	SEM	
Sticky rice starch	Repeated freezing and thawing				Repeated freeze-thaw cycles resulted in increased hardness and decreased viscosity of the gel.	After repeated freezing and blending, more pits appear.	Ye et al. (2018)
Wheat starch	Multiple freezing and thawing	Freeze-thaw treatment did not change the crystalline morphology of wheat starch; the relative crystallinity (RC) decreases, and the decrease of RC is more significant with the increase of F/T cycle.	The shortened molecular order of the samples treated with F/T is decreased significantly, and the decrease is more significant with the increase of F/T cycle.	With the increase of the number of freeze-thaw cycles, the repeat distance between the plate layers decreases gradually. Similarly, the thickness of the crystal (dc) sheet tends to decrease.	The freeze-thaw treatme nt of multiple cycles did not change the overall shape of the paste, but increased the paste viscosity of the starch	The freeze-thaw treatment increases the size and number of pores, and the pores, cracks or depressions on the surface are more obvious with the increase of the number of freeze-thaw cycles.	Xu et al. (2022)
Corn starch	Repeated freezing and thawing	The leach of that amylose slightly increases the relative crystallinity of the starch			The expanded particles are easily destroyed by mechanical forces resulting in viscosity destruction	Starch particles tend to aggregate into clusters, a trend that increases with FT treatment	Zhang et al. (2017)

Potato starch	Multiple freeze-thaw cycles	The leach of that amylose slightly increases the relative crystallinity of the starch			The formation of ice in the freezing process weakens the particles and promotes the expansion in the early stage of pasting, thus increasing the overall viscosity.	The surface morphology of the starch particles changes and a portion of the crystallinity is lost to form solid particles	Zhang and Lim (2020)
Sweet potato starch	Heating-freezing and thawing cycle treatment	The intensity of sweet potato starch diffraction peak is weakened after heating-freezing-thawing treatment				After heating and freeze-thaw cycling treatment, the morphology of round particles of sweet potato starch disappeared and changed into irregular block structure	Zhang et al. (2017)

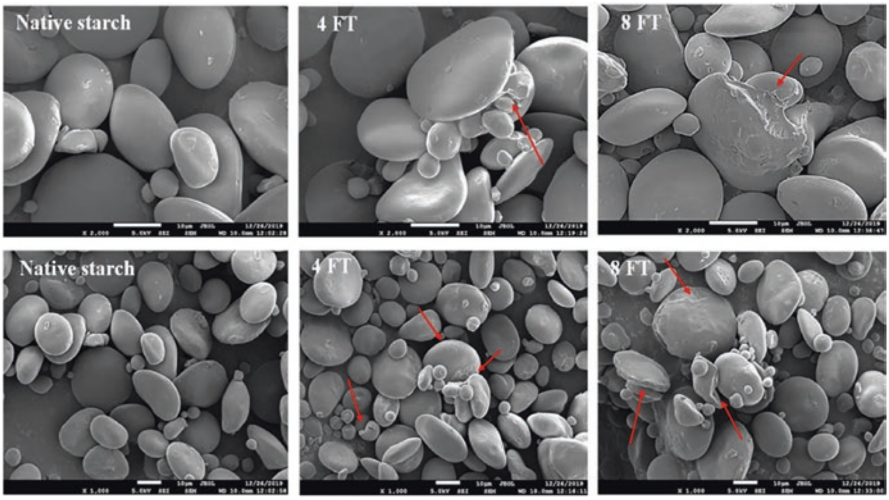

Fig. 12.2 SEM images of native and repeated freezing-thawing treated wheat starches (×1000, ×2000; scale bar, 10 μm; FT, freezing-thawing; 4, 8 FT represent the times of freezing-thawing cycles) (Wang et al., 2022b)

12.3.1 *Effect of the Freeze–Thaw Process on Starch Granules*

Freezing treatment is often used in the preservation and drying process of starch food, and the structural destruction of starch granules affects their properties at low temperatures. The micro-structures and surface characteristics of the starch granules after natural and freeze-thaw treatment are shown in Fig. 12.2 (Wang et al., 2022b). Native starch showed two types of granules, which are divided into larger elliptical A-type granules and smaller spherical B-type granules. The natural starch granules showed complete granules and smooth surface with some pores. After repeated freeze-thaw treatment, the surface of the starch granules became rough, and the external parts of the granules are grooved and damaged without changing the shape of the granules. These phenomena are more obvious, the surface visible obvious porosity, crack or depression, part of the damaged particles together. The above transformation of starch morphological characteristics may be attributed to the continuous formation and melting of ice crystals during the freezing and thawing (FT) process. In particular, repeated freeze-thaw can produce strong mechanical force inside and outside the starch particles, broaden the internal channels of starch particles and compressed particles, surface pitting, and finally lead to the loose grain structure. For example, after several freeze–thaw cycles, granule gap and the specific surface area of potato starch are significantly improved, but granule density is reduced. In the process of circulation, the migration and analysis of water molecules lead to a loose structure of starch and other internal spaces and channels will be generated, which may be caused the precipitation of amylose and water in the amorphous region. After freeze–thaw cycles, starch granules are insoluble in water and dense in space. The internal structure of the starch granules depends on the content of the bound water (Whistler et al., 1967). Zhang et al. (2014a) conducted multiple experiments on glutinous rice starch with different moisture contents after freeze-thaw cycles and found that ice crystals formed by water molecules caused damage to starch granules after repeated freezing-thawing. With increasing freeze-thaw times, the starch surface becomes rougher and the interspaces and channels within the granules become larger, resulting in concave holes and damage. This process may be caused by the pressure of ice crystal expansion on starch granules during the freezing-thawing process, resulting in increased surface holes (Zhang et al., 2014a). In addition, the loose internal structure of starch caused by repeated freezing and thawing may be related to the weakening effect of the starch double helix (Yan et al., 2012). For example, the double helix of potato starch is damaged during the thawing process of the freeze–thaw cycle, and the released water molecules recombine with the glucose hydroxyl group in the form of hydrogen bonds and form a new semicrystalline structure. This process changes the distribution of water in the granules and improves the water retention capacity of potato starch (Szymońska et al., 2003). In summary, the free water in the granules changes to the crystalline state of ice during freezing. Ice crystals occupy more space on the starch granules than the same amount of water, thus exerting greater mechanical force, causing starch granules to expand and swell and forming loose structures after thawing. This phenomenon also leads to an increase in granule size without damaging the integrity of the granules. After freezing and thawing, indentations and grooves are generated on the surface of starch granules due to the precipitation of amylose or ice crystals (Yu et al., 2018). Amylose exists in the amorphous region of starch, and polar fat substances and amylose molecules can form spiral cages. In addition, the amylose content also affects the oil absorption performance of starch granules. Freeze–thaw treatment destroys the amorphous area of starch and results in the reduction of amylose. Ice crystal pressure loosens the structure of the starch granules and increases the total water and oil absorption of the starch. However, increasing the contact angle on the rough surface of the starch enhances the hydrophobicity of the surface of the freeze–thawed starch. Therefore, compared with raw starch, the starch after freeze-thaw treatment had higher oil absorption (Wang et al., 2019).

12.3.2 *Effect of the Freeze–Thaw Process on Starch Gel*

As the main component of the human diet, starch affects the hydrophilicity, transparency, hydrolysis, gelatinization, thermodynamics, and stress characteristics of the gel after freeze-thaw treatment (Wu, 1988). Changes in mechanical properties and starch regeneration are related to changes in the gel network microstructure. With increasing number of freeze–thaw cycles, gel hardness (Seetapan et al., 2015), regeneration rate, and water separation rate are increased (Charoenrein &

Preechathammawong, 2010; Shifeng et al., 2012), whereas the digestibility, viscoelasticity and cohesion are decreased (Zhang et al., 2015b; Wang et al., 2013). The dehydration condensation caused by multiple freeze–thaw cycles accelerates the retrogradation of starch as well as the appearance of phase separation and ice crystal growth, thus forming a spongy structure (Teng et al., 2013). When frozen at low temperatures, ice crystals are formed due to some water freezing of the starch gel, promoting starch enrichment and association with molecular chains. During the thawing process, water is released from the pores of the gel after the ice crystals melt, resulting in dehydration shrinkage (Zhou, 2014). The dehydration shrinkage rate is an important index to measure the freeze–thaw stability of starch (Jia et al., 2012), and this information can be obtained by testing the separation degree of the starch paste phase. Thus high regeneration and dehydration shrinkage have a serious impact on food quality.

During the freeze–thaw process, the physicochemical properties of the starch gel, such as crystallinity and retrogradation properties, can be changed by controlling the number of freeze–thaw times and the freeze–thaw temperature, and then the physicochemical properties and quality of starch food can be regulated. With the increase in freeze–thaw times, the crystallinity, retroactive enthalpy, and resistant starch content of wheat starch gel are increased, and the transparency and water retention capacity of corn starch and waxy corn starch gel decrease (Gao et al., 2017). Additionally, temperature fluctuation accelerates the regeneration of starch molecules during the freeze–thaw cycle, which may be caused by the rearrangement of amylose and amylopectin in starch, thus increasing the rigidity of its structure (Funami et al., 2005). Zhao Zhongkai et al. (2015) found that the solubility of sweet potato starch first decreased and then increased with increasing temperature after the heating-freezing-thawing cycle, which may be due to the water absorption and expansion of starch during heating because the dissolution of amylose leads to an increase in starch solubility. As the heating temperature continues to increase, starch gelatinize into a gel, and its internal structure are changed, resulting in increased rigidity of starch gel. In freeze–thaw cycles, the lower the temperature, the greater the degree of regeneration. In addition, the viscosity characteristic values and regeneration capacity of starch decrease with increasing freeze–thaw cycles (Zhao et al., 2015). Before and after freezing-thawing, the appearance of starch gel is highly correlated with the stability of freezing and thawing. Amylose and lipids inhibited the expansion of starch granules and maintained the integrity of the expanded starch granules, while amylopectin is an important factor affecting the expansion of starch granules. Generally, a gel with a high starch content, a high starch concentration, and low water content have higher hardness or elasticity, which could be due to the high dehydration value of amylose gel after the first freeze–thaw cycle. With the increase in the number of cycles, the amount of water dewatering decreases (re-absorption of separated water), and the smooth gel is transformed into a rough, porous, and textured spongy gel (Saiyavit et al., 2002). In addition, a high amylose or phosphorus content contributes to the formation of dense and thick three-dimensional layered fiber network structures, inhibiting the expansion of starch granules. After freeze–thaw cycles, starch gels form honeycomb or layered structures, which may be due to the aggregation of starch molecules driven by freeze–thaw cycles, resulting in mandatory phase separation, ice crystal growth, and starch gel cavity size increases. The increased amylopectin content helps to maintain the gel structure. The expansion force, the water holding capacity, and the maximum iodine absorption peak of the wheat starch gel prepared by compression heat are greater than those of the native starch after the freeze–thaw cycle (Tang et al., 2013). which may be due to the amylose molecules are separated after the freeze–thaw cycle, and amylopectin is retained in the granules, leading to an increase in the expansion force of the starch gel (Tao & Wang et al., 2015).

12.3.3 Effect of the Freeze–Thaw Process on Starch Crystallization

The regular arrangement, packing and orientation of the double helix structure of starch can be organized in monoclinic or hexagonal crystal units to construct good microcrystalline orientation and closely arranged crystal lattice to finally produce an ordered crystal structure.The freeze–thawing treatment did not change the type of starch crystallization, but affected the degree and order of crystallization, XRD spectra of natural starch and freeze-thaw treated starch are shown in Fig. 12.3 (Wang et al., 2022b). All wheat starches showed an A-type polymorph with obvious peaks

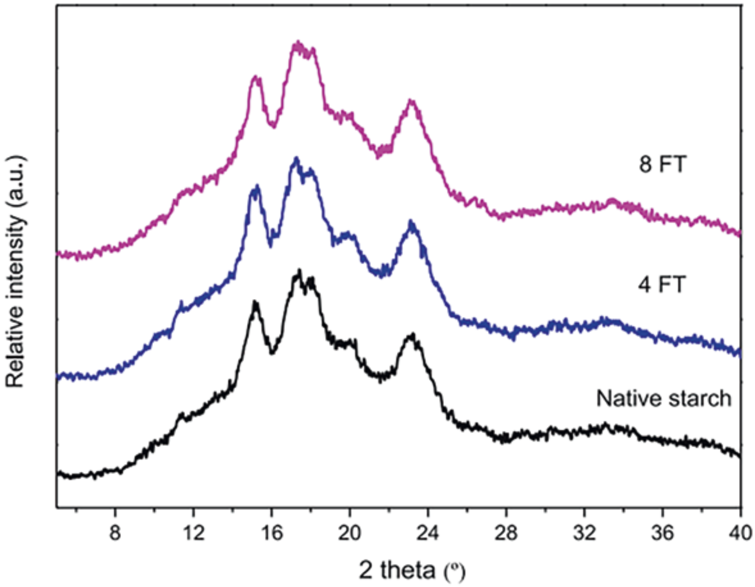

Fig. 12.3 X-ray diffraction patterns of native and repeated freezing-thawing treated wheat starches (FT, freezing-thawing; 4,8 FT represent the times of freezing-thawing cycles)(Wang et al., 2022b)

at 2θ of ca. 15°, 17°, 18°, and 23°, indicating that freeze-thaw treatment did not change the crystalline morphology of wheat starch. However, a significant decrease in the intensity of the diffraction peak can be observed, especially after the 8 FT cycle, which might be due to the destruction of intramolecular and intermolecular hydrogen bonds under the influence of ice crystallization and recrystallization during the F/T treatment, resulting in the amorphization of the ordered molecular structure, the destruction of starch crystals, and/or the decrease in the orientation of the double helix structure, leading to the significant decrease of relative crystallinity. The results again confirmed that multiple freeze-thaw cycles would be destroied (Shifeng et al., 2015). Wheat starch is a semicrystalline structure. The crystalline region is mainly composed of an amylopectin multilayer double helix structure, while the amorphous region of starch consists of amylose, disordered amylopectin, and amylopectin chain branch points. After repeated freezing and thawing, the crystallinity of wheat starch increased. Which finding could be attributed to the loss of amylose, fat, and protein caused by freeze–thaw cycles, leading to the rearrangement of the double helix in the amylopectin crystal region, thus improving the proportion of the crystallization region and large-size crystallization and crystal integrity. Szymonska et al. (2003) conducted several freeze–thaw treatments on potato starch and found that the surface morphology of starch granules changed significantly, resulting in increased crystallinity. Which could be due to the presence of water molecules in the outer and amorphous areas of starch granules that easily form hydrogen bonds with starch during thawing, which protects the structural arrangement of starch granules and leads to increased starch crystallinity (Szymońska et al., 2003). Some studies suggest that the increase in starch crystallinity is positively correlated with the number of damaged starch granules, and freeze–thawed starch may have a more easily recombined structure than natural starch. Many studies have confirmed that changes in amylose content significantly affect the crystallinity of starch. It is believed that amylose may hinder the molecular migration of amylopectin and restrict its structural recombination. After freeze–thaw starch amorphous area dissolution, loss of amylose chains causes amylopectin chain reordering, such as the reorientation of the double helix and the interaction between the double amylose helix. Thus, a more orderly and stable double helix structure is easily formed. Furthermore, the crystallinity and identifying characteristics of the crystal region are improved, which may be due to the different degrees of hydration in the amorphous region during the cooling process (Cheetham & Tao, 1998; Tao & Wang et al., 2015). Some studies have found a decrease in the crystallinity of starch after freezing-thawing. Decreased crystallinity may be due to the rapid aggregation of leached amylose molecules, which weakens the granule structure. In addition, frozen water induces amylopectin leaching accompanied by the loss of double helix, thereby affecting the structure of starch microcrystals (microcrystals derived from the interleaving of amylopectin and outer chain). For example, after freeze–thaw cycles of corn starch and glutinous rice starch, the crystallization of starch granules and the crystal order of the amorphous region of the double helix are irreversibly destroyed due to the extrusion of starch granules by expanded ice crystals, resulting in a reduction in crystallinity (Yu et al., 2018).

12.3.4 Effect of the Freeze–Thaw Process on Starch Gelatinization

The effect of freeze–thaw treatment on starch gelatinization depends on the degree of starch leaching, the content of damaged starch, and the internal structure of starch, which contribute to the interaction between starch chains. As the freeze–thaw cycle progresses, the crystallinity of the starch granules is improved and the stable structure formed requires more energy to be destroyed during gelatinization. Specifically, the gelatinization temperature and enthalpy value of starch are increased, and starch is more resistant to gelation. Crystallinity is generally positively correlated with gelatinization temperature, and the formation of the crystalline area of amylose leads to starch retrograde and final viscosity reduction. A higher decomposition viscosity indicates that granule rupture or starch has less tendency to resist shear force in the heating process and is more likely to be destroyed (Yu et al., 2018). Amylose content has a certain influence on the gelatinization property of starch, and waxy starch has a greater gelatinization enthalpy than ordinary starch because waxy starch has more crystals and fewer amorphous regions. After freeze–thaw cycles, waxy starch showed stronger compressive resistance due to its small granule size, its gelatinization enthalpy decreased with increasing damaged starch content, and which is not easily aggregated after freezing (Tao et al., 2015b). After freeze–thaw treatment, the amylose content of hard and soft wheat starch also resulted in different gelatinization characteristics. The more amylose lost in soft wheat starch (with a smaller flour granule size and less damaged starch), the more crystalline the double helices formed by self-association. After freeze–thaw cycles, the soft starch had a lower swelling capacity, peak value, valley value, decomposition and final viscosity than durum wheat starch (Tao et al., 2018). The gelatinization temperature of mutant sweet potato starch is 20 °C lower than that of native sweet potato starch and it is difficult to regenerate. Thus, mutant sweet potato starch has a higher swelling capacity, which can make the gel matrix compact and solid and increase the gel strength. The sizes of the frozen surimi ice crystals and gel voids after thawing are reduced compared to those of the natural sweet potato starch gel afters low-reviving sweet potato starch is added, and the hardness and viscosity are effectively maintained, showing minor structural damage. Starch regeneration is also reduced after mixing. During the thawing process, part of the water is retained in the starch granules of the surimi gel, and most of the water is regenerated slowly. Compared to natural granules, sweet potato starch could better maintain the original granule shape (Jia et al., 2018).

12.4 Causal Relations Between Starch Hierarchical Structure and Physicochemical Characteristics After Repeated Freezing-Thawing

The final quality of freeze–thawed starch is affected by its multiscale structure (morphology structure, lamellar structure, crystal structure, spiral structure, short-range ordered structure, etc.) and physical and chemical properties (expansion capacity, gelatinization, rheology, coagulation characteristics, etc.).

12.4.1 Lamellar Structure

Starch granules are mainly composed of crystalline lamellar (5–6 nm) and amorphous lamellar (2–5 nm) alternately formed semicrystalline growth rings (thickness 120–400 nm), which can be used to study the accumulation state of starch lamellar structures using small angle X-ray scattering technology (SAXS) (Zhao et al., 2018). According to the structural hierarchy of the polymer, starch molecules can be divided into three structural levels: near structure (primary structure), remote structure (secondary structure), and aggregated structure (tertiary structure). The near structure includes the chain structure, either straight chain or branch chain, glycosidic bond configuration, and the number of glucose units. The remote structure corresponds to the size and distribution of the starch molecule, and the internal rotation conformation and flexibility of the molecular chain. The aggregate structure corresponds to the internal structure formed by the geometric arrangement and stacking of the intermolecular spiral structure of starch, including the crystal state, the amorphous state, and the orientation of the crystal structure (Perezlloret et al., 2010). The thickness of the characteristic peak corresponding to the semicrystalline layer appears around the scattering vector $q = 0.68\ \mathrm{nm}^{-1}$ ($q = 4\pi\sin\theta/\lambda$). The thickness of the semicrystalline lamellar structure of different starch granules is approximately 9–10 nm (Xu et al., 2021). The repeated distance between the starch lamellar can be calculated according to the Woolf-Bragg equation ($d_{\mathrm{Bragg}} = 2\pi/q$) (Dongling et al., 2017; Zhang et al., 2013). Xuke et al. allowed a starch suspension with a moisture content of 60% (w/w) to be equilibrated at 25 °C for 12 h and then placed the starch sample in a sample cell for fixation. After freeze–thawing treatment, they found that all wheat starch doughs showed obvious scattering peaks at a q value of approximately $0.060\ \text{Å}^{-1}$, and their D_{Bragg} values decreased between 10.45 and 10.76 nm (Xu et al., 2021). The lamellar structure of the crystalline region is composed of the regular arrangement of the double helix structure of amylopectin, while the amorphous lamellar structure is composed mainly of amylose and amylopectin with branching nodes (Zhang, 2012). Freeze–thaw cycle treatment can reduce the area and intensity of the small angle XRD peak of starch and destroy the nanolamellar structure of starch. After processing, the slice thickness gradually decreases with increasing freeze–thaw cycles, and the crystal slice layer thickness and semicrystalline (d_c) thickness of the layer structure (d_L) decrease. The reason may be that the repeated formation and melting of ice crystals in starch granules during freezing and thawing lead to a disordered arrangement and irregular accumulation of crystal lamella (such as double helix and crystal structure) in the amorphous region (Xu et al., 2021).

12.4.2 Ordered Molecular Structure

The ratio of absorption peak intensity at 1045 cm^{-1} and 1022 cm^{-1} of the infrared spectrum of freeze–thawed starch can be used to indicate the short-range ordered structure of starch, and the higher the ratio, the higher the degree of ordering. The $R_{1045/1022}$ value of starch decreased with increasing number of freeze–thaw cycles, indicating that the short-range order degree of starch is damaged by freeze–thaw cycles, which could be due to the ordered double helix structure of amylopectin molecules is destroyed by freeze–thaw treatment (Sevenou et al., 2002).

The Raman spectrum of starch at half-peak width (FWHM) in the 480 cm^{-1} band is negatively correlated with the degree of the short-range ordered structure of starch. FWHM values of starch samples after freeze–thaw treatment increased with increasing cycles, indicating that freeze–thaw treatment may destroy hydrogen bonds in the crystallized and amorphous regions, resulting in the disorder of the short-range ordered molecular structure (Mutungi et al., 2012).

In addition, starch generally has an obvious endothermic peak (G) in a thermodynamic DSC test, which corresponds to the ordered molecular structure of melting (for example, ordered molecular structure of long and short range) (C et al., 2016). Endothermic enthalpy (ΔH) is positively correlated with the number of starch microcrystals or the double helix ratio. The ΔH of the starch granules decreased significantly after freeze–thaw treatment (especially after 8 freeze–thaw cycles), indicating that the ordered structure of starch is destroyed, which is related to the expansion force caused by the repeated formation and melting of ice crystals in the freeze–thaw process, leading to the destruction of hydrogen bonds and crystal lattices in the starch aggregate structure. In addition, freezing and thawing treatment increases the initial temperature of the endothermic peak (T_0) but decreases the end temperature (T_c). Moreover, T_o reflects the energy required for helical-helical dissociation, indicating the thermal stability of the ordered molecular structure (Wang et al., 2018). The higher T_o values implied that starch subjected to freeze–thaw treatment need higher thermal energy for gelatinization, and this change is probably attributed to freeze–thaw treatment that disrupted the less ordered molecular structure (less stable or smaller crystallites) of starch. In addition, the narrower ΔT ($T_c - T_o$) represents the uniformity of the ordered molecular structure, which can also lead to a better regularity of the ordered molecular structure and an enhancement of its thermal stability (i.e, increased T_o). Therefore, the increase in gelatinization temperature is closely related to a dense spiral structure and/or a more perfect crystal size and/or a larger crystal size (Wang, et al., 2022b).

12.4.3 Swelling Power and Solubility

The solubility and expansion force of starch reflect the interaction between the crystalline region and the amorphous region inside the starch granules, the hydrogen bonds in the crystalline region, and the strength of the interaction between starch molecules in the amorphous region. Starch solubility and expansion are related to starch morphology, granule size, amylose content, amylose ratio and nonstarchy components inside the granules (Sandhu & Singh, 2007). The expansion force (SP) reflects the water holding capacity of starch granules under shear-free conditions, and the expansion force of starch granules is mainly affected by amylopectin, which ultimately affects the use value and processing performance of starch in various food systems (Zhang et al., 2014b). The SP of natural starch and freeze–thaw treated starch increased in the temperature range of 55~95°C. The high temperatures weaken the hydrogen bonds between the starch chains, thus promoting the hydration of water molecules with free hydroxyl groups of starch. Starch after repeated freeze–thaw treatment exhibited a higher SP than native starch in the same temperature rise zone, and this trend became more pronounced as the number of freeze–thaw cycles increased. After freeze–thaw treatment of starch, the increase in SP may be due to the destruction of the multiscale structure of starch and the appearance of pores and cracks in starch granules after freezing-thawing. These changes make it easier for water molecules to penetrate inside the granules and combine with more starch molecules to form hydrogen bonds, thus increasing the permeability of water into the amorphous and crystalline regions of starch granules (Liu et al., 2019).

The solubility of starch shows a trend similar to that of the expansion force. Solubility reflects the interaction between the starch molecular chain and water molecules, which indicates the degree of dissolution during starch swelling. For example, after a sweet potato starch gel, formed after heating to 95 °C, is treated by freeze–thaw cycles, with increasing number of freeze–thaw cycles, the solubility of the sweet potato starch shows a tendency to decrease first and then increase, and the viscosity and rejuvenation value decrease, but slow digestion starch content (SDS) increases (Zhao et al., 2015). The gelatinization temperature has an important effect on the solubility of starch. The solubility of gelatinized corn starch at 50 ~60 °C is significantly greater than that of the native starch, but the solubility of gelatinization at 70 ~ 90 °C is less than that of the native starch. Most of the gel structure after gelatinization is in an amorphous state and produce more leaching of amylose, which makes it easier to absorb water during low-temperature gelatinization, thereby improving solubility. However, the hydrogen bonds between the recrystallized starch molecules in gelatinized starch are increased after higher gelatinization temperature, which is more difficult to break and results in lower solubility (Liu, 2006).

The proportion of straight-branched chains of starch is another important influencing factor. Waxy corn starch with lower amylose is less recrystallized after gelatinization, and waxy corn starch is more likely to absorb water and expand to form a gel than native starch at high-temperature gelatinization. At the same gelatinization temperature, the solubility of gelatinized corn starch increased with increasing number of freeze–thaws, while the gelatinized waxy corn starch gradually decreased. This may be due to two reasons: (1) Due to incomplete gelatinization of corn starch, residual starch granules are broken under alternating freeze–thawing treatments and more starch molecules are released to dissolve in water. (2) An increase in the number of freeze–thaw cycles causes severe damage to the starch gel structure, exposing more hydroxyl groups, making it easier to bind to water molecules and increasing solubility. The solubility of gelatinized waxy corn starch decreases with increasing freezing-thawing cycles, which shows that after freeze–thaw treatment, the waxy corn starch molecules can be recrystallized to form aging starch (Gao et al., 2017).

12.4.4 Paste Properties

The gelatinization characteristic of starch is very important for the processing characteristics of starch in food production and the quality of the final product. A series of continuous stages are involved in the starch gelatinization process. After the starch granules absorb water and swell, they lose their original ordered structure and produce a starch paste with high viscosity. After cooling, a three-dimensional network structure is formed, which depends primarily on the rearrangement ability of the starch molecular chain (Wang et al., 2020; Zhang, 2015a). Peak viscosity (PV) reflects the maximum expansion size of starch granules, that is, the water absorption and swelling capacity of starch granules, which is obtained through the entanglement of amylose and amylopectin and their interaction with the disordered shell of starch granules. Freeze–thawed starch has a higher PV than natural starch. Which could be due to the freeze–thaw treatment destroys the structure of the semicrystalline thin layer, crystal, double helix and short-range ordered molecules of starch granules, which may improve the swelling property and the tensile degree of the starch molecular chain, resulting in an increase in the swelling size and the larger peak viscosity (PV) of freeze–thawed starch. Second, due to holes and cracks in starch granules after freeze–thaw cycles, free water can enter more easily the interior of the granules, forming intramolecular and intermolecular hydrogen bonds with the stretched starch molecules, which can also increase its maximum viscosity (Wang et al., 2022b).

After the starch paste reaches the maximum viscosity, if accompanied by mechanical stirring, the viscosity of the starch paste is decreased, which is attributed to the disintegration of starch granules, and then the starch molecular chains (mainly amylose) dissolve into the medium and mix and become arranged (i.e., the linear orientation of the molecules) (Klaochanpong et al., 2015). The disintegration value (BV) reflects the shear resistance of the starch granules. The higher the BV is, the greater the integrity and lower rigidity of starch granules. Freeze–thaw treatment can increase the BV value of starch granules, which can result from damage to the multiscale structure of starch.

The final viscosity (FV) of the starch paste is very important to the final product quality. After being cooled for a certain period, starch paste viscosity increases, which is attributed to the formation of a three-dimensional network and the interaction between amylose and the disintegrating starch granules (Hongwei et al., 2019). The FV of the freeze–thaw treated starch is significantly higher than that of the natural starch, which may be due to the high expansion of the starch granules during heating and the complete stretching of the starch molecular chain, which accelerated the diffusion, collision and bonding of starch molecular chain and made the starch paste more inclined to transform into a solid state (Hwa et al., 2020; Zhang et al., 2015b).

The regeneration value (SB) reflects the degree to which starch molecules, particularly free starch molecules that have been released from starch granules, are re-associated or rearranged during the cooling process. The SB value increases with the number of freeze–thaw cycles, which may be due to the destruction of the granules, resulting in increased leaching of amylose, which facilitates the rearrangement and recrystallization of starch molecules (Xu et al., 2020; Miao et al., 2018).

12.4.5 Rheological Properties

The rheological properties of starch can reflect the microstructural changes of starch gel. The rheological behavior of starch gels is determined by the recombination and rearrangement of starch molecular chains, which ultimately affects the rigidity of the gel network (Hwa et al., 2020). The elastic modulus (G′) and viscosity modulus (G″) represent the elasticity and viscosity of the starch gel, respectively. G′ refers to the deformation degree of starch paste under the action of an external force, namely, the energy stored due to elastic deformation, which represents the self-recovery ability of starch paste after the action of an external force. G″ refers to the energy loss of starch paste as a result of viscous deformation (irreversible deformation) in the deformation process, which reflects the fluidity of starch paste. With the increase in freeze–thaw times of starch, the G′ and G″ of starch showed a trend of gradually increasing, mainly because repeated freeze–thaw destroyed the ordered arrangement of starch granules so that the starch granules easily absorbed water and expanded, and the starch molecular chains easily rearranged or regenerated, thus improving the hardness (Wang et al., 2022a).

The tangent of the loss angle tan δ (G″/G′) is often used to reveal the magnitude of gel stiffness. The lower the tan δ value is, the higher the rigidity of the starch gel. With increasing freeze–thaw cycles, tan δ values gradually decreased, indicating that freeze–thaw treatment increased the rigidity of starch gel. This may be due to the rearrangement of starch molecules (i.e., amylose and amylopectin), which form a rigid gel due to the increased entanglement and interaction of starch chains during cooling (Li & Yeh, 2001; Hwa et al., 2020).

In other words, the ice crystallization and recrystallization that occurred in the repeated freeze–thaw process destroyed the morphological characteristics, semicrystalline lamellar structure, crystal structure, ordered molecular structure, and spiral structure of short-range starch granules and promoted the entanglement and interaction between starch molecules, thus forming a firm gel network structure. As a result, freeze–thaw treated starch granules or gels exhibit greater rigidity than natural starch.

12.5 Factors Influencing the Freeze–Thaw Stability of Starch

12.5.1 Amylose, Protein, and Fat Contents and Amylopectin Chain Length

The amylose content in starch is positively correlated with the freeze–thaw stability of starch (Cristina et al., 2007; Srichuwong et al., 2012). The low mobility of long and amylose molecules can limit the dehydration shrinkage rate, and amylose can also form spiral complexes with phospholipids, which can limit the swelling of starch granules, keep starch granules intact, produce hard gels, and improve the freeze–thaw stability of starch. Amylopectin contributes to the swelling of starch granules, such as waxy starch with low amylose content and common wheat starch. After freezing and thawing, the retrogradation of these starches is lower than that of high-amylose paste at the same concentration. Although starch contains less fat and protein, it still has retarding and inhibitory effects on starch regeneration after freeze–thaw cycles. Lipids and proteins can inhibit starch granules swelling and thus maintain freeze–thaw stability. The interfacial interaction between starch granules and hydrated glutenin networks can be attributed to hydration. During freezing, water molecules in starch granules can enlarge the channels in the granule envelope and lead to leaching of components, resulting in the formation of rough surfaces and wide granular channels. This process can accelerate the infiltration of water into the granules and increase the hydroscopicity of freeze-treated starch (Tao et al., 2016). The removal of starch fat and starch-binding protein increases the expansion property and gelatinization viscosity of starch and accelerates the retrogradation of starch. Which could be due to the interaction between starch fat and starch-binding protein and amylose molecules (or side chains of branched molecules) inhibits the aggregation of branched molecules and prevents the formation of a double-helix structure between amylose molecules. This phenomenon further hinders the recrystallization of amylopectin molecules (Tao, 2017). Long et al. (2007) subjected defatted fern root starch to freeze-thaw treatment and found that its stability decreased and the water separation rate of the starch paste increased. These effects can be caused by the increased probability of interaction between starch molecules after defatted starch. In addition, retrogradation precipitated water formed ice crystals during the freezing process, causing damage to the structure

(Long et al., 2007). Kumar et al. (2018) mixed milk products (such as whey protein concentrate, whey protein, and skim milk powder) with oat starch. Compared to whey protein concentrate and whey protein, defatted milk powder reduced the surface swelling and decomposability of oat starch, resulting in an increase in the shrinkage of dehydration and a decrease in granule size. After the protein-starch mixture is subjected to a freeze-thaw cycle, the casein in the system can improve the structure of the starch granules and may form micelles through self-association, which can be adsorbed on the surface of the granules, limiting starch swelling and decomposition and improving its stability (Kumar et al., 2018). In the freeze–thaw process, the structure and degree of starch regeneration are also related to the length of amylopectin. Furthermore, texture characteristics are significantly related to the proportion of medium starch length in total starch, and the distribution of amylopectin length in starch is also affected to a certain extent. The high-branched structure and short-chain amylopectin could delay the retrogradation process (Wang et al., 2013). The increase in the content of longer amylopectin (Degree of Polymerization, DP > 18) increased the phase separation and starch phase regeneration, while starch with a short chain length (DP 6–11) contributed to the connection between lamellar structures (Yu et al., 2018).

12.5.2 Freeze–Thaw Rate

The freeze-thaw stability of starch varies with different freezing rates, and starch gel thawed at a low temperature has a lower dehydration shrinkage rate than starch gel thawed at a high temperature. It was found that rapid pre-cooling and freezing storage at low temperature (−60 °C) and ultralow temperature (−100 °C) could effectively reduce the regression enthalpy of the rice amylopectin gel and inhibit the regression, while low-temperature treatment (−20 °C and −30 °C) did not reduce the degree of regression. Freschi et al. (2014) found that the potato starch gel structure is greatly affected by the freezing rate, whereas the wheat starch gel structure is jointly affected by the freezing and thawing rates. In addition, the pore size generated by ice crystals increased with an increasing freezing rate (Jérôme et al., 2014). Waxy corn starch after freeze–thaw treatment at different rates showed that slow freezing at low temperatures caused more significant damage to starch granules than rapid freezing with liquid nitrogen (Zhang et al., 2014a). This may be because slow freezing can easily cause starch gel to regenerate and dehydrate under different freezing rates. In contrast, rapid freezing can cause water in starch to freeze quickly, resulting in smaller ice crystals and less pressure on starch granules (Su et al., 2020). Thus, reduced damage and inhibition of starch regeneration are noted. Generally, under different freezing rates, starch with a higher amylopectin content is generally unable to form a crystalline or rough gel surface structure due to steric obstacles, and exhibits better freeze-thaw stability (Saiyavit et al., 2002).

12.5.3 pH

Lower pH values depolymerize starch molecules and reduce viscosity (Majzoobi et al., 2016). Shorter starch chains are more likely to absorb more water. Thus, the free water ratio probably increases and leads to the formation of larger ice crystals during freezing. Then, the crystals melt and separate from the starch paste, resulting in an increase in dehydration shrinkage. When the pH value is high, the hydroxyl group in the glucose unit of the starch chain is ionized, which delays the aggregation and retrogradation of starch (Suortti et al., 1998), breaks the hydrogen bond between starch molecules, improves the solubility of the water, and strengthens the interaction between starch and water. The starch network binding water is not easily separated from the paste, resulting in a low dehydration shrinkage rate (Hedayati et al., 2016). The addition of hydrophilic colloid to starch can reduce the dehydration shrinkage caused by freeze–thaw cycles. For example, the addition of xanthan gum to starch gel can prevent entanglement of starch molecules, thus reducing the degree of contraction of dehydration after repeated freeze–thaw cycles, which is consistent with other starches (such as corn, sweet potato, yam, and wheat starch) (Ferrero et al., 2010; Lee et al., 2002). Under acidic conditions, the dehydration shrinkage rate of starch remains high. For example, xanthan gum is the most effective for improving the freeze–thaw stability of cassava starch gel when the pH value is approximately 7, and its effect is slightly lower when the pH value is approximately 9; the effect is poor when the pH is approximately 3. After freezing and thawing 2 times, the dehydration shrinkage volume almost doubles and basically stabilizes after 5 cycles. Under acidic conditions, the high dehydration shrinkage value of control samples may be attributed in part to the soft texture of starch gels (weak gel network structure), which is less resistant to centrifugal deformation (Yuan & Thompson, 1998).

12.5.4 Carbohydrates

12.5.4.1 Oligosaccharides

Small molecular weight sugar can be used as a functional component and improve the quality of food. Some studies have mixed natural starch with sugar to inhibit starch retrograde, but its effect on starch retrograde remains controversial. The addition of sucrose can reduce the water evolution rate of starch, which may be due to slow initial rate of water separation under the action of more hydrophilic compounds. Arunyanart & Charoenrein (2008) found that sucrose is an effective reagent to inhibit the dehydration shrinkage of rice starch gel after freeze–thaw cycles. When the mass fraction of sucrose is 20%, the freeze–thaw stability of starch gel is improved. It is possible that as the sucrose concentration increases, the freezable water content in the starch system may decrease, which is also related to the gel microstructure and the dehydration shrinkage rate. Different sugars have different degrees of inhibition on starch retrogradation. Regarding the order of inhibition of oligosaccharides on starch retrogradation, disaccharides (sucrose) exhibit greater inhibition than monosaccharides (glucose) (Chang et al., 2004). Under the simulated molecular dynamics model, disaccharides, such as sucrose, lactose, and maltose, have a strong interaction with starch during freezing, thus inhibiting retrogradation (Wang et al., 2016). Sucrose may inhibit starch regeneration by delaying the formation of the spongy structure of rice starch gel during repeated freeze–thaw cycles. Other researchers have also found that adding sucrose to sweet potato starch and rice starch gels can accelerate starch retrogradation. Which could be due to the sucrose promotes recombination of the starch chain after freeze–thaw cycles and forms a network structure, leading to an accelerated retrograde rate of starch (Suna et al., 2010).

12.5.4.2 Polysaccharides

Repeated freeze–thaw treatment can increase the size of the starch gel hollow cavity and eventually destroy the honeycomb structure. The addition of hydrocolloid (such as xanthan gum, guar gum, alginate, konjac glucomannan, etc.) can improve the microstructure of starch gel by reducing the size of the starch gel hollow cavity and increasing the thickness of the network membrane (Chen et al., 2015). Charles et al. (2016) added kudzu starch to cassava and sweet potato starch and found a reduction in the formation of ice crystals in the gel paste of the mixture, which reduced the freeze–thaw damage. Kudzu starch can regulate the gelation properties of starch (Charles et al., 2016). Ye et al. (2018a) found that inulin could enhance the water retention capacity of rice gel, reduce the amount of freezable water, and reduce the dehydration shrinkage of gel. In addition, inulin with a low degree of polymerization had a better effect. Inulin molecules are entangled with the leached amylose and amylopectin chains. This entanglement may reduce the interaction between amylose and amylopectin and thus inhibit gel coagulation (Ye et al., 2018b). Chen et al. (2015) studied the effects of gum arabic on the freeze–thaw stability of natural cassava starch, modified cationic cassava starch, and anionic cassava starch. The swelling force and the solubility index of natural cassava starch, cationic cassava starch, and anionic cassava starch are reduced after the addition of gum arabic. This finding may be due to the molecular attraction between different charges when anionic arabic gum encounters positive cationic cassava starch. The cationic cassava starch granules are tightly wrapped by arabic gum, which affects further swelling of the granules and prevents leaching of amylose. Gum arabic can reduce the formation of ice crystals and minimize the damage caused by freezing and thawing. It is suitable for starch-based food stabilizers or viscosity control agents, but it is not suitable as an additive in the product freezing process (Chen et al., 2015). Xanthan gum has been widely used to improve the physical properties of pastes and gels of various starches (sweet potato, yam, corn starch and wheat flour), such as reducing colloidal coagulation and dehydration shrinkage and improving the freeze–thaw stability of starch gels. The addition of xanthan gum can reduce the dehydration shrinkage rate of sweet potato starch gel, possibly because the addition of xanthan gum can avoid the extension of the spongy structure of the starch. This phenomenon is related to delayed amylose regeneration, which reduces the interaction between amylose. The amylose leached in the gelatinization process is easily soluble in xanthan colloids, which affects the binding between the colloids and other amylose chains (Lee et al., 2002).

12.5.5 Salts

Increasing salt concentrations can reduce the volume of ice crystals and recrystallization in starch, which may be due to the gelatinization of starch in a salt solution will be destroyed by salt or the electrostatic interaction between salt and starch hydroxyl groups. On the basis of texture analysis and thermodynamic analysis through scanning electron microscopy, Wang Guanqing et al. (2015) found that NaCl could reduce the water separation rate of corn starch gel after freeze–thaw cycles, indicating the addition of salt reduced the phase separation between starch and water and the degree of damage to the gel structure (Wang et al., 2015). The hardness and regeneration degree of the gel decreased with increasing NaCl concentration after freeze–thaw cycling. The zeta potential test showed that the addition of NaCl reduced the zeta potential of the system, indicating an interaction between NaCl and starch that inhibited starch regeneration. The stability and coagulation of starch paste are related to the retrogradation degree of starch. The effect of salt on starch retrogradation follows the principles described by Hofmeister. Salt-out ions (such as F^- and $SO4^{2-}$) are added to the starch paste. These ions tend to protect the hydrogen bond connection between starch molecules, thus reducing the swelling force, solubility, granule size, and transparency of starch. When the gelation temperature, gelatinization enthalpy, retrograde degree, and water extraction rate of starch are increased, the gel freeze–thaw stability is poor. When salt-soluble ions (such as I^- and SCN^-) are added to the starch paste, the dissolution of amylose in the starch paste is inhibited due to the destructive effect of salt-soluble ions. Thus, the damaging effect of ice crystals generated during freezing is small and the freeze–thaw stability is improved (Zhou, 2014).

12.6 Application and Progress of Freeze–Thaw Modification of Starch

Starch is often used as an important ingredient in food and industrial products. However, native starch is limited by its inherent properties, such as being insoluble in cold water, ease of quick regeneration during storage, poor film formation, lack of emulsifying ability, poor mechanical resistance, poor processing stability and other shortcomings. These factors limit their demand for modern food or industrial products, so various functionally modified starches have been developed (Zhang et al., 2017). Starch modification can not only improve the original properties of starch but also give it new functional properties. The main methods of starch modification are chemical, physical, biological and compound modification methods. Among them, chemical modification results in a change in the chemical structure of starch or introduces new groups or reagents, and its safety should be considered and evaluated when chemically modified starch is used in the food industry. The cost of enzyme chemical reagents required for biological modification is high, and the modification process is relatively complex and not conducive to production. However, the physical starch modification method, which does not introduce new groups or chemical residues, possesses numerous advantages,such as no pollution, high safety, and high efficiency (Wang et al., 2020).

Currently, the ultra-low temperature freeze–thaw modification method is regarded as one of the most promising physical methods in the food industry due to its safety and environmental protection advantages. Freeze–thaw denaturation is a mild and effective physical modification method for starch. The effect of freeze–thaw denaturation is related to the structural characteristics of starch granules, freeze–thaw temperature, number of repeated freeze–thaw cycles, and moisture content. Although the crystal morphology does not change significantly, the microforce generated by the formation of ice crystals can act on the surface of the starch granules, making its appearance sag, generating or collecting holes, changing the surface structure of the granules (including channels) and weakening the polarization cross. Therefore, these changes result in increased contents of damaged starch, amylose, protein, etc., in starchy foods (Tao et al., 2015b). In addition, the expansion force and thermal transformation behavior of the starch granules are affected, and the starch chain is induced to exude from the starch. At the same time, in the freezing process, the formation of ice can produce physical force, the hole size, granule size, and granule surface area can increase, and damaged starch chains could induce leaching (low molecular weight of amylose and amylopectin), which induces rearrangement of the surface and internal structure of starch granules (Zhang et al., 2017). The multiscale structure of starch (such as lamellar structure, crystalline structure, and short-range ordered structure) tends to be ordered, which improves the hygroscopicity, enzyme sensitivity, and digestibility of starch (Wang et al., 2020).

Sometimes, it is also necessary to change further structural characteristics in conjunction with other methods. For example, before freeze–thaw treatment, the starch is gently heated at an initial temperature (T_o) for 1 h and then freeze–thaw treatment could make the granules expand and induce the release of starch chains from the granules into the starch dispersion (40% dry solid, W/V), causing deformation of the granule structure. The repolymerization of released starch can effectively improve the shear resistance and thermal stability of the starch paste, improve the formation of a gel network, and increase the hardness, cohesion and elasticity (Zhang et al., 2017). In addition, the freeze–thawed starch suspension combined with the α-amylase and glucosidase mixture can further improve the properties of starch. The structure of starch granules obtained by this method is loose and water molecules can easily enter into the granules, which can improve the oil absorption rate, water absorption rate, solubility, number of holes, crystallinity, and thermodynamic stability of starch granules. However, it is not conducive to the formation of the elastic properties of the starch gel and is more conducive to the transformation of the solid properties (Zhao et al., 2018). Therefore, the development of freeze–thaw technology is undoubtedly introduced new hope and vitality into the development of the entire food industry.

12.7 Case Study

Modified starch obtained by freeze–thaw modification has many excellent properties, such as low gelatinization temperature, high paste transparency, high viscosity, good stability, low coagulation, excellent film formation, strong frost resistance, acid resistance, alkali resistance and mechanical resistance, which can be widely used in the food industry (Hu et al.,2010). In food processing, starch is usually used as the main ingredient in some high-oil-content foods, such as biscuits, fried chicken, instant noodles, etc. However, because excessive fat intake often leads to nutritional problems, including hypertension, hyperlipidemia, cardiovascular diseases, and obesity, it is necessary to reduce the oil content of starch foods to reduce fat intake, and the use of freeze–thawed starch can reduce the total oil absorption of food (Varela & Fiszman, 2011). For example, after two freeze–thaw cycles, starch can form a smaller specific surface area and a rougher surface to increase starch hydrophobicity and became part of the amylose decomposition to increase the hydrophilic group and reduce the hydrophobic group, reducing the ability of starch to combine with oil. In addition, the freeze–thaw cycles did not change the type of starch but reduced its crystallinity. The loose structure slightly increased the oil absorption of the internal structure; however, the influence of this part could be ignored, so the total oil absorption decreased (Wang et al., 2019).

Porous starch, as a new type of modified starch, has attracted the attention of researchers; it has a large specific surface area that wraps around the granules from the surface to the center of abundant pores. In the food industry, porous starch is widely used as an absorbent and encapsulation agent and for the protection of highly bioactive substances, including sunflower oil with high oleic acid, fragrance, and procyanidins (Li et al., 2016; Xu et al., 2017). Porous starch treated by freeze–thaw methods combined with enzymatic hydrolysis not only can exhibit an increased adsorption capacity, swelling capacity, and solubility of water and oil, but also enhanced sensitivity to digestive enzymes due to the openings and cavities in starch granules formed by freeze–thaw treatment. In short, freeze–thaw treatment in combination with other methods (such as biological enzymes) is an alternative method of obtaining porous starch (Yu et al., 2018).

In recent years, with the rapid expansion of the clean-label food market, there has been a strong demand to not use or use less chemicals (Wang et al., 2022c). To facilitate the ability of consumers to distinguish food additives, the European Union adopted the E-NUMBER (E code) numbering system to include hundreds of approved additives in the licensing list, including pigments, preservatives, thickeners, flavor enhancers, stabilizers, etc. Clean label food is in the product label E code as rarely as possible and maintains the natural attributes of food in the ingredient column of the product label (Kang, 2021). Freeze–thaw-modified starch can be used as a stabilizer, thickener or gelling agent for a variety of clean-label products, such as sauces, puddings, or jello. In addition, modified starch can also be directly labeled "starch" in food so that the food composition list is easier to familiarize with and be recognized by consumers (Zhang & Lim, 2020).

Although starch freeze–thaw technology has many advantages, starch freeze–thaw treatment has negative effects on the dough and its products (Xu et al., 2021). In the freezing-thawing process, the quality of frozen dough deteriorates, affecting the sales and consumption of products and seriously restricting the industry's development. Which could be due to the migration of water during freezing-thawing, the formation of ice crystals, and recrystallization, which may destroy the structure of gluten protein and starch polymer. This decreases the survival rate of yeast, resulting in defects in the final product that is prepared from the frozen dough, such as cracking, high hardness, small specific volume, and rough texture, which greatly restricts the industrial application of frozen dough. Therefore, the development of freezing-thawing starch is a continuous process, and the mechanism of quality change of frozen dough during freezing-thawing should be further elucidated in future research, fundamentally solving the problem of quality degradation of frozen dough and related quick-frozen food caused by freeze–thaw cycles. This study provides a theoretical basis for developing and preserving frozen dough and other flour products.

12.8 Conclusions

This chapter reviewed the effects of repeated freezing-thawing cycles on starch granule morphology, crystal state, gel mechanics, and physicochemical properties. The effects of starch chain length, nutrient content, freezing-thawing rate, pH value, carbohydrate content, and salt content on starch freezing-thawing stability are explored to reveal the influence on starch structure and properties. Water freezing-thawing volume, starch gelatinization and gel properties, and the interassociation of starch molecules influence freezing-thawing properties, which prevents the phase separation and regeneration of starch. However, the conclusions drawn by current studies are also subject to specific hydrocolloids, starches, concentrations, ratios between hydrocolloid and starch, and preparation and measurement methods, leading to differences in the final experimental results. As refrigeration technology develops and the pace of life continues to increase, the stability of rice, wheat flour, and starch foods through the process of transport, storage, and sales to final consumption represents an important issue. These foods are usually subjected to several rounds of freezing and thawing during these processes. As an important component of rice and flour, the stability of starch under freezing and thawing conditions is directly related to the stability of frozen product quality. It is beneficial to confirm this research hypothesis better, determine the freeze–thaw stability of starch in the future, and provide theoretical support for improving the quality of starch foods.

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Chapter 13
Pulsed Electric Field Treatment for the Modification of Starch

Sahil, Mitali Madhumita, and Pramod K. Prabhakar

13.1 Introduction

Starch is a natural biodegradable macromolecule found abundantly in tubers, roots, seeds, fruits, and leaves. It is composed of amylose, a linear chain polymer with α 1–4 glucose linkages and amylopectin, a branched chain polymer with both α 1–4 and α 1–6 glucose linkages. Starch granules showed complex multi-level hierarchical structures from nano to micro metre scale. Starches have a wide range of uses in the food and pharmaceutical sectors because of their distinctive structural and functional properties. The food industry uses it for various functions such as thickening, stabilizing, binding, emulsifying, gelling, encapsulation, fat replacers, etc. (Chen et al., 2018). However, before industrializing the starch for different applications, certain modifications are required because native starch has inherent problems like lower thermal stability, syneresis, and retrogradation (Ashogbon, 2021). Therefore, modification of starch is required to get some desired functions such as thickeners (sauces, gravies, and soups), gelling (puddings, pies, candies), emulsifiers (beverage emulsions), freeze thaw stability (frozen foods such as yoghurts), stabilizers (salad dressing), binders (comminuted meat products, bakery products), encapsulating agents (spray drying flavors), fat replacers (dairy, sauces, salad dressings). Broadly, these modification techniques are classified into physical, chemical, and enzymatic modification (Chen et al., 2018). Physical modification of starch offers its advantages of consumers acceptance over other modification methods since it is

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Sahil · P. K. Prabhakar (✉)
Department of Food Science and Technology, National Institute of Food Technology Entrepreneurship and Management, Sonepat, HR, India
e-mail: pramodkp@niftem.ac.in

M. Madhumita
Department of Food Technology, School of Health Sciences and Technology, University of Petroleum and Energy Studies, Dehradun, India

V. S. Sharanagat et al. (eds.), *Starch: Advances in Modifications, Technologies and Applications*, https://doi.org/10.1007/978-3-031-35843-2_13

a "Clean Label" ingredient that does not require any E/INS no. (International numbering system for food additives) on the food label, as well as providing improved functional properties.

Pulsed electric field (PEF) treatment is one among different physical modification techniques such as High hydrostatic pressure (HPP), High-pressure homogenization (HPH), γ-irradiation, cold plasma, and ultrasonication (Maniglia et al., 2021a, b; Schmiele et al., 2018). In PEF treatment, a high-intensity pulse electric field strength of 10–80 kV/cm is applied to starch suspension for a very small duration (usually μs to ms) which alters the structural conformations of starch molecules, hence affecting their functional characteristics. PEF modification of starch offers certain advantages, such as less energy consumption and shorter processing time compared to other physical modification methods (Zhu, 2018). In the past, researchers have used this technology for a wide variety of starch modifications like potato starch (Han et al., 2009a), corn starch (Han et al., 2009b), tapioca starch (Han et al., 2012b), waxy rice starch (Zeng et al., 2016), wheat and cassava starch (Maniglia et al., 2021a, b), wheat starch, potato starch, and pea starch (Li et al., 2019), and japonica rice starch (Wu et al., 2019). This book chapter intends to summarise the application of PEF treatment for both physical and PEF-induced chemical modification of starch, the effect of starch on structural, functional, and physiological properties, as well as their modification mechanism.

13.2 Pulse Electric Field System

The PEF system of food processing consists of a pulsed power supply, treatment chamber, and control and monitoring system, as shown in Fig. 13.1. Static (batch) mode or continuous flow-through chamber mode could be used for PEF treatment. A pulse power generator is employed to deliver high voltage pulsed over the treatment chamber. A pulse power generator consists of a power supply source, energy storage elements, power switches, and a pulse-forming network. The treatment chamber consists of two electrodes separated by a certain distance d, in cm (distance between electrodes), maintained in a specific position by insulators. Electric field strength (kV/cm) produced by PEF is pulsating (usually square and exponential decay waveform) and applied to the treatment chamber containing food materials. An in-line oscilloscope monitors and records the voltage, current, and waveform signals.

13.2.1 Various Parameters Affecting PEF Processing of Foods

PEF system has numerous parameters which influence the processing conditions for food applications. Majorly these associated parameters are classified into two electrical system parameters and process parameters. Electrical system parameters are

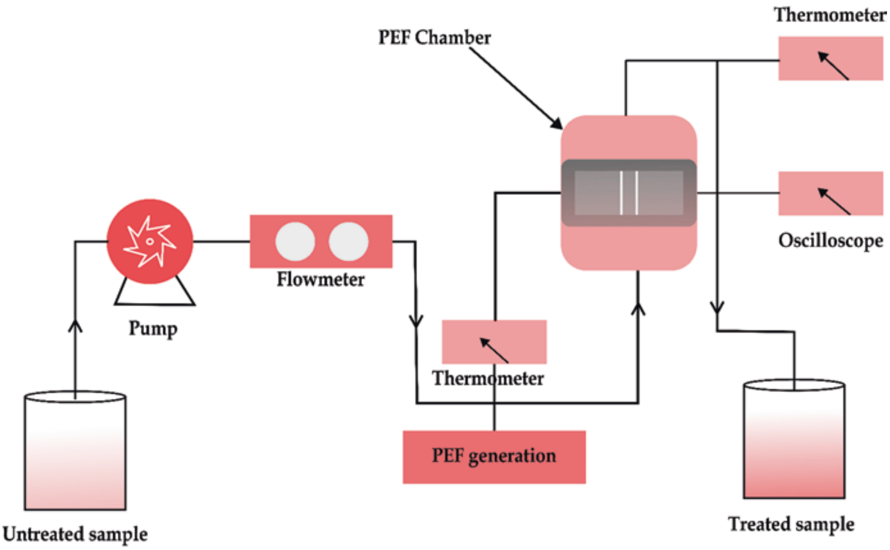


Fig. 13.1 Schematic diagram of continuous PEF processing system. (Adapted from Hong et al. (2018a, b))

high voltage pulse generator, treatment chambers, and control and monitoring system, and process parameters are electric field strength, pulse shape, pulse width, pulse frequency, treatment time, flow rate, and temperature variations. This section will discuss these parameters in detail.

13.2.1.1 Electrical System Parameters

13.2.1.1.1 High Voltage Pulse Generator

The production and transmission of high voltage repeating pulses are essential for an effective PEF processing system, along with precise control over voltage amplitude and pulse widths are very essential. Several methods exist for high voltage pulse electric field generation, from simple circuits to very sophisticated networks. This also depends upon the type of waveform required and the availability of the elements (capacitors, inductors, transformers, and switches). These circuits can be distinguished as follows:

- Basic pulsed power circuits: These circuits consist of capacitive circuits and inductive circuits as well as their series and parallel ringing circuits.
- Voltage multiplier circuits: When the on- and off-switches used in basic capacitive or inductive circuits have to maintain a full load of voltage above 100 kV with frequency ~ 1 Hz, voltage multiplier circuits having transformers (storage or pulse), MARX – generator, GREINACHER cascade, XRAM generator should be considered.

- Pulse forming networks (PFN): PFN allows the generation of rectangular voltage waveforms (monopolar or bipolar) using principles of transmission lines. Transmission lines contain smaller pieces having their capacitance or inductance.

Since this book chapter is focused on the application part of PEF technology, hence only a short description is given here. For a more detailed understanding of each type of pulse generator, readers are encouraged to study the description given (Loeffler, 2006).

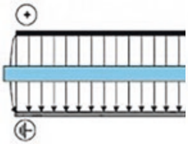
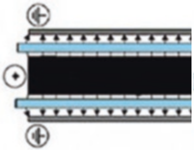
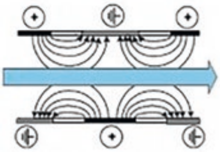
Power Switches Power switches are an important component of the pulse generator because this is at the interface between the load and devices, which stores energy. These are very expensive and control output pulse parameters and determine the modulator's speed and power ratings. Earlier material (gas/liquid) filled switches such as spark gaps, thyratron, and ignitron were mostly used in PEF processing due to their low cost and simple design. But due to today's technological advancements, semiconductor switches such as metal oxide semiconductor field effect transistors (MOSFET) and insulated gate bipolar transistors (IGBT) are very popular in PEF food processing applications. Detailed characteristics of the advantages and disadvantages of each switch type were given by Arshad et al. (2020).

13.2.1.1.2 Treatment Chambers

The treatment chamber is the heart of the PEF processing system. Since the PEF process efficiency, which mainly depends upon peak field intensity and homogeneity of treatment, is affected by the treatment chamber design. Treatment chambers based on the nature of the food (solid/liquid) and quantity of liquid processing are majorly categorized into two batches and continuous. According to electrode configuration, there are three common types of treatment chambers, i.e. parallel, coaxial, and co-linear (Kandušer et al., 2017). Characteristics of these three electrodes configuration are summarised in Table 13.1.

The parallel plate electrode configuration provides the most uniform electric field configuration, with a large electrode effective area that results in the chamber having lower electrical resistance. This may cause electrode corrosion at their interface with the liquid and also some food safety issues due to the release of metal ions into liquid food (Morren et al., 2003; Zeng et al., 2016). In the coaxial chamber configuration, the sample moves axially between two hollow cylinders, and the radial direction of the electric field is from the inside of the cylinder to the outside. This configuration can be utilized for treating materials at high electric field intensities requiring little temperature rise since there is less probability of arcing (Huang et al., 2013). Arshad et al. (2021) developed an improved coaxial chamber, which uses a static mixer to disrupt laminar flow inside the treatment zone, hence rectifying the heterogeneous electric field distribution, which otherwise causes heterogeneous temperature distribution of liquid food samples such as in fruit juices, milks etc. In the co-linear configuration, there are multiple electrode rings with alternative potentials which are isolated by multiple rings. There is a large treatment capacity

Table 13.1 Different electrode configurations (parallel plate, co-axial, co-linear) common characteristics used for PEF food processing. Adapted from Arshad et al. (2020) with permission from the publisher

Characteristics	Parallel plate configuration	Co-axial configuration	Co-linear configuration
Flow direction relative to electric field's direction	Right angle	Right angle	Parallel
Electric field distribution	Uniform	Almost uniform at large radii	Non-uniform
Electric field strength	$\frac{V}{d}$	$V \big/ r\ln\left(\frac{R_o}{R_i}\right)$	Estimation through numerical simulation
Number of electrode	2 (maximum)	2 (maximum)	2 (minimum) with an insulator
Equivalent resistance	$R = \sigma \frac{d}{A}$	$R = \frac{\ln(r_o / r_i)}{2\pi\sigma l}$	–
Equivalent capacitance	$C = \frac{\varepsilon_o \varepsilon_r A}{d}$	$C = \frac{2\pi\varepsilon_o\varepsilon_r l}{\ln(r_o / r_i)}$	–
Electrical resistance (Ω)	Low	Low	High (hundreds)
Cross-sectional geometry for continuous flow			

with heterogeneous electric field strength distribution, and the design of an insulator between electrodes is used to control the temperature in a co-linear configuration (Góngora-Nieto et al., 2002).

In PEF-induced starch modification, researchers mainly used parallel plate electrode configuration with different gaps between electrodes in both batch and continuous modes of operation, although one or two researchers used co-linear cylindrical configuration also.

13.2.1.1.3 Control and Monitoring System

The control and monitoring system helps in controlling, measuring, and keeping track of the processing variables such as voltage, treatment chamber current, temperature, and product flow rate. It is the central part of the technology, which helps in the continuous monitoring of the PEF process with all data and graphs related to uniformity in field intensity and pulse waveform about their shape, frequency, and treatment time during the whole process.

13.2.1.2 Process Parameters

13.2.1.2.1 Electric Field Strength

For PEF-induced modification of starch, there must be a requirement of minimum necessary and sufficient intensity, which brings changes in starches' structural and functional properties. This minimum intensity is termed critical PEF treatment intensity (E_c); this may include electric field strength, no. of pulses, frequency, pulse width, treatment time, pulse energy, and temperature (Giteru et al., 2018). E_c also relies on the PEF system configuration, chamber geometry, electrodes, and electric field delivery rate. Using the specific energy input (Q_{sp}) kJ/Kg, E_c can be described for the batch (Eq. 13.1) and continuous system (Eq. 13.2) (Jaeger et al., 2012; Wiktor et al., 2015) as:

For batch system

$$Q_{sp} = \frac{U.I.n.\tau}{w} = \frac{U^2.n.\tau}{R.w} \tag{13.1}$$

For continuous system

$$Q_{sp} = \frac{f}{u}\int_0^{\tau} U(t).I(t).dt \tag{13.2}$$

where U (V) represents electric voltage, I (A) is treatment chamber current, w (g) is sample weight, R (Ω) is material resistivity, τ (μs) is the pulse width, f (Hz) is frequency, u (mL/s) product mass flow rate, n (dimensionless) is no. of the pulse. However, most earlier studies haven't considered E_c while discussing their findings because of difficulties in assessing PEF parameters since they depend on the engineering design of PEF equipment. Table 13.3 shows that electric field strength between 10–50 kV/cm for monopolar and 1–10 kV/cm for the bipolar square pulse is commonly used for starch modifications.

13.2.1.2.2 Pulse Shape

During the PEF processing of foods, usually rectangular, exponential pulse waveforms with a mix of short and long-duration pulses in monopolar and bipolar modes are used. Rectangular pulses with quickly rising and descending edges are found to be most effective since low temperature increment along with the highest effective area, as shown in Fig. 13.2. However, producing a precise rectangular-shaped waveform is difficult and quite expensive. In contrast, exponential decay waveform is simpler and easy to produce. However, in the exponential decay waveform, the

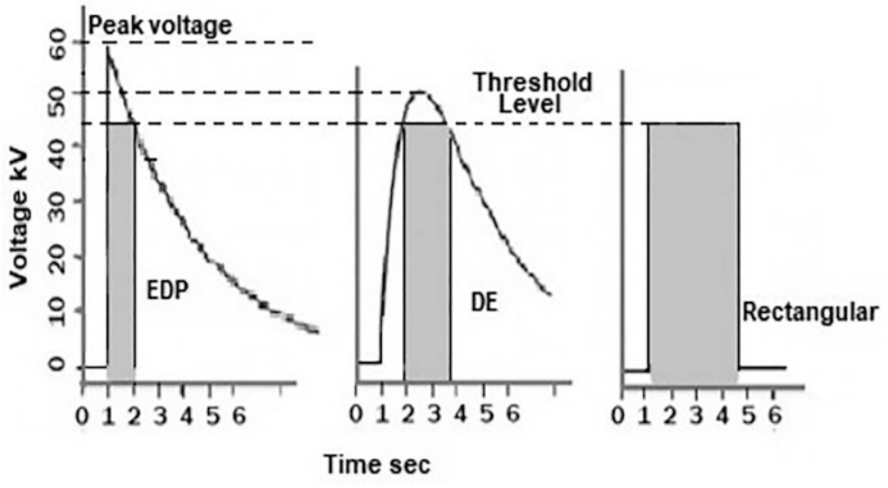


Fig. 13.2 Different waveforms (ED = exponential decaying, DE = double-exponential, rectangular) effective area (shaded) for PEF food processing. (Adapted from Arshad et al. (2019))

high-intensity electric field has a narrow useful peak, while the remainder is a long tail of the low-intensity field. Arshad et al. (2019) developed a double exponential (DE) waveform system to get rid of the trade-off between exponential decay and rectangular pulse waveform. Because DE's effective area lies between exponential decay and rectangular pulse, as shown in Fig. 13.2, DE is more efficient than exponential decay as it reduces the power loss of the electrical system at a reduced cost.

The applied pulse's shape is controlled by the treatment chamber containing food samples impedance and the pulse-forming network. Also, the shape of the pulse is affected by the changing conductivity of the food samples. Biopolar modulator designing is very complex and more expensive than monopolar one. But they offer a certain advantage in keeping the quality of electrodes for a longer time since swapping anode/cathode prevents the charge accumulation, hence slowing down the electrode degradation by the electrolysis.

13.2.1.2.3 Pulse Width

Pulse width is influenced by both sample resistivity and discharge circuit. It is an inverse relationship with electric field strength, i.e. we can get similar results with high field intensity with narrow pulse width and smaller field intensity with broader pulse width. Additionally, treatment intensity at a fixed electric field strength increased either by increasing pulse width or frequency. Typically, a pulse width between 1–50 μs is used for starch modification applications. Table 13.3 shows the different pulse widths used by researchers for different starches.

13.2.1.2.4 Pulse Repetition Rate

Pulse repetition rate, also known as pulse frequency, contributes to treatment time and pulse width. Solid-state power switches are recommended for higher frequency. However, higher switching losses exist when operating at higher frequencies in a high-voltage pulse generator. Usually, starch modification is done between 100 and 1000 Hz frequency range, as shown in Table 13.3.

13.2.1.2.5 Flow Rate

Starch suspension flow rate is important for continuous PEF processing processes. It defines the total residence time inside the treatment chamber. Therefore, it has an important impact on designing treatment chambers and pulsed power supplies (Arshad et al., 2020). A uniform velocity profile distribution is required for homogenous treatment and uniform temperature distributions. Usually, homogeneous electric field distributions require turbulence in the treatment chamber.

13.2.1.2.6 Temperature Variations

Although PEF processing is non-thermal, a fair amount of heat is generated from converting electrical energy into heat, causing ohmic heating. This heating leads to additional conformational changes in starches, which causes some swelling, slight gelatinization, or weak gel-like structure formation. Han et al. (2009a, b) reported PEF treatment results in the gel-like structure of corn and potato starch particles during the PEF treatment. Although in both cases, authors mentioned the controlling of temperature below 50 °C using water-bath during PEF treatment. However, the rapid nature of the process could make peak temperature undetected. Hence, heating could be considered a reason for this and other PEF treatment effects. The heating of starch granules played an important role in their modification. Since heating starch in aqueous systems increases the motions of molecular chains and helical structure, which weakens their crystalline structures.

13.2.2 Mechanism of Starch Conformational Changes

The mechanism elucidating starch conformational changes by the PEF system is not clearly understood yet. So far, researchers have given only two hypotheses, i.e., electrochemical and electrolysis reactions and biomolecule polarization, to get a basic initial understanding of the phenomenon. These are discussed in the following section.

13.2.2.1 Electrochemical and Electrolysis Reactions

From an electrochemical viewpoint, the PEF system functions as an electrolytic cell containing two metal electrodes connected to the high-voltage pulse generator, which is in direct contact with an aqueous solution (electrolytic). A double layer consisting of charged particles or oriented dipoles will be formed at the electrode-solution interface due to the electro-neutrality condition, which behaves as an electrical capacitor, even without external electric field application. This double-layer formation results in a potential drop ($\Delta\Phi_{dl}$) in (mV) magnitude across the electrode-solution interface when no external voltage is supplied (Morren et al., 2003). The use of an external electric field during PEF treatment raises the charge density and thickness (x_{dl}) of the ionic layer causing an increased potential drop ($\Delta\Phi_{dl}$). When this double-layer potential crosses the threshold voltage or standard reduction potential (E^{o}_{red}) of reactive species reaction potential (~ 1–2 V), two independent electrochemical half-cell reactions oxidation half-cell reactions and reduction half-cell reactions occurred (Pataro et al., 2017). During monopolar pulse application, these oxidation and reduction half-cell reactions happened at the surfaces of high voltage electrode (behaving as the anode) and the grounded electrode (behaving as the cathode).

The following equations give some instances of these electrochemical reactions that take place at the electrode-solution interface.

Cathodic half-reactions:

$$Cl_{2(gas)} + 2e^- \rightarrow 2Cl^- \tag{13.3}$$

$$O_{2(gas)} + 4H^+ + 4e^- \rightarrow 2H_2O_{(liquid)} \tag{13.4}$$

$$O_{2(gas)} + 2H^+ + 2e^- \rightarrow 2H_2O_{2(liquid)} \tag{13.5}$$

$$2H^+ + 2e^- \rightarrow H_{2(gas)} \tag{13.6}$$

$$2H_2O_{(liquid)} + 2e^- \rightarrow H_{2(gas)} + 2OH^-_{(aq)} \tag{13.7}$$

Anodic half-reactions:

$$M_s \rightarrow M^{n+}_{(aq)} + ne^- \left(\mathrm{M} = Fe, Ni, Pt, Cr, Mn \ldots.\right) \tag{13.8}$$

$$2H_2O_{(liquid)} \rightarrow O_{2(gas)} + 4H^+ + 4e^- \tag{13.9}$$

$$2H_2O_{(liquid)} \rightarrow 2H_2O_{2(liquid)} + 2H^+ + 2e^- \tag{13.10}$$

These electrochemical reactions cause partial electrolysis of water around electrodes, producing gases (hydrogen and oxygen) as well as ions (H^+ and OH^-).

Moreover, these electrochemical and electrolysis reactions cause disaggregation or aggregation of suspended starch particles. Wang et al. (2016) reported konjac glucomannan-tungsten (KGM-T) hydrogel formation at the anode during 10–40 V direct current (dc) application. Authors attributed this to the reaction of isopropyl-tungstic acid ions with the –OH (C-6 position) on KGM for bridge formation. These isopropyl-tungstic acid ions formed between water electrolysis protons (H^+) products and tungstate (WO_4^{2-}) molecule ionization.

13.2.2.2 Starch Molecules Polarization

External electric field application during PEF treatment could alter the starch suspension system's electronic relaxation stabilization energy (charge). This perturbates the electronic configuration of starch molecules; hence destabilization occurs. The destabilization upsets the dipole moment configuration of starch, which results in change in the dielectric constants of starch and hence, the polarization of starch molecules (Giteru et al., 2018).

13.2.3 Energy Consumption During PEF Treatment of Starch

Energy requirement during food processing is an important consideration for the application of any novel technologies at an industrial scale. However, very limited studies exist on energy consumption during starch modification by PEF. Han et al. (2012b) calculated the energy requirement during the PEF modification of tapioca starch, shown in Table 13.2.

Authors used the following (Eq. 13.11) for energy input to PEF equipment

$$Q_{in} = E^2 \bullet t \bullet \sigma \tag{13.11}$$

$$t = n.\tau \tag{13.12}$$

$$\mathrm{n} = \frac{V.f}{u} \tag{13.13}$$

Table 13.2 Energy consumption analysis during PEF treatment of tapioca starch at different electric field strengths. Reproduced from Han et al. (2012b) with permission from the publisher

Electric field intensity (kV/cm)	Solution temperature rise (°C)	Input energy, Q_{in} (J/g)	Dissipated energy, Q_s (J/g)	Starch energy consumed, $Q_{in} - Q_s$ (J/g)
30	4.7 ± 0.1	28.85 ± 0.03	19.59 ± 0.05	9.26 ± 0.04
40	6.3 ± 0.2	51.29 ± 0.07	26.58 ± 0.09	24.71 ± 0.08
50	7.3 ± 0.1	80.14 ± 0.09	30.78 ± 0.11	49.36 ± 0.10

where Q_{in} represents energy input (J/m^3), E is electric field strength (V/m), σ is the samples electrical conductivity (S/m), and t is total treatment time (s), which is calculated using the number of pulses (n) and pulse duration, τ (μs) (Eq. 13.12). And, the number of the pulse is calculated using (Eq. 13.13) where V is the treatment chamber volume (mL), f is the pulse frequency (Hz), and u represents starch suspension flow rate (mL/s). The energy dissipated in the solution during treatment (Q_s) is given by (Eq. (13.14), where T is the increase in solution temperature (°C), and C_s is the solution-specific heat (J/kg/ °C) which is given by Eq. 13.15. C_w is the water specific heat (4200 J/kg/ °C), and X is the tapioca starch moisture content (Eq. 13.15).

$$Q_s = T \bullet C_s \tag{13.14}$$

$$\mathrm{C_s} = \mathrm{C_w} - \frac{Cw.X}{100} \tag{13.15}$$

As shown in Table 13.2, it was concluded that increasing energy input (Q_{in}) to the PEF system increased the starch energy consumption ($Q_{in} - Q_s$), since at 50 kV/cm, energy used for starch modification was 62%, which is much higher as compared to 30 kV/cm, where 31% input energy used. Therefore, more energy is used for starch modification at higher electric field strength. More studies are required to validate this concept of energy utilization during PEF treatment fully.

13.3 PEF Assisted Physical Modification of Starch

13.3.1 Structural Changes

13.3.1.1 Starch Morphological Changes

PEF treatment significantly changes the morphology of starch granules. Table 13.3 shows an overview of different starches modified by different PEF treatments. Zeng et al. (2016) observed through scanning electron microscope (SEM) analysis that some surface roughness emerged on the native irregular polygon structure of waxy rice starch at low EFI, 30 kV/cm. With increasing intensity, some pits on the surface and granules aggregation were observed. However, at high EFI, 50 kV/cm, complete distortion of granules and congregation were observed, as shown in Fig. 13.3.

Similar results were seen for tapioca starch (Han et al., 2012b); for potato starch Han et al. (2009a). Maniglia et al. (2021b) observed from optical microscopy that changes in the internal structure of cassava starch showed a reduction in birefringence but no changes in granule morphology. Whereas some damage and fractures on the wheat granule's surface. Li et al. (2019) observed no morphology changes for potato, wheat, and pea starch observed under polarized light microscopy, which might be related to the low EFI (2.86–8.57 kV/cm) applied as compared to other researchers.

Table 13.3 An overview of physical modification of different starches by PEF

Starches	Crystal type	Starch suspension	PEF conditions	Major outcomes	References
Corn starch	A type	8%	Electric field strength: 30, 40 & 50 kV/cm Wave form: Square Pulse duration: 40 μs Pulse frequency: 1008 Hz	Destruction of starch granules, congregation, and gel like structure formation at higher intensity Crystallinity decreased after treatment. Peak, breakdown, and final viscosity decreased	Han et al. (2009b)
Maize starch	A type	8% (w/w)	Electric field strength: 30, 40 & 50 kV/cm Wave form: Square, bipolar Pulse duration: 10 μs Pulse frequency: 1 k Hz Electrode gap: 0.30 cm Number of pulses: 20.16 Treatment time: 424, 848 and 1272 μs	Molecular weights (M_w, M_n) decreased with increasing field intensity and treatment time. Decreased gelatinization temperatures (T_o, T_p) and enthalpy (ΔH) with increasing field intensity and treatment time.	Han et al. (2012a)
Rice starch (waxy)	A type	10% (w/w)	Electric field strength: 30, 40 & 50 kV/cm Wave form: Square, bipolar Pulse duration: 40 μs Pulse frequency: 1 k Hz	Surface roughness and aggregation of granules at higher intensity No interruption of glycosidic bonds RDS content increased, while SDS and RS decreased	Zeng et al. (2016)
Japonica rice starch	A type		Electric field strength: 2.86, 5.71 & 8.57 kV/cm Wave form: Square Pulse width: 6 μs Pulse frequency: 600 Hz Residence time: 90 s Electrode gap: 3.5 cm	Sunken areas on outer structure of starch granules after PEF Rearrangement of short-range structures at higher intensity Molecular chain broken at higher intensity. RDS content increased, while SDS decreased and no significantly changes in RS	Wu et al. (2019)

(continued)

Table 13.3 (continued)

Starches	Crystal type	Starch suspension	PEF conditions	Major outcomes	References
Glutinous rice starch	A type		Electric field strength:3 kV/cm Pulse duration: 40 µs Pulse frequency: 1 k Hz Number of pulses: 50–300	Partial gelatinization of rice sample after 200 & 300 pulses Increased swelling degree after treatment Decreased gelatinization temperature (T_o) and enthalpy (ΔH)	Qiu et al. (2021)
Tapioca starch	C type	8% (w/w)	Electric field strength: 30, 40 & 50 kV/cm Wave form: Square, bipolar Pulse duration: 10 µs Pulse frequency: 1 k Hz Electrode gap: 0.30 cm Number of pulses: 21.37 Treatment time: 214 µs	Damaged and dissociated starch granules Transformation of crystalline structure to amorphous after PEF Decrease in PV and BD viscosity	Han et al. (2012b)
Wheat and cassava starch	A type pattern for both	8% (w/v)	Electric field strength and energy input: 15 kV/cm- 15 kJ/kg, 25 kV/cm- 25 kJ/kg & 25 kV/cm- 50 kJ/kg Wave form: Square, monopolar Electrode gap: 4 mm Co-linear cylindrical chamber	Depolymerization of starches, more pronounced for cassava Relative crystallinity first increased and then decreased at high field intensity for both starches Increased gel firmness for both starches, more pronounced effect for wheat starch Retrograde ability lowered for cassava starch, while increased for wheat starch at high intensity	Maniglia et al. (2021b)

(continued)

Table 13.3 (continued)

Starches	Crystal type	Starch suspension	PEF conditions	Major outcomes	References
Potato starch	B type	8% (w/w)	Electric field strength: 30, 40 & 50 kV/cm Wave form: Square, unipolar Pulse duration: 40 μs Pulse frequency: 1008 Hz Electrode gap: 0.30 cm	Dissociated starch granules, with gel like structure formation Crystallinity decreased at higher field intensity Decreased PV and BD viscosity	Han et al. (2009a)
Potato starch in potatoes			Electric field strength: 0.5, 0.7, 0.9 & 1.1 kV/cm Wave form: Square, bipolar Pulse width: 20 μs Pulse frequency: 100 Hz Number of pulses: 900–6250 Electrode gap: 80 mm	No change in crystallinity of isolated and on potato surface starches Narrow gelatinization temperature range for starch on potato surface Digestibility of starch on potato surface decreases at high field intensity, while no changes for isolated starch	Abduh et al. (2019)
Wheat, potato and pea starch	Wheat- A type, potato-B and pea- C type		Electric field strength: 2.86, 4.29, 5.71, 7.14 & 8.57 kV/cm Wave form: Square, bipolar Pulse width: 6 μs Pulse frequency: 600 Hz Resident time: 90 s Electrode gap: 3.5 cm	No change in morphology of three starches Molecular weight changes in wheat and pea starch, while potato remained stable Degree of short range ordered structure reaches maximum with increasing intensity, then decreases for all starches Increase in RDS content, while SDS and RS decreases in all three starches	Li et al. (2019)

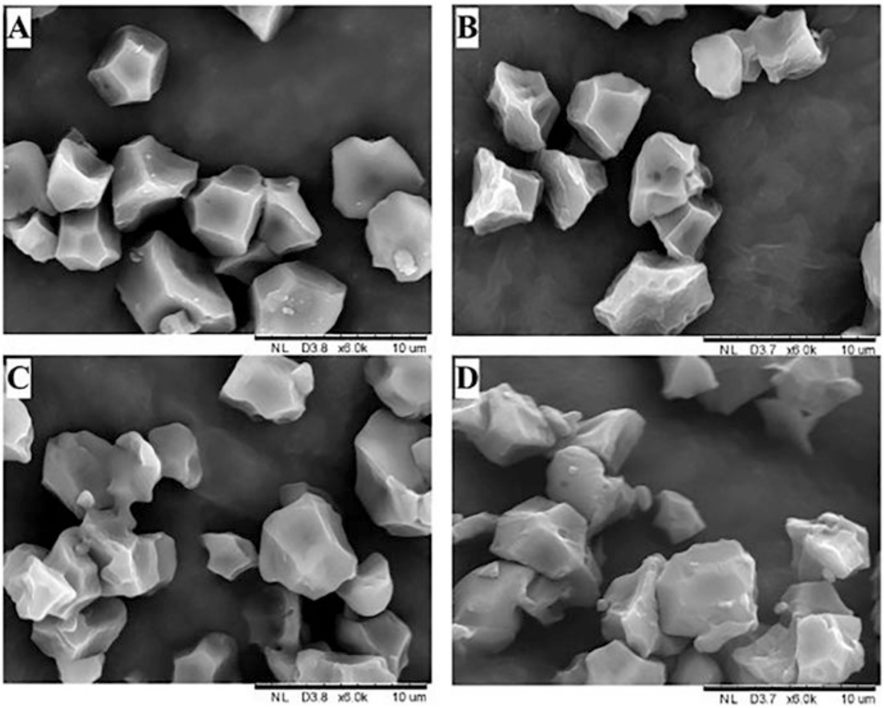


Fig. 13.3 SEM images of PEF treated waxy rice starch: (**a**) Native, (**b**) 30 kV/cm, (**c**) 40 kV/cm, and (**d**) 50 kV/cm. (Adapted from Zeng et al. (2016) with permission)

13.3.1.2 Crystalline Structure Changes

X-ray diffraction (XRD) analysis is used to investigate changes in the long-range crystalline structure of starches. Starch crystallinity is majorly governed by: (1) the amount of amylopectin, (2) the length of the amylopectin chain, (3) double helix orientation within crystallites, and (4) crystallite size. Zeng et al. (2016) observed for waxy rice starch that relative crystallinity (RC) decreased from 38.1% to 28.7% with increasing EFI (30–50 kV/cm), which might be related to disorganization of amylopectin crystalline as displayed by decreased in gelatinization enthalpy. Similar results of RC decrease were observed for wheat and cassava starches (Maniglia et al., 2021b); for tapioca starch from 24.2% to 7.2% (Han et al., 2012b). Interestingly, for japonica starch, Wu et al. (2019) observed that RC first increased at low EFI (2.86–5.71 kV/cm), whereas when EFI reached a critical point (8.57 kV/cm) then it decreased. Similar results were seen for wheat, potato, and pea starch. But more decreases in RC were observed for potato starch which might be related to the phosphate ions presence, which transmit a better electric pulse (Li et al., 2019).

13.3.1.3 Lamellar Structure Changes

Small-angle X-ray scattering (SAXS) technique was used to characterize the semi-crystalline lamellar structure of starch. Fractal structure (D) is used to quantify the irregularities of substances, and for starches, it is classified as a mass dimension (D_m), which used an indicator of the degree of smoothness and surface dimension (D_s), which is used to predict compactness. For waxy rice starch, Zeng et al. (2016) observed 9 nm lamellar decreased with increasing PEF intensity, suggesting the destruction of amorphous starch inside amorphous growth rings. Also, semi-crystalline lamella thickness (d) increased with increasing PEF intensity. Wu et al. (2019) observed that for japonica rice starch, d and I increased with increasing EFI, then decreased, indicating that higher EFI caused perturbation to double helix structure in semi-lamella and difference in electron cloud density between amorphous and crystalline regions. Li et al. (2019) observed that wheat, potato, and pea starch showed mass fractals structure and PEF treatment significantly influenced the wheat and potato starch, scattering and self-similar fractal dimension structures, but not for pea starch, as shown in Fig. 13.4.

13.3.1.4 Molecular Order Structure Changes

Fourier transform infrared spectroscopy (FTIR) is used to obtain short ranged molecular order structure changes in less than 2 μm beneath the surface of starch granules. The region between 1200–900 cm^{-1} bands provide information about the fingerprint region of starches. The ratios of band intensity at 1040 cm^{-1} and 1020 cm^{-1} ($R_{1040/1020}$) could be applied to the short-ranged molecular ordered structure. The higher $R_{1040/1020}$ ratio, the more the short ranged ordered structure. For wheat and cassava starch, Maniglia et al. (2021) observed that PEF decreased the $R_{1040/1020}$ ratio indicating affecting short ranged ordered structure as destruction of crystalline structure. Similar results were seen for japonica rice starch, where until 5.71 kV/cm, there was no significant changes in $R_{1040/1020}$ but further increasing EFI disrupted the short ranged molecular structure (Wu et al., 2019). Interestingly, $R_{1040/1020}$ for wheat, potato, and pea starch increased with increasing EFI and then decreased after reaching maximum as compared to control. Also, potato starch showed more variations than wheat and pea starch (Li et al., 2019).

13.3.2 Functional and Physiological Changes

13.3.2.1 Thermal Properties

Thermal properties of starch are closely related to the structure of granules, like gelatinization temperatures and enthalpy related to the crystallinity degree and amylopectin structure. Thus, any alterations in the structure of starch affected their thermal properties. Han et al. (2012a) observed gelatinization temperatures (T_o, T_p) and

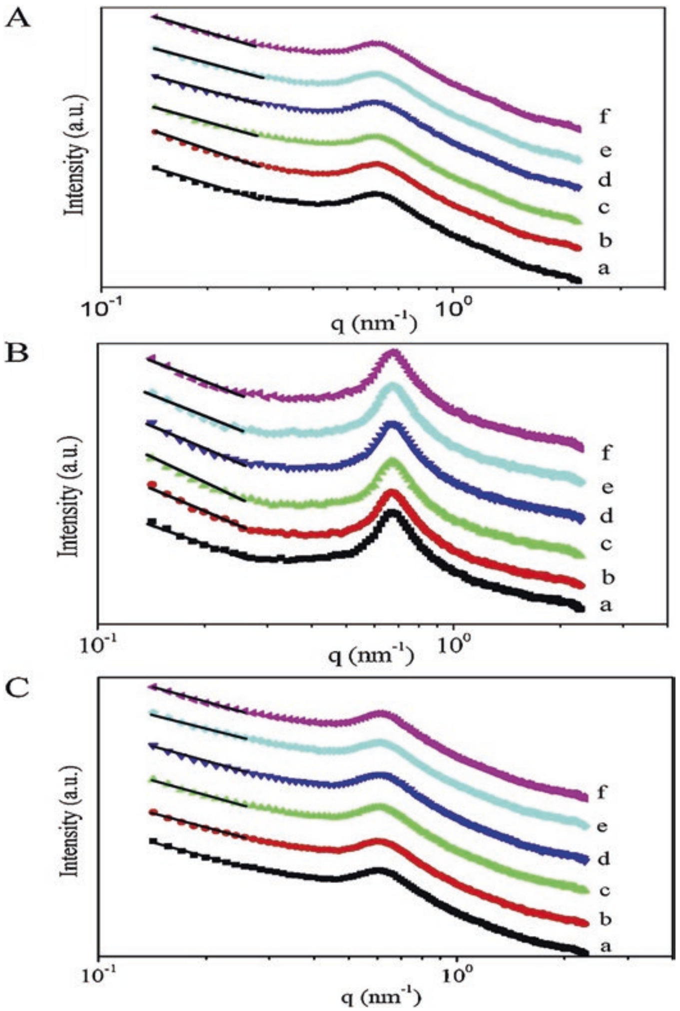


Fig. 13.4 Double logarithmic scale SAXS curves of wheat starch (**a**), potato starch (**b**), and pea starch (**c**) treated with PEF.: a control (0 kV/cm), b, c, d, e, and f refer to 2.86, 4.29, 5.71, 7.14, and 8.57 kV/cm, respectively (Li et al., 2019)

gelatinization enthalpy (ΔH) decreased for maize starch with increasing EFI intensity. Also, narrow gelatinization temperature range with increasing intensity and treatment time, indicating strong cohesion between crystallites and homogeneous stability. Similar results were observed for potato starch, where significant decrease in T_o and ΔH with increasing PEF treatment intensity (Han et al., 2009a); for tapioca starch (Han et al., 2012b); for cassava and wheat starch, a significant decrease in T_p and ΔH (Maniglia et al., 2021b). Whereas, PEF didn't cause any effect on thermal stability of starches, like Han et al. (2012a) observed for maize starch no chemical degradation after PEF from 30 to 50 kV/cm.

13.3.2.2 Pasting Properties

Starch pasting properties could be classified as: peak viscosity, breakdown, final viscosity, and setback viscosity. Peak viscosity (PV) is related to water absorption of the granules. Breakdown viscosity (BD) is related to the thermal stability of the paste, final viscosity is the viscosity obtained after cooling in the last stage, and setback viscosity (SB) is related to the retrogradation degree (Chen et al., 2014; Vandeputte et al., 2003). Han et al. (2009a) reported increased pasting temperatures (PT) from 58.9 °C (native) to 59.7 °C (50 kV/cm) of potato starch after PEF treatment. In addition, PEF treatment decreased the PV and BD of potato starch, indicating increased thermal stability with increasing EFI from 30 to 50 kV/cm. Also, PEF treatment reduced the retrogradation tendency of potato starch paste during cooling. Similar observations were reported for the PEF treatment of corn starch (Han et al., 2009b). Maniglia et al. (2021b) found PEF treatment (15 and 25 kV/cm) significantly reduces PV for both wheat and cassava starch, indicating lowering swelling (water holding capacity). While non-significant changes were observed in PT, BD, and final viscosity for both starches. In addition, PEF significantly reduces SB for cassava starch but increases for wheat starch, indicating lower retrograde ability for cassava starch, while higher for wheat starch. Qiu et al. (2021) found increasing PEF treating pulses from 50 to 300 at a constant 3 kV/cm of glutinous rice starch, results in decreasing PV. Whereas in-significant changes in PV, trough viscosity, PT, and BD were found for low EFI (2.86, 5.71, 8.57 kV/cm) PEF treated Japonica rice starch.

13.3.2.3 Digestibility Changes

Starch digestibility is a very complex process. And based on digestibility, starch can be classified into different proportions, such as rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). RDS is the portion of starch that can be digested in the first 20 min, SDS between 20–120 min, and RS is the remaining portion that cannot be hydrolyzed in the small intestine by the enzymes and passed to the large intestine. The digestibility of starch is significantly affected by the PEF treatment.

Wu et al. (2019) found that RDS content increased while SDS content decreased, and no significant changes in RS of Japonica rice starch with increasing PEF intensity (2.86 to 8.57 kV/cm). Li et al. (2019) studied the effect of PEF treatment on the digestibility of different crystalline-type starches (Type A: wheat starch, Type B: potato starch, Type C: pea starch). Authors found that RDS content increased significantly from 37.9% (control) to 44.16% (8.57 kV/cm), SDS content decreased from 39.46% (control) to 35.37% for wheat; similarly, for potato starch, RDS content increased from 24.78% (control) to 35.95% (8.57 kV/cm), SDS content decreased from 45.68% (control) to 39.59% (8.57 kV/cm); and for pea starch, RDS content increased from 50.88% (control) to 59.40% (8.57 kV/cm). Thus, PEF increased the overall digestibility of wheat, potato, and pea starch.

13.4 PEF Assisted Chemical Modification of Starch

PEF treatment, apart from the physical modification of starch, also assisted in the chemical modification of starch. PEF assisted dual chemical modification of starch offers advantages of both techniques. Since the new chemical group could be easily inserted in the starch molecule by chemical modification by eliminating problems that occurred during traditional chemical modification, like low reaction yield, reagent overdose, and time-consuming process, through increasing accelerating mobility of ions by PEF. Therefore, an increased degree of substitution (DS) could be easily seen in PEF assisted chemically modified starch. Hong et al. (2018b) found that increasing PEF treatment EFI from 1.25 kV/cm to 5 kV/cm, has increased DS of acetic anhydride (AA) substitution in potato starch from 0.075 to 0.096 as compared to conventional (only chemical) treatment. Similarly, the highest DS of 0.131 was found at 2 kV/cm PEF treatment during cassava starch acetylation with AA (Hong et al., 2016b). Table 13.4 gives an overview of PEF assisted chemical modification used for different starches.

13.4.1 Structural Changes

13.4.1.1 Morphological Changes

Hong et al. (2018a) studied the esterification of maize starch with different amylose content (< 1%, 23%, 50% and 80%) with AA using conventional and PEF (2.5 kV/cm at 1000 Hz frequency) assisted dual modification methods. It was found that the smooth polygonal surface of native maize starch transformed into a deformed shape with bumps which was higher for starch with (>50% amylose content) and dual modified than conventionally esterified. Similarly, Chen et al. (2021) found oval-shaped granules with smooth surfaces of potato starch changed after PEF assisted Octenyl succinic anhydride (OSA) modification having minor imperfections with a rough surface with conventionally modified. The reasons behind these surface imperfections or shape deformations were the combined treatment of chemical (acetylation/esterification) and irreversible electroporation by PEF treatment.

13.4.1.2 Crystalline Structure Changes

Esterification of starch granules, whether conventionally or PEF assisted chemical modification, involved changes in the granule's crystallinity. Hong et al. (2016b) reported RC decreased from 33.3% to 29.7% for PEF assisted AA acetylation of cassava starch with increasing DS from 0.085 to 0.131. Similarly, for PEF assisted chemical modification (OSA) of potato starch, RC decreased from 18.11% to 14.46%, increasing EFI from 2 to 6 kV/cm (Chen et al., 2021). This decreased in

Table 13.4 An overview of PEF assisted chemical modification of starches

Starches	Chemical agent	Starch suspension	PEF conditions	Major changes	References
Potato starch	Acetylation, acetyl group	30, 35 & 40% (w/w) Electrical conductivity: 11 mS/cm	Electric field strength: 2, 3.5 & 5 kV/cm Wave form: Square, bipolar Pulse duration: 40 µs Pulse frequency: 1000 Hz Electrode gap: 0.30 cm	DS increased with increasing field strength Deformation of granules with increased bulges and surface roughness Lowered breakdown and setback values compared to non-PEF assisted acetylation PEF assisted acetylation decreased solubility, swelling power, retrogradation and freeze thaw stability	Hong et al. (2016a)
Potato starch	Esterification, Octenyl succinic anhydride (OSA) 3%	35% (w/w)	Electric field strength: 2, 3, 4, 5 & 6 kV/cm Wave form: Square, bipolar Pulse width: 40 µs Pulse frequency: 1000 Hz Electrode gap: 0.30 cm	Increased surface debris, lost envelop protection and complete morphology lost at high field intensity Molecular structure disorder increased with PEF intensity Greater decrease in gelatinization temperatures (T_o, T_c) and enthalpy (ΔH) of PEF assisted esterification than conventional	Chen et al. (2021)
Potato starch	Esterification, acetic anhydride 6%	35% (w/w) electrical conductivity: 11 mS/cm	Electric field strength: 1.25, 2.5, 3.75, & 5 kV/cm Wave form: Square, bipolar Pulse width: 40 µs Pulse frequency: 1000 Hz Electrode gap: 0.30 cm	DS increased with increasing field intensity and highest DS of 0.096 at 5 kV/cm Deformation of starch granules as increased protrusions and pits Increased SDS content	Hong et al. (2018b)

(continued)

Table 13.4 (continued)

Starches	Chemical agent	Starch suspension	PEF conditions	Major changes	References
Wheat starch	Esterification, acetic anhydride 6% (w/w)		Electric field strength: 2.5 kV/cm Pulse frequency: 1000 Hz	Reduced gel hardness for all starches Decreased peak viscosity for TS and AS, while for BS increased	Hong et al. (2020c)
Cassava starch	Acetylation, acetic anhydride 6, 8 & 10% (w/w)	35% (w/w) electrical conductivity: 11 mS/cm	Electric field strength: 1.5, 2 & 2.5 kV/cm Wave form: Rectangular, bipolar Pulse duration: 40 µs Delay time: 460 µs Pulse frequency: 2000 Hz Electrode gap: 0.30 cm	Maximum DS 0.131 at 2 kV/cm, 51 ms and 10% acetic anhydride Increased solubility and swelling power of starch Lower peak viscosity, breakdown and setback value than native starch Increased freeze thaw stability and better modification as compared to conventional	Hong et al. (2016)
Maize starch	Esterification, acetic anhydride 6% (w/w)		Electric field strength: 2.5 kV/cm Pulse frequency: 1000 Hz	Crystalline structure of waxy starch changed from A to B type by PEF assisted dual modification Higher crystallinity and short range ordered structure for PEF assisted dual modification than conventional Solubility increased with increasing amylose content and with PEF assisted RDS, SDS decreased and RS increased for normal, 50% & 80% amylose content, while reverse trend observed for waxy	Hong et al. (2018a)

(continued)

Table 13.4 (continued)

Starches	Chemical agent	Starch suspension	PEF conditions	Major changes	References
Maize starch	Esterification, acetic anhydride 6% (w/w)		Electric field strength: 2.5 kV/cm Pulse frequency: 1000 Hz	DS increased with increasing amylose content and PEF assisted esterification compared to conventional one Higher amylose (> 50%) starches with PEF assisted esterification enhanced elasticity of dough sheets Decreased RDS and RS of cooked noodles with adding PEF dual modified starches compared to conventional	Hong et al. (2020a)

RC was caused by the disruption of crystalline structure of amylopectin due to OH groups substitution by the OSA groups, hence inter- and intramolecular hydrogen bonding decreased and also RC. Whereas changes in crystal pattern were observed for waxy maize starch as the A-type pattern changed into B- type with a reduction in RC from 22.8% to 17.6% after PEF assisted chemical modification (Hong et al., 2018a).

13.4.1.3 Lamellar Structure Changes

PEF assisted chemical modification (acetylation) changed the fractal structure of cassava starch from surface to mass as the dimension of the fractal structure increased from 1.74 to 2.69 with increasing DS 0.085 to 0.131 (Hong et al., 2016b). Hong et al. (2018a) reported that PEF-assisted acetylation of waxy maize starch decreased the thickness of alternative repeating distance of amorphous and crystalline lamella, as changes in first peak intensity from SAXS analysis. This was due to crystalline amylopectin branches damaged by the PEF treatment. Hong et al. (2020b) investigated the behavior of different-sized wheat granules, large (A-type) and small (B-type), on esterification by conventional and PEF assisted dual chemical modification. Results reported that diffraction intensity at $q = 0.68\ nm^{-1}$ increased after PEF (2.5 kV/cm for 1000 Hz) assisted dual modification while decreased for conventional esterified. This indicates the destruction of starch granules by PEF treatment.

13.4.1.4 Molecular Order Structure Changes

FTIR spectroscopy is used to evaluate the occurrence of chemical modification by determining the presence of characteristics of carbonyl group peak in the FTIR spectra and starch granules short ranged molecular order structure. Hong et al. (2016a) reported the presence of an acetyl group band at 1730 cm^{-1} in PEF assisted acetylation of potato starch. Reduction in $R_{1040/1020}$ ratios with increasing EFI from 2–6 kV/cm and characteristics peaks for ester carbonyl stretching (C=O) and carboxyl groups (COONa-) were observed at 1728 cm^{-1} and 1567 cm^{-1} (Chen et al., 2021). Whereas, higher $R_{1040/1020}$ were reported for higher amylose content maize starches than conventional ones during PEF assisted dual modification of maize starch, indicating increased short-ordered structure (Hong et al., 2018a).

13.4.2 Functional and Physiological Changes

13.4.2.1 Solubility and Swelling Power

Hong et al. (2016a) reported for PEF-assisted esterified cassava starch that solubility (%) and swelling power (g/g) increased up to 17% and 33.9 g/g as compared to native cassava starch, 10.5%, and 24.2 g/g. This increase in solubility and swelling power is related to the electrostatically binding of water molecules by acetyl groups. For different amylose-content maize starches, Hong et al. (2018a) observed higher solubility for PEF-assisted esterification of waxy and high amylose maize (>80%) starch as compared to native and conventional esterified starches. While swelling power of PEF-assisted esterified starches was lowered than conventional esterified starches but higher than native starches. PEF assisted esterification has been reported to decrease potato starch's solubility and swelling power (Hong et al., 2016b).

13.4.2.2 Freeze Thaw Stability

Freeze-thaw stability is measured in terms of % syneresis. Hong et al. (2016b) reported PEF-assisted acetylated cassava starch to have better freeze-thaw stability as compared to conventional chemical acetylated starch. This is due to amylose chains re-association prevention by acetyl groups, preventing water loss. Similarly, for large A-type and small B-type wheat granules, low syneresis value was obtained after PEF-assisted dual modification as compared to native and conventional chemical acetylated (Hong et al., 2020b). Whereas, lower freeze-thaw stability was observed for potato starch acetylation assisted by PEF with high DS (Hong et al., 2016a).

13.4.2.3 Thermal Properties

Chen et al. (2021) reported gelatinization temperatures (T_o and T_f) and enthalpy (ΔH) of PEF assisted OSA treated potato starch decreased more as compared to conventional esterified potato starch and this decrease more with increasing EFI intensity. Similar results were seen for PEF assisted esterification of cassava starch (Hong et al., 2016b). Thermo-gravimetric analysis (TGA) analysis was used to measure starch thermal stability. Hong et al. (2020b) reported that PEF assisted esterification of wheat starch granules decreased their thermal stability with increasing DS compared to native and conventional esterified. Hong et al. (2016b) observed PEF-assisted esterified cassava starch with high DS lower thermal stability as compared to conventional esterified. Also, higher amylose content maize starches showed poor thermal stability as compared to waxy maize starch (Hong et al., 2018a, b).

13.4.2.4 Pasting Properties

Chen et al. (2021) reported PEF-assisted OSA esterification of potato starch decreased PV with increasing EFI from 0 to 4 kV/cm, indicating the lower swelling performance of PEF-assisted OSA esterified granules. While BD first decreased from 0 to 4 kV/cm, then increased with increasing EFI from 5 to 6 kV/cm, indicating lower granules destruction at lower EFI (0 to 4 kV/cm), whereas higher destruction at 6 kV/cm. SB was lowered as compared to native starch showing lower retrogradation, which was caused by steric hindrance caused by the addition of a chemical group, hence preventing the re-association of amylose molecular chains. Similar observations were made by Hong et al. (2016a) for PEF assisted esterification of potato starch.

13.4.2.5 Digestibility Changes

Hong et al. (2020) observed for PEF-assisted acetylation of A-type and B-type wheat granules that digestibility increased, lowered RS (37.44%) for A-type as compared to conventional esterified (39.61%) and native large A-type granules (48.46%). Similar results were seen for PEF-assisted esterification of potato starch as with increasing EFI from 1.25 kV/cm to 5 kV/cm, RDS increased from 36.75% to 55.65%, SDS from 8.70% to 14.12% (Hong et al., 2018b).

13.5 PEF Modified Starch Applications in Food System

An innovative application of PEF-modified starch is used as a potential for 3D food printing (Maniglia et al., 2021b). Figure 13.5 depicts the different 3D-printed shapes produced from wheat starch and cassava starch hydrogels. Results from texture

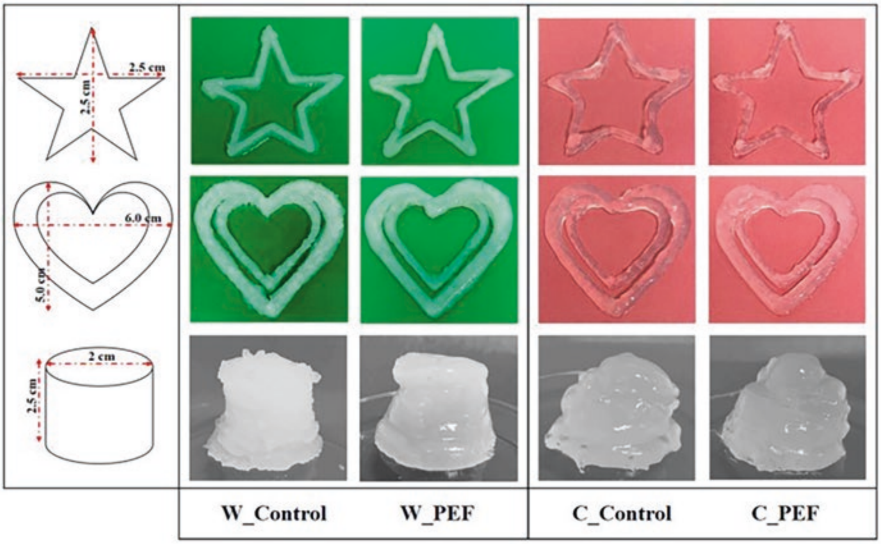


Fig. 13.5 Different shaped 3D printed hydrogel structures; Control (W_Control and C_Control) and PEF modified starches (W_PEF and C_PEF). (W = wheat starch, C = cassava starch). (Adapted from Maniglia et al. (2021b))

analysis showed that PEF-treated wheat starch hydrogels found higher hardness, and lower adhesiveness, whereas there was not significant ($p < 0.05$) difference in cohesiveness (structure retention between first and second chew), springiness (hydrogels recovery between the end of first chew and starting of second chew), and chewiness (hydrogels masticating energy). Whereas, for cassava starch, not many differences were observed between the control and PEF treated. Therefore, the authors concluded that PEF treatment improved the printability of wheat starch hydrogels, which also depends on the source of starch.

Hong et al. (2020c) used PEF-assisted esterified total, A-type, and B-type wheat starch for noodles production. It was observed that adding esterified starch with PEF assistance significantly changes the noodles cooking characteristics, as the water absorption rate increased as compared to traditional esterified starch. Cooking loss of noodles also decreased after the addition of PEF-assisted esterified starch and this result is more prominent in B-type granules compared to total and A-type, which could be caused by the tiny size of B-type granules which linked closely with the gluten network. Also, the hardness and springiness of noodles got reduced more after adding esterified starch with PEF assistance than conventional esterified. Qiu et al. (2022) used PEF treatment at 3 kV/cm with 100–400 pulses to develop refrigerated shelf-stable, lower retrogradation tendency glutinous rice products (rice gels and rice cakes). Pores created at rice surfaces by the PEF treatment, helps in both moisture retention and increased diffusion of other components such as sugar, oil, and gums.

13.6 Conclusion

Several studies in this chapter have conclusively demonstrated the use of pulsed electric field processing to modify starches' structural and functional properties. PEF treatment-induced electrochemical and electrolysis reactions and starch polarization effects initiate changes in the structural properties such as morphology, crystalline structure, lamellar structure, and molecular order structure based on both physical and chemical prospects. These structural changes then affect thermal properties, pasting properties, freeze-thaw stability, and digestibility of starch. PEF process parameters such as electric field strength, pulse number, treatment time, temperature, and inherent material properties (pH, conductivity, concentration) both influenced these changes. These above studies were performed in the laboratories. Industrial facilities for PEF induced starch modifications have not been realized yet. Therefore, there is a great need to do technical feasibility analysis and cost estimation from transferring batch process to continuous and pilot scale. Furthermore, comparative studies between starch modification by PEF and other existing technologies could be an area of future research.

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Chapter 14
Cold Plasma Treatment of Starch

Uday S. Annapure and Thirumdas Rohit

14.1 Introduction

The properties of native starches do not fit some of the industrial applications. Some disadvantages are poor physicochemical, functional properties and colloidal sols, inability to withstand high pressures, temperatures, and shear (Thirumdas et al., 2017a). In the past, starches have been subjected to physical, chemical, and enzymatic modifications to enhance their properties. Recently, genetic modification is also practiced as another method of starch modification. However, physical modification is advantageous over chemical methods due to lack of chemicals, residue-free, consumer safety and environmental concerns. The commonly used physical modification methods are pre-gelatinization starches, annealing, conventional extrusion cooking process, and high moisture treatment. Recently, the non-thermal physical methods have gained considerable interest as the alternative methods for starch modification are ultrasound, irradiation, pulsed electric fields, ozonation, cold plasma, and high-pressure processing (Castanha et al., 2019; Lal et al., 2021; Liu et al., 2016; Teixeira et al., 2018). Thirumdas et al. (2017a) reported that the starch modifications using the novel methods result in the formation of fissures and pores, mainly affecting the functional properties. A similar kind of modification was observed when the starch is exposed to cold plasma at varying voltages and times. The different reactive species formed in the gaseous plasma are responsible for the

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U. S. Annapure (✉)
Department of Food Engineering and Technology, Institute of Chemical Technology, Mumbai, India

Institute of Chemical Technology, Jalna, Maharashtra, India
e-mail: us.annapure@ictmumbai.edu.in

T. Rohit
Department of Food Process Technology, College of Food Science and Technology, Professor Jayashankar Telangana State Agricultural University, Hyderabad, India

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changes, and the formed reactive species immediately return to their native/stable state without leaving any chemical residues on the treated samples. Cold plasma is considered an eco-friendly non-thermal technology that can be exploited for starch modification.

14.2 Cold Plasma: Fundamentals and Chemistry

Plasma is the 4th state of matter after solid, liquid, and gas. Plasma is partially or completely ionized gas containing electrons, free radicals, positive and negative ions, neutral and excited atoms, UV radiations, and visible light. The presence of charged species like negatively charged electrons and both positively and negatively charged ions differentiate it from the gaseous state. The cold plasma technology has been applied in several research areas like microbial decontamination to the prevention of foodborne infections, enzymatic inactivation, aflatoxin degradation, seed germination enhancement, and insect disinfestation to improve the cooking qualities of rice and pulses and partial hydrogenation of oils (Devi et al., 2017; Sadhu et al., 2017; Sarangapani et al., 2017; Thirumdas et al., 2018; Thirumdas, 2022; Sutar et al., 2021). The samples can be directly exposed to the plasma gaseous plume and indirectly by activating liquids that are applied to the sample (Thirumdas et al., 2018).

Plasma can be generated by applying electrical power and voltage, magnetic fields, radio-frequency waves, and magnetic radiations to a gas. The rate of energy exchange depends on the mass ratio of colliding bodies. Sharma (2020) reported that collisions are categorized into two stages, i.e. the first stage collisions are related to the electrons while the second stage collisions are heavy particle collisions. In the case of larger body collisions like ions, the ratio of masses is equal to 1, and a complete transfer of energy occurs between the colloid bodies. Similarly, during the collision between smaller particles like electrons, the mass ratio is much lesser than 1, facilitating only a smaller fraction of energy exchange. Based on the overall temperature, the plasma is divided into cold/non-thermal plasma and thermal plasma. In cold plasma, the electron temperatures (Te) are higher compared to the massive chemical species temperatures (Ti), attributing to lower temperature (Ti >> > Te) to the plasma. Since the overall temperature of the generated plasma is near or below ambient temperature, it is designated as cold plasma (Mandal et al., 2018). Whitehead (2016) reported that the electron temperatures are nearly about 10^5 K, whereas the temperature of heavier particles is close to 300 K. The highly energetic electrons can initiate several chemical reactions via ionization, excitation, and dissociation.

The reactive gas species formed in plasma depends on the mode of generation (corona discharge, fluorescent tubes, radio-frequency plasma reactor, dielectric barrier discharges, and microwave discharges), type of feed gas used, and operating parameters (Coutinho et al., 2021). The reactive species of plasma, particularly the reactive oxygen species (ROS) and reactive nitrogen species (RNS) have the potential to cause significant changes in the substrate. Some of the reactive species formed in the atmospheric air plasma are O, O^{2-}, $^1O_2$, OH^*, O_3, N_2, N^{2+}, N, NO, and NO_2, N_2O (Berardinelli et al., 2021). Whitehead (2016) reported that the electrical energy supplied to atmospheric air results in excitation followed by the dissociation and ionization of nitrogen and Oxygen. Apart from the reactive species formed from the primary collision, the other important active radical formed from the subsequent collision is hydroxyl ions (OH^*). The OH^* radical possesses a strong oxidation capacity that involves several advanced oxidation reactions. The type of reactive species formed in the plasma also depends on the pressure applied for the plasma generation. Ionization mechanisms occurring during plasma generation involve (i) direct ionization by electron impact (ii) stepwise ionization by electron impact (iii) heavy particles collisions (iv) photo and surface ionization (Fridman & Friedman, 2013).

Plasma chemistry is very complex as the half-life of many reactive species formed is very short. Ionization, dissociation, excitation, and ion-molecule interaction are the main processes responsible for the formation of different reactive species (Misra et al., 2019). Of the different reactive species formed in the plasma, stated that atomic oxygen (O), hydroxyl radicals (OH), and ozone (O_3) are the important reactive oxygen species, whereas nitrogen oxide radicals and excited nitrogen molecules are important reactive nitrogen species. However, the half-life of ROS like hydroxyl radicals and singlet oxygen ($^1O_2$) has a shorter life but can create significant microbial destruction (Thirumdas et al., 2017a).

14.2.1 Cold Plasma Sources

The nature of chemical reactions taking place in the atmospheric pressure plasma could be different compared to low-pressure plasmas. Misra et al. (2011) reported that the atmospheric pressure plasma is beneficial for applications in food and agriculture due to the ease of operating at ambient pressure. However, operating and controlling the gaseous atmosphere for plasma generation under a vacuum is easier compared to atmospheric gas plasma (Choudhury, 2017). The basic setup for low-pressure plasma generation is easier, but it is restricted to batch processing only. When the applied voltage for the plasma generation is higher than the gas breakdown voltages, the dielectric property loss makes them a conductor. A wide range of plasma generation sources like glow corona discharge, atmospheric plasma jets, dielectric barrier discharge (DBD), Radio Frequency (rf) discharge plasma, inductively coupled plasma, and gliding arc discharge are currently underuse (different types of plasma sources are given in Fig. 14.1a, b). Liu et al. (2021) reported that depending on the applied electric field intensity, several modes of discharges like Townsend, streamer, glow, and corona discharges are generated.

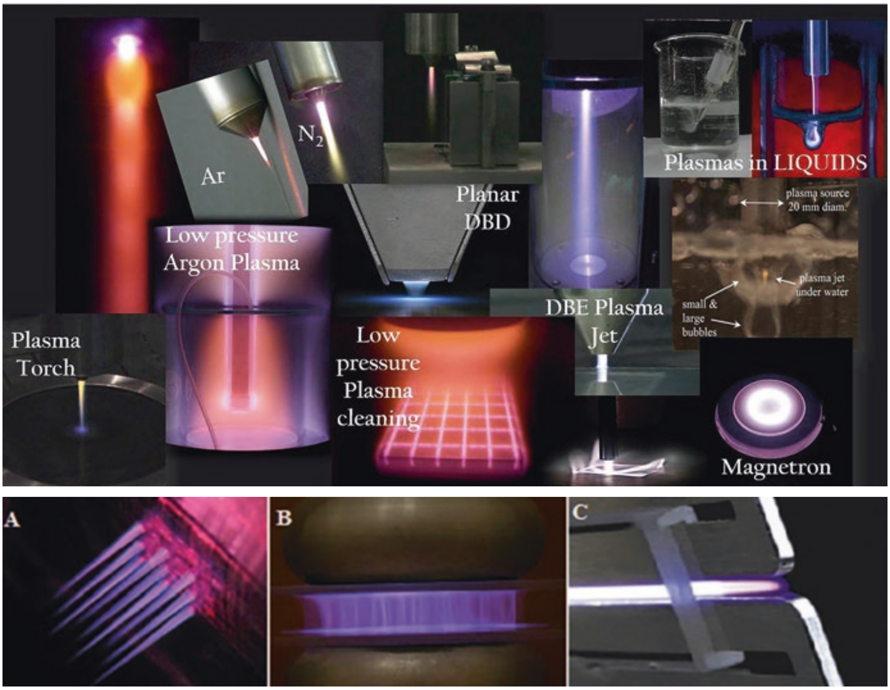

Fig. 14.1 (**a**) Different types of plasma generation sources. (Source: Pictures taken from Plasma Processes, Materials and Surfaces: National Institute for Laser, Plasma and Radiation Physics of Bucharest, Romania). (**b**) Images showing plasma discharges (A) Array of microjet (B) DBD discharge model, (C) Atmospheric pressure plasma jet. (Source: Adopted from the Misra et al. (2016). Cold plasma in food and agriculture: fundamentals and applications. Academic Press)

14.2.1.1 Glow Corona Discharge

Generally glow discharge plasma is produced from the alternating current, pulsed voltage, direct current, radiofrequency, and microwave frequencies (Okyere et al., 2022a, b). The glow discharges are produced in a closed chamber with internal electrodes. Bogaerts et al. (2002) reported that when a potential difference is applied across the electrodes, the electrons are driven by the electric fields that result in a collision with other particles resulting in excitation, ionization, and dissociation. The above authors have also commented that the excitation is immediately followed by de-excitation resulting in the release of energy responsible for the glow discharge. The continuous emission of electrons at the cathode increases the number of collisions attributed to self-sustained glow discharge plasma. Conrads and Schmidt (2000) reported that the presence of magnetic fields perpendicular to the electric fields widens the path length of electrons attributing to higher ionization efficiencies with high plasma densities. Based on the polarity at the high voltage electrode, the corona discharge is classified into positive and negative discharge (Lu et al., 2016).

Microwave glow discharges are caused by electromagnetic waves above 300 MHz frequencies. The most commonly used frequency for domestic, scientific, and industrial purpose is 2.45 GHz can have an electron density of 10^{11} cm^{-3} (Conrads & Schmidt, 2000). A microwave plasma reactor consists of a magnetron (power source), a circulator, a matching circuit, and a microwave-to-plasma applicator. Lebedev (2010) reported that these kinds of discharges are required for quasi-equilibrium and non-equilibrium plasma generation. The plasma generation using electromagnetic waves is an electrode-less type of discharge. Microwave discharges can be generated at high and low levels of power, and low and atmospheric pressures, in both pulsed and continuous waveforms.

Radiofrequency (rf) plasma is generated using a wave frequency of 13.56 MHz using parallel electrodes. The two common rf plasma sources used are capacitively coupled plasma (CCP) and inductively coupled plasma (ICP). The basic setup of the plasma source involves the use of coils and electrodes to apply the potential difference in ICP and CCP, respectively. Conrads and Schmidt (2000) reported that to have a sustainable discharge using the rf system the generator along with the combination of impedance matching network to the electrodes. In the presence of applied frequency, the ions move towards the electrodes and generate secondary electrons, resulting in glow discharge. In CCP the electrodes are often covered with an insulating material like dielectrics. Chen (2007) reported that the electric fields applied to heat up the electrons attributed to high electron energy resulting in ionization forming a large avalanche of ions. The uniformity of plasma discharge depends on the plasma density, rf power, mean free pathway, and collisionality rate. The rf power consumed is used for the calculation of plasma parameters and governs the chemical reactions and processes.

14.2.1.2 Dielectric Barrier Discharge (DBD)

In DBD plasma reactors, the electrodes are covered by a layer of dielectric materials to avoid sparks. The schematic setup of a DBD plasma reactor is given in Fig. 14.2. The common insulating materials used in DBD plasma reactor are glass, quartz, polymers, and ceramic (Li et al., 2019). However, the stability of the discharge depends on the insulation capacity and dielectric constant of the insulating materials. The design of the electrodes and dielectric materials plays an important role in stopping arc formation during plasma generation (Okyere et al., 2022a, b). DBD plasma is one of the widely used methods to generate non-thermal plasma that is easily prone to different chemical reactions at atmospheric pressures (Li et al., 2019). Conrads and Schmidt (2000) reported that a voltage of 1–100 kV with a frequency range of 50 Hz – 1 MHz is required to have a sustainable discharge and can be operated at a varying pressure of 0.1–1 bar. Working DBD at atmospheric pressure facilitates a wide range of applications in food and agriculture and ease of scale-up.

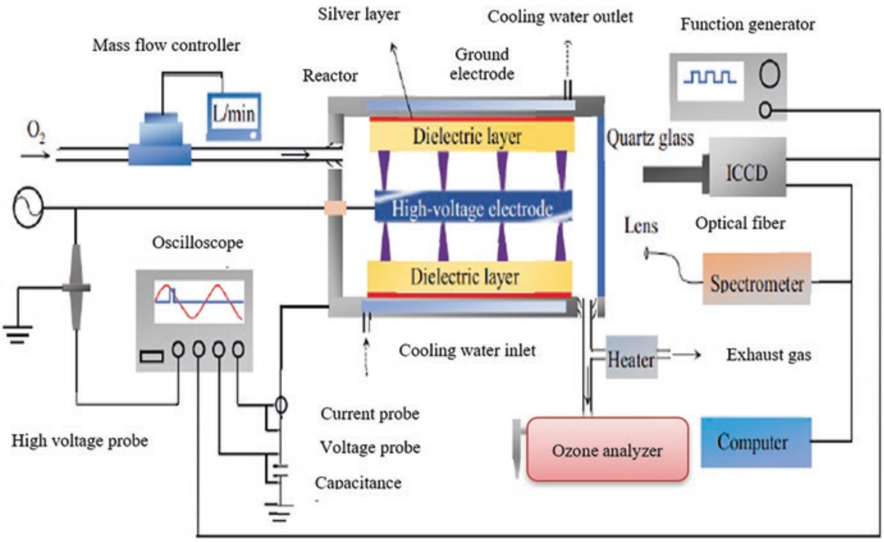

Fig. 14.2 Schematic diagram of the DBD plasma setup. (Adopted from the Liu et al., 2021)

14.2.1.3 Atmospheric Pressure Plasma Jets

Plasma jets can be generated using sinusoidal voltage excitation at various frequencies (Hz to MHz) with an electron density up to 10^{15} cm^{-3} at low gas temperatures and power consumption (Hong, 2021). Schutze et al. (1998) reported that the Langmuir probe measurement showed a concentration of 1010 cm^{-3} charged species in the jet plasma plume. Unlike other plasma sources wherein the plasma is generated in between the electrodes but in these plasma jets, the plasma is produced in the open air. The jet flames released into the air can be applied to various surfaces of different sizes with a few millimeters to a centimeter gap. The plasma jet source consists of a dielectric tube, single/double electrodes of varying shapes, and a power source. Park et al. (2000) reported that the plasma jet is a type of capacitively coupled electrode without a dielectric cover that is employed to produce a stable discharge at atmospheric pressures. Helium is one of the most frequently used feed gases for the generation of atmospheric plasma jets. Few researchers have suggested that the use of cheap gases like air and nitrogen could make the use of plasma jets economical and can be directly applied to many objects without the limitation of size.

14.3 Modification Mechanisms and Methodology

Starch modification using cold plasma is a type of physical method of modification that has drawn considerable interest among the researcher's societies. Thirumdas et al. (2017a) postulated different types of starch modification mechanisms by cold plasma. Crosslinking, depolymerization, etching, and starch oxidation were the possible ways that the starches have been modified. The reactive species formed in the cold plasma are responsible for the modification via the above-reported mechanisms. The changes in the different starch properties instigated by the above mechanisms are given in Fig. 14.3. An 80.6% crosslinking at the C2 site of the pyranose unit of starch chains was observed, attributing an increase in the number of glycosidic bonds (Zou et al., 2004). Thirumdas et al. (2017b) reported that the reducing end of the glucose molecule, easily prone to reactive species of plasma, undergoes cleavage and forms a new bond (i.e. carbon-oxygen-carbon), resulting in crosslinking. An increase in the peak 924 cm-1 in the FTIR spectrum of cold plasma processed starch indicates the relative increase in C-O-C linkages (Wongsagonsup et al., 2014). Deeyai et al. (2013) confirmed the extent of crosslinking after the plasma treatment from the data interpretation of 1H NMR analysis. Similarly, Okyere et al. (2019) observed an increase in α 1,6 glycosidic linkage of glucose units in plasma-modified waxy starches. Taslikh et al. (2022) studied the extent of crosslinking ratio in starch after the cold plasma treatment. The above authors have observed a 5.2% increase in the crosslinking ratio in the plasma-treated corn starch. Similarly, the other important mechanism of starch modification is due to the oxidation of starches by the chemical reactions caused by the plasma treatment. The oxidation of starch due to plasma reactive species resulted in the conversion of C-O and O-C-O bonds to O=C–OH (Bie et al., 2016). The carbonyl groups are formed in wheat starch after the oxygen plasma treatment due to the oxidation of OH groups, particularly present at C-6 (Khorram et al., 2015).

The bombardment of electrons has resulted in depolymerization and chain scission of the amylose branches and amylopectin side branches. Thirumdas et al. (2017a) reported that the chain scission led by the plasma reactive species has resulted in smaller fragments of the glucose chain like maltose, maltotriose, and maltotetraose. The hydroxyl radicals formed in the plasma from the breakdown of water molecules can damage the starch crystallinity due to depolymerization.

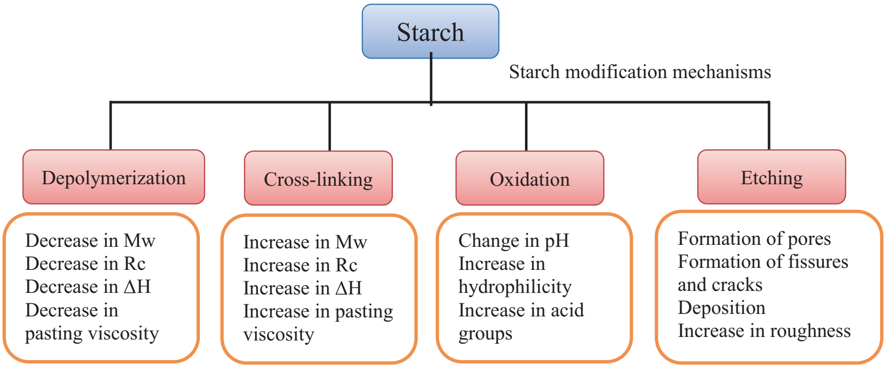

Fig. 14.3 Starch modification and their effects on the properties

Chaiwat et al. (2016) observed a decrease in the moisture of starch after the plasma treatment due to the breakdown of water molecules associated with the starch helical structure. A similar observation was also reported by Zhang et al. (2015) that the reactive species of plasma decomposed the water molecules into H^+ and OH^- present in the crystalline structure of starch. Bie et al. (2016) found that the average molecular weight of amylose and amylopectin chains was increased after the treatment indicating depolymerization of starch molecular structure. The depolymerization has resulted in several changes in the structural and functional properties of starches (Banura et al., 2018; Thirumdas et al., 2015).

The plasma etching of starch granules caused by the cold plasma has enhanced the surface area, which led to many changes in the functional and rheological properties. The chemical reactions between the reactive species formed in the plasma and the starch lead to the formation of etching volatile products that are easily removed, resulting in plasma etching. The etching has opened new binding sites that enabled the formation of bonds. The increase in the surface area is directly correlated to an increase in the hydrophilicity of the starch granules. Thirumdas et al. (2017a) reported the increase in morphological surface energy of starch granular structure due to plasma etching, enabling an increase in hydrophilicity. Zhang et al. (2015) observed an increase in hydrophilic moieties attached to crystal units of the starch after the nitrogen and oxygen glow plasma treatment. Hernández-Torres et al. (2022) reported that oxygen plasma is mainly used for etching purposes.

14.4 Effect of Modification on Starch Properties

14.4.1 Starch Granular Morphology

The granular morphology of different starches depends on the botanical origin. Particle morphology of the starch granules is one of the important physical parameters that affect many physicochemical properties, flowability, and packing. Any kind of damage that occurs to the starch morphology due to the modification treatments could lead to changes in the properties. The extent of starch damage to the granular morphology can be analyzed by particle size reduction. The average granular size of different starches ranges from >1 μm in quinoa and amaranth starches to more the 100 μm in potato starches (Fuentes et al., 2019). Several scientific pieces of evidence have shown that the cold plasma treatment did not alter the size and shape of the starch granules. However, the etching effect of plasma has resulted in fissures, cracks, and uneven surfaces on the surface morphology (Thirumdas et al., 2017a). Banura et al. (2018) also reported a similar kind of observation in rf air plasma processed corn starch. The investigation of the atmospheric air plasma treatment effect on corn starch granules resulted in surface etching (Wu et al., 2019). Another investigation carried out on glow discharge plasma-treated kithul starch granules by Sudheesh et al. (2019) reported the formation of fissures due to the plasma treatment. In a similar kind of investigation, exposure of banana starch to glow electrical discharge led to the formation of fissures (Wu et al., 2018). Similarly, the formation of fissures and coat deposits on maize starch was also reported by Sifuentes-Nieve et al. (2021). Scanning electron microscopy micrographs have revealed the presence of fissures and cracks on the surface topography of starch granules (Thirumdas et al., 2017a). Zhu et al. (2017) reported that the dielectric air plasma discharge partially fractured the starch granules, enlarging the cracks on the surface and leading to channels. Similarly, several cracks were found on the surface of banana starch granules by the DBD plasma treatment reported by Yan et al. (2020). Surface corrosion of starch granules by helium plasma was reported by Zhang et al. (2015). Taslikh et al. (2022) also observed the development of more cracks, pits, and holes in the cold plasma processed corn starches. A DBD plasma treatment for 9 min resulted in etching that has been attributed to the formation of small cracks in the potato starch (Guo et al., 2022). The reactive species can reach the interior regions of the starch granular structure through the fissures and cracks formed on the surface. This could further result in the depolymerization of amylose chains and amylopectin side branches, modifying the starch properties (Fig. 14.4).

14.4.2 Molecular Weight (Mw)

Several scientific pieces of evidence supported that the starches exposed to cold plasma suffered degradation of amylose and amylopectin chains. The size exclusion chromatography and gel permeation chromatography analysis showed a decrease in the average molecular weight of starch after the plasma treatment. Bie et al. (2016) observed a decrease in molecular weight from 19.34×10^6 to 0.98×10^6 g/mol in plasma-treated corn starch. The ammonia gas plasma treatment of cassava starch has reduced the molecular weight from 9.35×10^7 to 41.59×10^7 g/mol (Lii et al., 2002). A similar decrease in the average molecular weight of oxygen plasma exposed potato starch from 4.270×10^7 to 4.716×10^6 g/mol was reported by

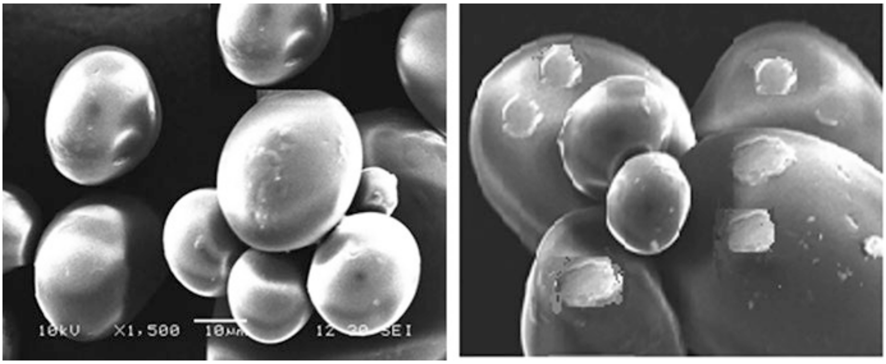

Fig. 14.4 SEM images showing the starch surface abrasion by cold plasma. (Adapted from Thirumdas et al., 2017)

Sudheesh et al. (2019). The decrease in molecular weight is due to the formation of smaller fragments resulting from the depolymerization caused by plasma reactive species. Thirumdas et al. (2017b) reported that the bombardment of high energetic particles of plasma on the amylose chains and amylopectin side chain could result in chain scission resulting in depolymerization. With an increase in the size of the starch granules, the extent of the decrease in molecular weight also changed. The decrease in molecular weight can also be explained by measuring the amylose content of the starch. Sudheesh et al. (2019) reported a decrease in the amylose content of kithul starch after the nitrogen glow discharge plasma was generated at 15 W power levels for 30 min. Similarly, Ge et al. (2022a) observed a decrease in the amylose content of cold-water-soluble rice starch after DBD plasma. Further, the above authors have also found that there is an increase in the ratio of long amylopectin chain conversion to smaller chain lengths. The above reforms have been attributed to the decrease in the molecular weight of the starch. Banura et al. (2018) reported a decrease in the amylose content of corn starch due to chain scission when exposed to rf low-pressure air cold plasma. However, the depolymerization rate depends upon the type of plasma reactor, operating parameters for the plasma generation, and the botanical origin of starches.

Based upon the type of reactive species formed in the plasma, the chemical reaction occurring between the reactive species and starch components may greatly vary. Many scientific reports have shown that plasma treatment decreases the average molecular weight. Contrary to this, another study conducted by Zhang et al. (2015) on helium gas plasma-treated potato starch observed an increase in molecular weight from 6.11×10^7 to 10.42×10^7 g/mol. The molecular weight of rice amylose content increased up to 3.82×10^5 g/mol from 0.75×10^5 g/mol after exposure to DBD plasma (Sun et al., 2022). The increase may be the result of the crosslinking of starch chains led by the reactive species. An increase in the H-O-H linkages in the plasma-treated starches was reported (Sudheesh et al., 2019). Wongsagonsup et al. (2014) reported an increase in C-O-C linkages plasma treated in tapioca starch. A similar increase in the C-O-C linkage areas in DBD-treated aria starches (Carvalho et al., 2021). Zuo et al. (2004) stated that the decrease in the OH groups could be related to crosslinking of starches. Khorram et al. (2015) observed an increase in crosslinks at the C2 site in wheat starches exposed to argon glow discharge plasma.

14.4.3 Multi-scale Structural Changes

The plasma-induced starch modification mechanisms like depolymerization and crosslinking have led to structural alterations like the disruption of lamellar regions and change in the degree of crystallinity. The growth ring consists of blocks of repeating units of amylose and amylopectin chains attributing to the amorphous and crystalline regions (Han et al., 2020). The above authors have also reported that physical treatments like HPP, electromagnetic radiations, and cold plasma are known to affect the amylose and amylopectin present in both the amorphous and crystalline regions and the packaging mode of the crystal structure of the starch lamellar regions. Kuang et al. (2017) reported that the double helix arrangement of amylopectin branches contributes to the semi-crystalline lamellar region. The reactive species of plasma caused partial decomposition of amylopectin chains primarily present on the starch granular surface resulting in damage and loose rearrangement (Ge et al., 2022a, b). Bie et al. (2016) observed an increase in the distance between the alternating repeating units after the plasma treatment. Any change in the starch lamellar structure is analyzed using small-angle X-ray scattering. Zhang et al. (2015) reported that the plasma treatment affected the starch granular lamellar structure disrupting the compactness components of the starch granules. The cold plasma treatment has decreased the amylopectin chain length in the semi-crystalline region of rice starch (Ge et al., 2022a, b). Han et al. (2020) reported that the amorphous regions of the starch are prone to the reactive species generated in the plasma. The above authors have also stated that the reactive species has transformed the lamellar amorphous into the lamellar non-amorphous regions by affecting the interhelical water molecules. The degree of destruction occurring to the supramolecular characteristics of the starch depends on the botanical origin of the starches (Guo et al., 2022). Zhang et al. (2014) observed a greater destruction of potato starch than corn starches when exposed to the oxygen gas plasma.

Any damage to the lamellar regions of the starch supramolecular structure will result in a change in the crystallinity. The widely used method for determining crystallinity and crystalline order is X-ray diffraction (both small and large wide-angle scattering) (Okyere et al., 2022a). The XRD analysis of different starches has shown predominant peaks at diffraction angles at 15°, 17°, 18°, and 23°, mainly attributing to the A-type crystalline pattern and V-type has peaked at 20° (Sun et al., 2022). Zhou et al. (2019) observed an A-type pattern with diffraction peaks at 15.2°, 17.1°, 18°, 23° in waxy and normal maize starches. The diffraction peaks at 5.5°, 14°, 17°, 20°, 22°, 24° of potato starch show the crystalline pattern of B-type (Guo et al., 2022). The other methods used to analyze the crystalline pattern and lamellar regions of starches are 1H Nuclear Magnetic Resonance Microscopy and polarized light microscopy (Zhu, 2017). The packing of amylopectin chains attributes common crystalline patterns observed in different starches: A, B, C, and V-type. This V-type pattern is a single helical chain, whereas A, B, and C-type patterns have a double helices structure. Okyere et al. (2022) reported the plasma treatment resulted in the reorganization of double-helical patterns to a less organized crystalline structure. NMR spectroscopy analysis revealed that the plasma treatment altered the crystallinity due to changes in the proportion of single and double helical structures (Zhu, 2017). Zhu (2018) reported that the polymorphic form of the starch pattern is determined by the internal molecular organization of amylopectin chains and branches. Okyere et al. (2019) observed a V-type single helix pattern in the carbon dioxide and argon gas plasma treated in the waxy maize and potato starch. Thirumdas et al. (2017a) reported that the B-type pattern is highly susceptible to the reactive species and free radicals generated in the plasma compared to the A-type crystallinity pattern.

Argon gas plasma treatment decreased the crystallinity of potato starch by 5.5%. Sun et al. (2022) observed a decrease in the crystallinity of plasma-treated rice starch. The authors have also concluded that the combination with microwave treatment can further decrease the crystallinity of the starch. The atmospheric plasma jet treatment decreased the relative crystallinity of waxy maize starch (46.7–42%) and normal maize starch (40.1–35.5%), respectively (Zhou et al., 2019). Similarly, the cold plasma treatment reduced the relative crystallinity of rice starch from 29% to 20% by the disruption of intramolecular and intermolecular hydrogen bonds. A similar kind of investigation by Ge et al. (2022a) on the DBD-treated water-soluble rice starch found the relative crystallinity decreased from 31.59% to 20.69%. The DBD treatment of potato starches has decreased the relative crystallinity to 27.01% from 29.07% without any effect on the crystalline pattern compared to the native starch. Rice starch exposure to rf low-pressure air plasma at 40 W power for 10 min resulted in a 5.5% reduction in crystallinity (Thirumdas et al., 2017b). Thirumdas (2017a) reported that the disordering of the starch crystalline structure is also due to the breakdown of water molecules associated with the helical structure by the reactive species of plasma. The cold plasma disturbed the crystalized order of starch by depolymerization of amylopectin branches (Yan et al., 2019). Han et al. (2020) reported that oxygen as a feed gas can further cause damage to the crystal structure of starch by the formation of reactive radical species (ROS) like hydroxyl radicals resulting from the breakdown of water molecules attached to the helical structure.

14.4.4 Thermal Properties

In the presence of heat and water, the starch lamellar structure allows the water to move into the granular structure, breaking the intermolecular bonds that help form a chain-like network. Kumar and Khatar (2017) reported that heating the starch opens the molecular chains to allow them to colloid with other chains aligning parallel to each other resulting in network formation. Biliaderis et al. (1986) stated a theory for explaining the gelatinization of rice starch using a three-phase model that explains the amylopectin's amorphous and crystalline nature. The breakdown of hydrogen bonds and hydration of amylose and amylopectin in the amorphous region results in crystallinity loss and structural integrity (Ee et al., 2020). Thirumdas et al. (2017a) reported that during the gelatinization of starch, there is a conversion of ordered crystal lattice structure into a disordered structure resulting in loss of crystallinity. Li and Zhu et al. (2017) reported that the short amylopectin short chains result in more disordered packing of double-helical structure in the starch granules. Wang et al. (2021) postulated a few steps occurring during the starch gelatinization as follows (a) the water entering into the starch granular structure through the porous structure binds to the hydroxyl groups of chains in the amorphous region (b) the water imbibition results in the slight swelling of starch granules and with the application of heat, the water enters into the crystalline region breaking the double-helical structure this led to irreversible changes like loss of birefringence pattern, loss of maltese cross, amylose leaching, the starch paste becomes transparent (c) the further rise in temperature, the starch granules swell maximum leading to the rupture and the viscosity increases to maximum. Jane et al. (1999) reported that the gelatinization of starch results in the swelling of granules, loss of birefringence, loss of crystallinity, leaching of amylose, and an increase in viscosity. However, gelatinization depends on the botanical origin, starch morphology, shape and size, amylose/amylopectin ratio, chain length, chain distribution, etc. Several methods used to study gelatinization properties are DSC, NMR, X-ray scattering, FTIR, polarized light microscopy, etc. (Ai & Jane, 2015).

The rf low-pressure air plasma treated rice starch at 60 W- 10 min decreased the peak temperature reducing the thermal properties (Thirumdas et al., 2017b). Bie et al. (2016) observed a decrease in the enthalpy of gelatinization of cassava starch after the helium and oxygen gas glow plasma discharge treatment. Similarly, a decrease in the gelatinization enthalpy was also observed by Banura et al. (2018) in low-pressure rf air plasma processed corn starches at 60 W power level for 20 min of treatment. Guo et al. (2022) reported a change in enthalpy from 14.38–13.08 J/g in potato starch after the DBD plasma treatment. The decrease in the gelatinization temperatures could be the depolymerization of amylose and amylopectin chains in the crystalline lamellar regions of the starch granular structure. The mechanism of the depolymerization and change in the crystalline nature was discussed in the previous section. In a few other studies, the plasma treatment increased the gelatinization parameter of different starch varieties. For example, an increase in gelatinization temperature of high amylose corn starch after being treated with HDMSO rf plasma at 90 W for 10 min is reported by Sifuentes-Nieves et al. (2019). Okyere et al. (2019) reported a decrease in the gelatinization enthalpies of maize, rice, and potato starches when exposed to low-pressure rf plasma at 120 W for 1 h of treatment time. Yan et al. (2020) obtained similar results in atmospheric plasma jet-treated maize starch. The increase in the gelatinization properties could be the result of crosslinking of starch chains that increased the relative crystallinity, which requires more heat for the complete destruction of crystalline structure (Thirumdas et al., 2017b; Okyere et al., 2022). Okyere et al. (2019) and Ai and Jane (2015) reported that the presence of long branch-chain length (BCL) double helical chains could increase the gelatinization enthalpies of starches. Few studies have shown that the same operating conditions have changed the gelatinization properties differently in the different starches. The change in gelatinization temperature depends on the botanical origin of starches. Banura et al. (2018) observed that the operating parameters like the type of reactor, feed gas, power levels, and same treatment time has decreased the gelatinization enthalpy in corn starch whereas an increase is found in tapioca starch. Similarly, Yan et al. (2020) reported that the atmospheric plasma jet has increased the gelatinization enthalpy in normal maize starch and decreased it in the waxy maize starches. The decrease in the gelatinization enthalpies in plasma-treated different rice varieties (Basmati rice, brown rice, basmati rice flour, and parboiled rice) was also reported (Sarangapani et al., 2016; Thirumdas et al., 2015, 2016a, b). In conclusion, the type of reactive species formed, the operating parameters, and the botanical origin of starch could significantly change the gelatinization parameters.

14.4.5 Pasting properties

Analyzing the pasting properties of starches helps to find out the changes that occur with heat application, wherein the water molecules break down the association between the amylose and amylopectin chains (Dereje, 2021). The changes mainly happen after the post-gelatinization of starches affect the pasting properties. Some of the important pasting parameters obtained from the rapid visco-analyzer (RVA) or rheometer are peak viscosity (PV), trough viscosity (TV), setback viscosity (SV), breakdown viscosity (BD) and final viscosity (FV) need to be assessed. Thermal treatment of starch results in gelatinization, the disordering of molecular structure, and an increase in viscosity. Okyere et al. (2022) reported that the pasting properties of starches depend on the botanical origin, amylose and amylopectin ratio, and molecular structure. Starch granular structure loses its crystallinity when heated above the gelatinization temperatures and results in the swelling of granules and an increase in viscosity (Ai & Jane, 2015). Sun et al. (2021) reported that the rearrangement of gelatinized starch molecular structure on cooling results in the formation of starch gels. The process of viscosity formation and development of starch gels is called starch pasting (Ai & Jane, 2015). Zhu (2017) reported that the cold plasma treatment affected the starch pasting, flow, and dynamic rheology. The DBD air plasma treatment of rice starch has decreased the TV, PV, FV, and SV of starch (Ge et al., 2022a, b). Okyere et al. (2019) observed a decrease in the pasting temperature after the rf argon gas plasma processed waxy rice and potato starches. The above authors found the PV of rice and maize starches decreased from 253.8 BU to 160.8 BU, 406.3 BU – 354.3 BU, respectively, after 60 min of treatment at 120 W power levels. A similar decrease in the PV in potato starch was reported by Zhang et al. (2015). Likewise, a decrease in the FV, TV, and PV of atmospheric plasma jet-treated corn starch was observed by Wu et al. (2019). Chaiwat et al. (2016) reported an increase in PV and a decrease in the BD in the argon glow discharge plasma-treated cassava starch. Thirumdas et al. (2017b) analyzed the peak viscosity of rf plasma-treated rice starch using a rapid visco analyzer; the treatment reduced the peak viscosity due to crosslinking of starch chains. However, the above authors observed increased breakdown and final viscosities. A similar increase in BD and FV of nitrogen glow discharge plasma-treated kithul starches is reported by Sudeesh et al. (2019). Zhang et al. (2022a) observed that low-pressure DBD plasma treatment increased the PV and viscosity difference (maximum viscosity and lowest point viscosity) of the tapioca starch.

The rheological properties of a starch solution are characterized by measuring the storage modulus G′, loss modulus G″ and tan δ (Sun et al., 2021). The DBD plasma treatment of potato starch resulted in an increase of G’ and G” from 57.27 Pa to 63.91 and 1229 to 17.82 Pa respectively (Guo et al., 2022). The above authors have stated that crosslinking of amylose and amylopectin network tend to form rigid gels resulting in higher pasting parameters. Wongsagonsup et al. (2014) observed that a 50 W argon jet plasma treatment of tapioca starch decreased the tan δ has resulted in the formation of stronger gels due to crosslinking. However, Thirumdas et al. (2017b) and Sudheesh et al. (2019) reported a decrease in the G′ and G″ in the plasma-treated rice and kithul starches, respectively. The plasma etching made the starch surface more hydrophilic, facilitating the easy penetration of water molecules and significantly changing the starch pasting properties (Zhou et al., 2019). Carvalho et al. (2021) reported that the plasma treatment increased the carboxyl and carbonyl content, which is attributed to the change in the pasting properties. Thirumdas et al. (2017, b) reported a similar finding: the plasma treatment increased the carboxyl groups on the starch and resulted in oxidation. Several authors have found a decrease in the pH of the plasma treatment (Lii et al., 2002; Thirumdas et al., 2015; Thirumdas et al., 2017a).

14.4.6 Solubility, Water Absorption, Swelling Capacity

The starch modification using plasma has changed several functional properties like water absorption index, swelling characteristics, gel hydration properties, solubility, and turbidity. Any damage to the disintegration of the starch granular structure affects the starch modification. De La Hera et al. (2013) reported that the water absorption index (WAI) is an indicator tool to assess the structural damage caused to the starch granules. Thirumdas et al. (2017b) analyzed the WAI of rice starches after the rf low-pressure plasma treatment and found that there is an increase in the WAI values. Similarly, Kalaivendan et al. (2022) modified the starch properties of mango seed kernel using a pin to plate type plasma reactor operated at 230 V for 30 min and observed an increase in the WAI. The authors have also stated that the WAI results of modified starches that were observed at 70 °C are equal to the values obtained at 80 °C for unmodified starches. A 3.47% increase in the water uptake of atmospheric cold plasma processed corn starches were reported by Laricheh et al. (2022) after 6.99 min of treatment.

The atmospheric cold plasma treatment enhanced the corn starch solubility by 15.6% (Laricheh et al., 2022). Pin to plate type plasma reactor doubled the solubility of mango kernel starch compared to unmodified starch when processed at 230 V for 30 min (Kalaivendan et al., 2022). A similar increase in the solubility of low-pressure rf plasma-treated rice starch was previously reported by Thirumdas et al. (2017b). Ge et al. (2021) found an increase in the solubility of cold plasma treated red adzuki bean starches. The destruction of the crystalline nature of starch structure by reactive species of plasma has led to an increase in the molecular water association with the starch helical structure, attributing to enhanced solubility (Ge et al., 2022a, b). The formation of porous structures on starch granules by any mechanism increase solubility, swelling capacity, and viscosity (Alcázar-Alay et al., 2015). It is evident from the mechanisms mentioned above that the reactive species of plasma can create pores on the starch granular surface. This could be responsible for the increase in the solubility of the starch. Gao et al. (2019) and Thirumdas et al. (2017a, b) reported that partial depolymerization of starch chains resulted in the formation of small fragments that are easily leached out, enhancing starch solubility. The starch oxidation by plasma species also increased syneresis and solubility (Pal et al., 2016; Thirumdas et al., 2017b). However, the enhancement of the functional properties depends on the operating parameters of the cold plasma reactor. Some of the key findings of the properties modified using cold plasma technology are given in Table 14.1.

14.4.7 Starch digestibility

The rate of starch digestibility can be categorized into the 3 types i.e. fast digestible starch that occurs less than 20 min, slow digestible starch that takes place more than 20 min whereas the undigestible resistance starches that are not hydrolyzed in the stomach and small intestine.The resistance starches are not accessible to the digestive enzymes that are passed to the large intestine can be fermented by the gut microbiota (Bello-Perez et al., 2020). The interaction between the plasma reactive species and starch molecular chains have resulted in the alterations like depolymerization, chain scission, oxidation reactions can alter the starch digestibility (Thirumdas et al., 2015). Lambert et al. (2018) observed an increase in the enzymatic digestibility of corn starch afterRF argon gas plasma treatment for 30 min at 30–70 W power level. A DBD plasma generated at 14 kV increased the starch digestibility of aria starch as a result of depolymerization of starch chains (Carvalho et al., 2021) Similarly, Goa et al. (2019) reported a 48.29% rise in starch digestibility of sorghum starch after the DBD plasma treatment. The above have reported that the plasma treatment resulted in formation of holes and pores on the surface of starch granules making easy accessible to the digestive enzymes in to granules attributing increase in starch digestion. The CO_2 gas cold plasma treatment increased the slow digestability starch of waxy granular potato starches by 10.24% and resistance starches by 85.08% (Okyere et al., 2022).

On otherhand, the cold plasma treatment has decreased the starch digestibility. The decrease in rate of digestibility is due to cross-linking and oxidation reactions. The cold plasma treatment at different treatmen times (1, 3, and 9 min) caused change in the internal structure of treated starch resulting in the decrease of starch digestability (Ge et al., 2022b). Ao et al. (2007) reported that an increase in the starch branches and crystallinity resulted in the lower rate of starch digestibility.

14.5 Applications of Cold Plasma-Treated Starches

Cold plasma technology, has gained significant attention in recent times, for its ability to modify the physicochemical properties of biopolymers. Native starch has limited applications due to low water solubility, and other functional, physicochemical and thermal properties. Cold plasma results in surface etching and improves the water holding capacity of starches. Furthermore, a decrease in crystallinity,

Table 14.1 Recent finding on the different starch modification published in 2021–22

Botanical source	Plasma source	Parameters	Salient features	References
Corn	DBD	Power: 90 W Feed gas: HDMSO Time: 10 min	Polymerization of starch Etching is observed Increases in ΔH	Sifuentes-Nieves et al. (2021)
Aria	DBD	Power: 20 kV Feed gas: Air Time: 15 min	Decreases in pH values due to formation of acid groups Increase in rapidly digestible starches Increase in amylose content	Carvalho et al. (2021)
Red adzuke bean	Atmospheric plasma	Power: Feed gas: Air Time: 10 min	Cross linking of starch chains Decrease in swelling power Solubility increased	Ge et al. (2021)
Potato	DBD	Power: 50 V Feed gas: – Time: 9 min	Reduction in relative crystallinity Occurrence of cross-links between starch molecules	Guo et al. (2022)
Tapioca	DBD	Power: 30 kV Feed gas: Air Time: 20 min	Cross-liking of starch chains Increase in pasting (PV, TV, BV) and rheological properties	Zhang et al. (2022a)
Rice	DBD	Power: 40 V Feed gas: Air Time: 2, 6, 10 min	Increase in amylose content Increase in resistant starch Decreases in relative crystallinity	Sun et al. (2022)
Rice	DBD	Power: 40 V Feed gas: Air Time:1, 3, 6, 9 min	Decrease in amylose content Improvement in solubility, swelling power Decrease in relative crystallinity	Ge et al. (2022)
Mango seed kernel starch	Pin-to-plate	Power: 230 V Feed gas: Air Time: 30 min	Decrease in pH Reduction in turbidity Increase in hydrophilicity	Kalaievendan et al. (2021)

gelatinization temperature and gelatinization enthalpies have also been reported in several studies. This can be attributed to a variation in the amylose content of the starches, which further influences the swelling power, and retrogradation properties of starch. While some studies have suggested an increase in digestibility of starches after the plasma treatment, an increase in resistant starch has also been reported in several studies. The variation of the physicochemical properties depends upon the source of the starch and the kind of plasma system being used to modify the respective starch. Therefore, cold plasma starches hold great potential in batter systems, where increased hydration of the plasma-treated starches can be exploited effectively. The high water holding capacity and the decreased syneresis can slow down retrogradation of starches during storage, thus increasing the sensory shelf-life of the product. However, a detailed study of the physicochemical properties is necessary, as cold plasma has varying effects on the starch system, depending upon the source and structure of the starch. Apart from depolymerization, cold plasma can be used for inducing cross-linking reactions. Plasma-activated water holds great scope in "green" modification of starches.

14.6 Conclusion

Starches obtained from the different botanical sources are often having some drawbacks for better food applications due to their poor physicochemical properties. Cold plasma is an alternative technology for modifying the properties of native starches. In general, cold plasma can modify starches through multiple mechanisms like oxidation, depolymerization, crosslinking, and plasma etching. However, these mechanisms mainly depend on the molecular configuration of starches and their interactions with the different reactive species formed in the plasma. The molecular configuration of starches included the packing of amylose chains and amylopectin side branches and its distribution in the amorphous and crystalline regions. The formation of different reactive species (both oxygen and nitrogen species) depends on the type of plasma reactor, operating conditions like voltage applied, treatment time, feed gas, and pressure applied (sub-atmospheric or atmospheric pressure). Optimizing the operating conditions can greatly modify the starch properties by affecting its structural integrity and crystallinity, leading to significant changes. The plasma etching resulted in the formation of pores, cracks, and fissures on the granular surfaces that easily facilitated the movement of water inside and easy leaching out of components of starch. Some properties that were significantly affected are pasting, thermal, and functional properties like solubility, water absorption, and swelling power. A decrease in the molecular weight of the starch without any change in the starch morphology is reported. The depolymerization of starch chains resulted in the formation of smaller fragments, leading to changes like gelatinization temperatures, crystallinity, rheology, and other functional properties. The plasma treatment increased the gelatinization temperatures and relative crystallinity in a few starches. The nature of the plasma source and feed gas employed determines the type of chemical reactions that would have changed the properties differently. The study of NMR and FTIR showed the crosslinking with the formation of new hydrogen bonds. In addition to the above properties, plasma treatment significantly affected the gel hydration properties and a few other visco-elastic properties.

Scaling up the plasma setup at the industrial scale is another challenging task. It is easier to set up the atmospheric pressure plasma reactor than the low-pressure plasma reactor. The atmospheric pressure plasma type can be used for continuous treatment and applied to a wide range of food substrates. Approval from the regulatory bodies to commercialize the application of cold plasma processing in the food sector is a great challenge. We hope in the near future the approval of cold plasma technology as a chemical-free and eco-friendly technology.

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Chapter 15
Microwave Irradiation of Starch

Shahrul Razid Sarbini and Mohd Alhafiizh Zailani

15.1 Introduction

Starch is an abundant biopolymer that can be isolated from various plant source such as cereals, tubers, fruits, and legumes. Despite its abundance, native starches owing to their properties have limited industrial and domestic use. Thereby, various modifications have been implemented to improve the functionality, physicochemical and functional food properties of the starches. Mainly starches can be modified through enzymatic, chemical, or physical modification techniques. Implementations of dual treatments between these techniques are also being explored. In this chapter, the focus will be on the physical modification of starches, especially for the microwave heat treatment method.

Among the physical modification methods, microwave heat treatment is a technique that has potential to improve the properties of starches by altering their structure. The microwave heat treatment is considered as a more efficient method than the conventional heat moisture treatment for the physical modification of starch (González-Mendoza et al., 2022; Zailani et al., 2022). Microwave treatment can improve the physicochemical properties such as the amylose content, pasting properties, gelatinization temperature, solubility and swelling power of the starch (Anderson & Guraya, 2006; Li et al., 2018, 2019a; Sun et al., 2022; Zheng et al.,

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S. R. Sarbini (✉)
Faculty of Agricultural Science and Forestry, Department of Crop Science, Universiti Putra Malaysia Bintulu Sarawak Campus, Bintulu, Sarawak, Malaysia
e-mail: shahrulrazid@upm.edu.my

M. A. Zailani
Faculty of Agricultural Science and Forestry, Department of Crop Science, Universiti Putra Malaysia Bintulu Sarawak Campus, Bintulu, Sarawak, Malaysia

Centre for Pre-University Studies, Universiti Malaysia Sarawak,
Kota Samarahan, Sarawak, Malaysia

V. S. Sharanagat et al. (eds.), *Starch: Advances in Modifications, Technologies and Applications*, https://doi.org/10.1007/978-3-031-35843-2_15

2020). Furthermore, the treatment also improves the functional properties of the starch such as water and oil binding capacities, emulsifying properties, gelling properties, and its use as clouding agent (Li et al., 2019a; Zheng et al., 2020). Improvements in these properties enable better usage of the treated starch for various industrial processes. Microwave modification has also been used together in combination with other modification methods, i.e., chemical, and enzymatic modification. The combination of the microwave with other treatments generally improves the properties of the starches (Lin et al., 2019; Sun et al., 2022; Surendra Babu et al., 2018). Some have shown a better enhancement when compared to single treatments (Sun et al., 2022).

Microwave treatment has resulted in improving the functional food properties of starches. Functional properties in general term describes the behaviour of ingredients during preparation and cooking wherein properties like water absorption, oil absorption etc. are discussed. However, another expression of functional food properties refers to how that food benefits the health of the consumer wherein digestibility properties are discussed. In this context, functional food properties which can be exhibited by starch is a high resistant starch content which exhibit the function performed by dietary fibers in human digestive system (Li et al., 2020; Zheng et al., 2020). Resistant starches are a potential prebiotic substance that benefits the good gut microbiota such as lactobacilli and bifidobacterial (Zaman & Sarbini, 2016). Furthermore, resistant starch has a low glycemic index which is suitable for consumption by diabetic patients. Exploration of modified starch in the health field needs to be conducted. For example, the effect of these modified starches on cancerous cells, inflammation, allergy, and more. In this chapter, the microwave modification mechanisms towards starch and its influence on the physicochemical properties of the starch are elaborate and discussed.

15.2 Modification Mechanism and Methodology

Modification of starches using microwave irradiation is currently still being investigated and evaluated. Therefore, variations in the modification technique of starches using microwave treatment are reported. This includes the difference in the preparation of starch prior to the treatment itself, i.e., starch in powdered form with varying moisture content or in suspension form with various starch concentrations. Microwave irradiation radiates within the range of 0.3–300 GHz. However, the frequency used in the domestic microwave oven is normally 2.45 GHz which has been used by many researchers in their evaluation of the microwave heat treatment effects on starches. The frequency influences the strength of power used in the treatment influencing the effect on starches and can lead to a fast-heating rate of starches. The microwave treatment with its fast rate of heating changes the submicroscopic structure of the treated starch (Fan et al., 2014).

In the microwave modification technique, the power applied is measured as wattage per gram of sample used (Fig. 15.1). Varying the power applied on starches

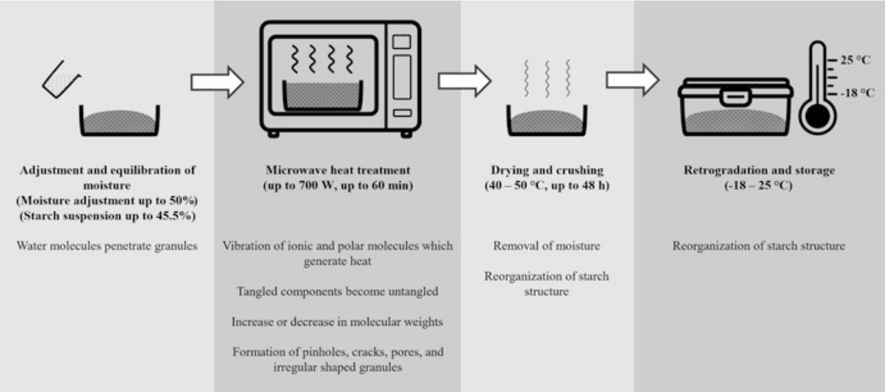

Fig. 15.1 Steps in microwave heat treatment of starch

showed varying effects on the starch granules in terms of their structure and functional properties (Xu et al., 2019). At higher wattage per gram, the damage to the structure of starch granules is more severe than at lower wattage per gram (Xu et al., 2019; Zailani et al., 2021). The changes are probably caused by the higher amount of energy causing the polar and ionic molecules to become more excited. This phenomenon causes an increase in the vibration between the components in the starch granules, especially on amylopectin. Due to this high vibration, the disruption of the hydrogen bond increases which weakens the structure of the starch granules. Changes from the ordered crystalline structure to a less ordered amorphous structure can be the reason behind the severe damage inflicted on the starch granules at higher wattage used in the treatment (Xu et al., 2019). Apart from that, the higher energy used in the treatment may cause the breakage of glycosidic bonding of the amylopectin (Yang et al., 2017). The breakage of the bond will affect the structure of the starch which may also lead to the rupture of the granule structure.

Other than the wattage used for the treatment of starches, moisture content also plays a role in the treated starch. Prior to the microwave treatment, the moisture of dry starch is adjusted to a fixed value or alternatively starch sample are prepared in a suspension form (Luo et al., 2006; Ma et al., 2015). For both preparations, distilled or deionized water is used in addition to the existing moisture in the starch samples. The moisture-adjusted starches are prepared up to 50% moisture content with the lowest being 20% moisture content (Anderson & Guraya, 2006; Li et al., 2019). Among the moisture-adjusted treatments, the most commonly used moisture level is at 30% (Xu et al., 2019; Zailani et al., 2021). Meanwhile, for suspension preparation, they are prepared between 6% to 45.5% starch in distilled water (Li et al., 2019b; Wang et al., 2021; Zailani et al., 2022). Different suspension concentration produces starch with different properties such as swelling power, transparency, relative crystallinity, thermal properties, and digestibility (Li et al., 2019a).

Treatment duration is also an important factor that can influence the physicochemical and functional properties of starches. Microwave treatments can be completed in seconds to minutes (Kumar et al., 2020). Longer treatment duration causes more damage to the starch granules structure (Zailani et al., 2021). This is influenced by the increase of chances for the glycosidic bond breaking and/or unwinding of helical chains of amylose and amylopectin. Apart from that, the treatment durations also influence the final temperature of the treatment. The temperature of treatment will lead to differences in the physicochemical and functional properties of the treated starches (Zailani et al., 2022). Different duration of the treatments affects different parts of starch structure and components. Treatment with 5 min duration causes degradation in the amorphous region of the starch granules while additional treatment duration exceeding 10 min damages the crystalline region of the starch (Yang et al., 2017).

Another vital factor that leads to changes in the properties of microwave-treated starches is the retrogradation periods. This includes both duration and the temperature used during the retrogradation period. The retrogradation can be conducted at temperatures from −18 °C up to 25 °C (Huong et al., 2021). The retrogradation of starch occurs during the cooling process and storage is influenced by conditions such as temperature used during cooling and storage duration. Upon microwave irradiation, the treated starch expands rapidly. This is followed by the swelling, especially for starch suspension. Recrystallization occurs during ageing or storage (Li et al., 2018). Recrystallization is associated with retrogradation which involves the reorganization of the starch structure by the changes that occurred on the composition of amylose and amylopectin. Higher amylose content increases the rate of retrogradation of the starch due to its susceptibility to retrogradation than the amylopectin chains (Park et al., 2019).

Thus, these four parameters (applied power, moisture level, duration and retrogradation period) are vital in influencing the physicochemical and functional properties of starch when treated using microwave heat treatment besides the origin of the starch. The changes are based on morphology and structure changes of starch granules in which the microwave treatment incurred damages at various levels on the starch granules.

15.3 Effect of Microwave Modification on Starch

Modification of starches using microwave heat treatment showed various changes in the properties of the starch (Table 15.1). This includes the physicochemical, morphology, functional, and functional food properties. The changes in the physicochemical properties of the starches are mainly linked to the changes that occurred in the morphology of the starch granules. Some of the functional and functional food properties are also linked to the morphology of the treated starch. Changes on the surface of starch granules are common, especially for heat-treated starch. Apart from the morphology, the changes in the internal structure of the

Table 15.1 Microwave heat treatment methods and their effects on various starches

Starch	Sequence for treatment method	Outcome	Reference
Indica rice	Starch suspension (20%) Microwave at 8 W/g for 3 min Cooled to 25 °C Stored at 4 °C for 72 h Oven dried at 40 °C overnight Ground	Decrease RS, increase SDS and RDS	Li et al. (2020)
Lotus seed	Starch suspension (8% w/v) Microwaved at 300 W for 8 min Stored at 4 °C	Amylopectin chains, decrease DP 25–36 (B2) and DP > 36 (B3) by 1.71% and 1.54% respectively. Increase DP 6–12(A) and 13–24 (B1) by 0.85% and 2.39% Lower melting enthalpy Increasing melting enthalpy as days of storage increases More-ordered structure	Wang et al. (2021)
Millet	Moisture adjusted to 30%, 35%, 40%, 45% or 50% Put into vacuum package bags, vacuumed, and kept for 24 h Microwaved for 1 min at 700 W Sample frozen by liquid nitrogen and freeze-dried	Decrease in pasting parameters as moisture content increase Weaker gel network strength during cooling phase Negative correlation between swelling power and moisture content Increase T_{onset}, T_{peak}, $T_{conclusion}$ ΔH decrease as moisture increases Retained A-type but crystallinity decreases D1-D5 Higher hydrolysis degree and increases as moisture increases Degree of starch damage increases as moisture increases Formation of porous surface, wide cracks, and deep cavities	Liet al. (2019a)

(continued)

Table 15.1 (continued)

Starch	Sequence for treatment method	Outcome	Reference
Millet (Long Gu 25)	Starch suspension (10%, 15%, 20%, 25%, 30%) Equilibrate for 24 h at 25 °C Microwave for 30, 60, 90, or 120 s Freeze using liquid nitrogen Vacuum freeze dried Ground to pass sieve (75 µm) Store 25 °C	Swelling power decreases Transparency increases (30 s & 60 s for conc up to 25%) Transparency decreases as duration treatment increases Gelatinization temperature increases (T_{onset}), no changes to T_{peak} and $T_{conclusion}$ (10% and 15%) Higher gelatinization temperature for 20%, 25% & 30% Gelatinization enthalpies decrease & decreases as concentration increases Higher hydrolysis percentage than native at all conc, increase hydrolysis percentage as duration increase until 60 s Change pasting curve from b-type to c-type, Granules destroyed, formation of smaller granules and lamellate gel blocks	Li et al. (2019b)
Non waxy rice	Moisture adjusted to 20% Stored in glass container at room temperature Heated to melting temperature and maintained the temperature for 60 min Air cooled to room temperature Ground and sieved (60 mesh)	Slight increase in digestibility at higher microwave power Pasting parameters increases Slight aggregation on granule	Anderson and Guraya (2006)

(continued)

Table 15.1 (continued)

Starch	Sequence for treatment method	Outcome	Reference
Normal maize & potato	Moisture adjusted to 30% Microwaved at 2.06 or 6.63 W/g for 5 min Oven dried overnight at 40 °C Ground and sieved (mesh-100)	Granules destroyed (higher power > lower power) Potato starch change crystal type from B to A No change for maize A type Decrease relative crystallinity (higher power > lower power) Gelatinisation temperature increases Gelatinisation enthalpy decrease Decrease double helix as power increase for both Single helices decrease as power increase for potato, increase for maize Degree of branching decrease as microwave power increase RS decreases, RDS increases, SDS increases (higher power > lower power)	Xu et al. (2019)
Normal maize, waxy maize, & amylomaize V	Moisture adjusted to 30% Microwaved for 20 min at 1 W/g	No changes on granules size and shape Formation of porous and cavity on granule surface Decrease swelling power Decrease solubility Increase T_{onset}, T_{peak}, and $T_{conclusion}$ Decrease ΔH Decrease syneresis	Luo et al. (2006)

(continued)

Table 15.1 (continued)

Starch	Sequence for treatment method	Outcome	Reference
Potato	Starch suspension (45.5%) Microwave for 100 s at 300 W by water bath method Final temperature of 80.4 °C Centrifugation at 3000 rpm for 5 min Sediment washed with distilled water (triplicates) Moisture adjusted to 60% Placed in sealed container Heated at 55 °C for 16 h with constant shaking Cool to ambient temperature Stored at 4 °C for 18 h. Died at 45 °C for 24 h Ground and sieved (mesh-100)	Increase amylose Increase RS Irregular, rough and non-uniform granule surface Increase enthalpy of gelatinization	Li et al. (2018)
Potato	Microwave treated at 300 W for 1, 3, or 5 min.	Lower moisture content No colour changes for color parameter a* and b* but decrease for L* parameter between 1- and 3-min treatments Increase water absorption capacity and as duration increases Reduce oil absorption capacity Relative crystallinity increases for 1 and 3 min but decrease for 5 min No changes on granules for 1 min Cracks deformed shape, and indentation of granules (3 and 5 min) Pasting temperature increases	Kumar et al. (2020)
Potato	Starch suspension (3%) Microwaved heated in 3 steps 1000 W (70 s) 350 W (50 s) 650 W (25 s)	Decrease T_{onset}, T_{peak}, and $T_{conclusion}$ Decrease ΔH	Ma et al. (2015)

(continued)

Table 15.1 (continued)

Starch	Sequence for treatment method	Outcome	Reference
Proso millet	Starch suspension (25%) Microwaved for 10 min at 500 W Freeze dried Ground and stored	Increase amylose Decrease amylopectin Increase RS Increase water binding capacity Increase solubility at 60 °C and 70 °C but decrease at 80 °C and 90 °C Average molecular weight decrease Decrease pasting temperature Decrease pasting parameters	Zheng et al. (2020)
Rice	Moisture adjusted to 40% Equilibrate for 2 h Microwaved at 500 W for 30 and 90 s in vacuum microwave	Maintain integrity of granules with slight changes Increase amylose Crystallinity decreases Solubility increase Swelling power decrease Decrease peak viscosity Increase RS	Sun et al. (2022)
Sago	Washed with distilled water (1:1.75) Stored at 4 °C overnight Microwaved at 180 W for 15 min Stored at 4 °C overnight Dried at 50 °C for 24 h	Changes on starch granules shape Increase amylose Decrease RS (uncooked sample) Higher swelling power and solubility at 65 °C and 75 °C Increase water and oil binding capacities Decrease paste clarity	Zailani et al. (2022)
Sago	Starch suspension (20%) Stored at 4 °C overnight Microwaved for 15 min at 180 W Filtered Dried at 50 °C for 48 h	Increase amylose Decrease RS (uncooked sample), increase RS (cooked sample) No changes on swelling power Increase solubility at 95 °C Decrease water binding capacity Increase oil binding capacity Decrease paste clarity	Zailani et al. (2022)
Taro	Moisture adjusted to 25% Equilibrate at 4 °C for 4 days Microwave at 180 W for 5 min Dried at 45 °C for 24 h	Decrease syneresis Increase solubility Decrease crystallinity No changes in amylose content	Deka and Sit (2016)

(continued)

Table 15.1 (continued)

Starch	Sequence for treatment method	Outcome	Reference
Waxy maize	Moisture adjusted to 30% w/w Microwaved at 160 W/g for 5, 10, or 20 min	Increase B1, B2, and B3 chains but decrease A chain (5 min) No changes on the crystalline region Chain A decreases as duration of treatment increases (damage of crystalline region) No change on A type crystalline but decrease relative crystallinity and molecular weight as duration increases ΔH decreases as duration increases Increase gelatinization temperature Syneresis decreases as duration increases Syneresis increases as number of cycle increases	Yang et al. (2017)
Waxy rice	Moisture adjusted to 20% Stored in glass container at room temperature Heated to melting temperature and maintained the temperature for 60 min Air cooled to room temperature Ground and sieved (mesh-60)	Slight increase in digestibility in a uniform increasing manner Decrease all parameter for viscosity except setback viscosity Slight aggregation on granule	Anderson and Guraya (2006)

starches also influence these properties. Color changes (L*) of starch are also observed after being treated by microwave irradiation. Color changes could be caused by the caramelization of the starch which occurs at the initial stage of the treatment (Kumar et al., 2020).

15.3.1 Morphology and Structure of Microwave Treated Starch

Looking into how microwave works in heating the starch matrices bring insights on how it is then affecting the starches. Microwave heat treatment changes the starch granules by penetrating the granule structure with its rapid heating feature. Microwaves affects the polar and ionic substances in the starch which gets vibrated as they receive sufficient energy from the microwave irradiation (Wang et al., 2019). Microwave heating exhibit both rapid heating and vibration (electromagnetic) effect. The rapid heating by the microwave treatment inhibits the destruction of the existing hydrogen bonding between the starch and the water molecules. Although the rapid heating by microwave treatment has protective effect on starch–water H-bonds, strong vibration during microwave treatment leads to destruction of the H-bonds. It has been found that destructive effect of strong vibration exceeds the protecting effect by the rapid heating (Fan et al., 2013). The vibration led to the unwinding of entangled amylose and amylopectin chains. The unwinding of double helices and the un-entanglement of these starch components changes the morphology and structure of the starch granules (Zhong et al., 2020). Another opinion on the cause for the changes in morphology and structure is the expansion or swelling of the starch while being treated with microwave radiation. Swelling of the starch granules during the microwave heat treatment followed by the drying and cooling process causes changes in the structure. Water molecules entrapped in the granules turns into steams which are released as it reaches the maximum pressure limits of the structure of the granules (Kumar et al., 2020). This creates fractures, pinholes, and irregular surfaces of granules.

Based on many studies it can be said that starches with a B-type crystalline structure are more vulnerable towards the microwave heat treatment than type A (Xu et al., 2019; Zhang et al., 2021). The starch granule with a B-type has a larger surface area than the A-type (Zhang et al., 2021). This provided a weaker structure of the starch crystal and affected the double helices packing in the structure of the granules. Besides that, the B-type has a higher amount of inter-helical water molecules due to its arrangement of the double helix chains in the structure (Xu et al., 2019). These extra water molecules absorb more microwave energy hence increasing the vibration of the starch components. The vibration caused by the microwave treatment at certain energy levels can cause the breakage of bonds present in the starch components.

Breakage of bonds of the starch components changes the starch granule's structure. Additionally, this process also influences the amount of amylose content in the starch. Since the bond connects the side and main chains, their breakage produces shorter molecules which may lead to the formation of low molecular weight amylopectin and amylose molecules (Yang et al., 2017). The changes in the amylose and amylopectin due to the disruption of the amorphous and crystalline structure causes the formation of pinholes, pores, irregular-shaped granules and possible cracks or openings on the starch granules. This degradation influences some properties of the starch such as causing the formation of weaker gel strength of the treated starch (Li et al., 2019a).

15.3.2 Changes on Amylose and Amylopectin Chains

Amylose and amylopectin are made up of D-glucose monomer units. They are bonded by α-(1,4)-glycosidic bond forming a long chain biopolymer. Besides α-(1,4)-glycosidic bond, amylopectin also has α-(1,6)-glycosidic bond which links the main chains to the side chains of the amylopectin. Microwave irradiation can reduce the molecular weights of these components (Zhong et al., 2021). Sufficient microwave power can cause the α-(1,6)-glycosidic bonding to break. The α-(1,6)-glycosidic bonds which are available within the amorphous region of the starch structure are easier to cleave than the α-(1,4)-glycosidic bonds by the microwave treatment (Wang et al., 2021). Preference for the α-(1,6)-glycosidic linkage to break is associated with its position which enables mobility and less influence by steric hindrance (Tao et al., 2020; Yang et al., 2017). However, there are also works reporting the increase in the average molecular weight of microwave-treated starches (Sun et al., 2022). For this phenomenon, it has been suggested that microwave irradiation causes the polymerization between the starch component which increases the molecular weight via crosslinking (Sun et al., 2022). The differences in the treatment outcome can still be due to the botanical origin of the starch, technique used for isolation of starch, and treatment parameters.

15.3.3 Gelatinization Properties of Microwave Treated Starch

Gelatinization temperatures can be measured and recorded using a differential scanning calorimeter (DSC) (Yang et al., 2017). Typically, native starch has low gelatinization temperature as reported for barley starch (Jane et al., 1999). However, the temperature of gelatinization depends on various factors such as the source of starch, composition of amylose/amylopectin ratio, temperature, moisture content, presence of trace substances. For example, high amylopectin content was reported to influence the high gelatinization temperature of waxy starch (Jane et al., 1999).

Treatment by microwave irradiation causes mostly a decrease in the enthalpy of gelatinization, ΔH, for the treated starches Ma et al., 2015). The changes in ΔH value are associated with the degree of double helix and order of the starch. The disruption caused by microwave irradiation affecting both degrees of order and double helix leads to poor crystalline region order. The poor order of the crystalline region is a result of the unwinding of double helix chains in the crystalline region (Yang et al., 2017). The decline in the crystalline and amorphous region stability of the starch decreases the ΔH value (Shiotsubo & Takahashi, 1984). Apart from that, the ΔH is said to have a negative correlation with B3 chains ($DP > 36$) (Yang et al., 2017). Meanwhile, the gelatinization temperature is reported to increase following microwave treatment. The increase in gelatinization temperature is also observed as the microwave power used increases (Xu et al., 2019). This is possibly linked to the decrease in DP6–12 chains and the increase in B3 chains (Yang et al., 2017). The availability of amylopectin with short side chains disrupts the crystalline lamellar structure while amylopectin with long side chains leads to the formation of longer double helices which requires a higher temperature for complete dissociation (Jane et al., 1999; Park et al., 2007).

15.3.4 Functional Properties

Changes are also observed on various functional properties of microwave treated starch such as swelling power, syneresis, water binding and oil binding capacities and solubility in water. The reported effect of microwave treatment on the swelling power of starch is contradictory. There are some reports which mentions reduction in swelling power of starch after microwave treatment (Luo et al., 2006; Sun et al., 2022). This reduction has been associated with the formation of double helices during the retrogradation process of microwave treated starch. The formation of double helices reduces the available binding sites for water molecules to bind which results in a decrease in swelling power (Shiotsubo & Takahashi, 1984). Besides that, the formation of double helices between amylose and amylopectin also restricts the expansion of the starch swelling. Other than the swelling power, water binding capacity is also linked to the degree of the double helix. The mechanisms for the reduction in water binding capacity is similar to the swelling property. On the other hand, some researchers have reported an increase in swelling power (65–75 °C) and water-binding capacity (Zailani et al., 2022; Zheng et al., 2020). This can be linked to the low moisture content of microwave-treated starches. This is supported by the low water activity of the treated starch (Kumar et al., 2020). Low moisture content allows the water molecules to bind the available sites which result in the increase of binding capacity and swelling power of the starch. These properties are directly associated with the power of the microwave and the duration of treatment used.

The oil binding capacity of starch is related to the ability of the starch to retain flavors and influences the mouth feels of the food products. Oil binding capacity has also displayed different results when starch has been treated with microwave. The variation in the oil binding capacity of starch can be explained by two postulations. First, the oil binding has been linked to the availability of hydrophobic sites on the starch components. The hydrophobic sites or zones on the starch components are dependent on the degree of the double helix of the starch (Kumar et al., 2020). The formation of double helices between amylose and/or amylopectin chains can cancel the polarity of their hydroxyl groups. This is possible when the functional groups are used to form the hydrogen bond, which results in the double helices. The second postulate for the variation in oil binding capacity is linked to the morphology of the starch molecules. As mentioned earlier, microwave heat treatment causes the formation of pores and pinholes on the surface of starch granules. These pinholes and pores are responsible for the oil binding through capillary action (Sirivongpaisal, 2008). Various results on microwave treatment on starch have indicated decrease in the oil binding capacity although the treatment causes damages on the starch granules. Moreover, the size of pores and pinholes also effect the oil binding capacity. If the pores or pinholes are too large, this may reduce the capillary action hence reducing the oil binding capacity. Having pores and pinholes at the right size is essential to ensure a higher oil binding capacity of starch.

Syneresis of microwave-treated starch also displays various outcomes. Syneresis was reported to have a negative correlation with B3 chains (Yang et al., 2017).

Syneresis of starch gels can be affected by the strength of gel formed. Generally, amylopectin can form starch gels with a softer texture than the higher amylose gels which tend to be stiffer. Removal of water molecules becomes easier from starch gels with higher amylopectin (Luo et al., 2006). Because the amylose chain can retrograde at a higher rate than amylopectin, this also explains why gels with higher amylose content, after microwave heat treatment can have lower syneresis value than their native counterparts (Deka & Sit, 2016; Luo et al., 2006). The reorganized amylose chain limits the amount of water held in the starch forming hard gels with lower syneresis as the result. While lengthy and branches of side chains of amylopectin can entrap more water molecules resulting in soft gel which will lead to higher syneresis.

15.3.5 Digestibility of Microwave Treated Starch

The degradation of starch due to the microwave treatment also reduces the starch endurance towards enzymatic digestions resulting in a more digestible starch. Digestibility properties of starch are their important functional food properties, i.e., resistant, and slowly digestible starches. These categories of starches are beneficial to human health benefits as they have a lower glycemic response when consumed. This is advantageous for diabetic consumers in controlling their blood sugar levels. However, reported studies on the effect of microwave treatment on resistant starch content of treated starches are varied in nature. Some reported a decrease in the resistant starch content yet, some studies revealed that the resistant starch content of treated starch is enhanced on microwave treatment (Li et al., 2018; Zailani et al., 2023; Zheng et al., 2020). The increase is linked to the storage condition of the treated starch. During storage, the retrogradation or re-structure of the starch occurs which in some increases the resistant starch content (Huong et al., 2021). The conditions influencing the changes include temperature and duration of storage. The increase in resistant starch is associated with the development of compact structure during microwave treatment which is followed by the retrogradation process during the cooling period and storage of the starch (Huong et al., 2021). The formation of the dense irregular structure due to rearrangement can effectively resist the enzymatic hydrolysis resulting in the improvement in resistance toward digestion (Kumar et al., 2020). The retrogradation ensures the formation of highly ordered molecules in the starch structure. This is attained by the formation of double helices in the starch between its components (Huong et al., 2021).

Double helix structures are believed to contribute toward the resistance properties of starch towards digestion by restricting the swelling of starch granules reducing their accessibility to digestive enzymes (Ai & Jane, 2015). Besides that, microwave treated starch possessed a unique enhancement within its structure where there is a molecular reassembly feature present during the digestion period as described by Li et al. (2020). In the report, Li et al. (2020) suggests that during the digestion process progress, reassembly of molecular structure of the treated starch is stronger than the native starch. This enhancement led to the generation of higher slowly digestible starch content (Li et al., 2020). However, more studies are required to unveil the mechanism behind the formation of stable structure that slowed the digestion process. Other than that, the heat treatment by microwave causes weak crystallites to melt and the formation of stronger crystallites occurs during the cooling and storage period (Li et al., 2019a).

15.3.6 Effect of Dual Treatment with Microwave Irradiation on Starch

Apart from a single microwave heat treatment of starch, a combination of microwave treatment with other modification methods is possible. The dual treatments involve the use of chemical reagents or enzymes as addition or pre-treatment to microwave irradiation. Besides that, the use of other physical treatment methods has also been explored. Examples of dual treatments are the combination of phosphorylation with microwave treatments and microwave with autoclave heat treatments (Table 15.2). This combination of treatments has altered the physicochemical and functional properties of starches. Phosphorylation followed by microwave treatment help in improving the amylose content of the starch (Surendra Babu et al., 2018). Generally, dual modification that involves the use of microwave treatment has a similar effect as single microwave treatment. On top of it, the changes applied by the microwave treatment further enhance the effect of other treatments. The damage incurred on the starch granules surface and structure are held responsible for the effect. In chemical treatment, the damaged structure by microwave assists in increasing the reaction sites. The unwinding of double helices increases the sites for reaction to occur between the available hydroxyl groups with the reagent used for the modification. The formation of extra shorter amylopectin and amylose chains also increase the chances for the reaction to occur. With less availability of the bulky long chains, this reduces steric hindrance which eventually increases the chances for reaction. This is seen in various works by the increases in the degree of substitution for microwave pre-treated starch (Zhao et al., 2018). The enzymatic treatment combined with microwave showed that microwave treatment improved the degree of the order and double helix of enzymatic treatment (Huong et al., 2021). The reorganization of the granule components after the microwave treatment possibly is the key towards the improvement. This kind of improvement can be also observed for the microwave treatment combined with other physical treatments.

Table 15.2 Dual treatments with microwave heat treatment and the outcomes of the treatment

Starch	Dual treatment	Sequence for treatment method	Outcome	Reference
Banana	Phosphorylation + microwave	Starch suspension (62.5%) in sodium sulphate solution (1.25%) pH adjusted to 11.5 Maintain temperature at 25 °C Addition of phosphoryl chloride ($POCl_3$) (0.05% or 0.10%) Stirred for 1 h pH adjusted to 5.5 Sediment washed with distilled water Dried at 40 °C for 7 h Ground Treated starch suspension (30%) Microwave for 90 or 180 s	Increase amylose content for 0.1% $POCl_3$ starches Decrease ash and moisture content Decrease water absorption capacity at 90 °C Decrease swelling for 0.1% $POCl_3$–180 s Decrease solubility Decrease syneresis	Surendra Babu et al. (2018)
Canna	Citric acid + microwave	Citric acid solution 20%, 30% or 40% of starch dry weight pH adjusted to 3.0 Sprayed onto starch and mixed Sealed in vacuum bag for 30 s Microwaved at 400 W for 5 min at 55 °C Ground Heated in far-infrared oven for 1 h at 140 °C Cooled and washed with distilled water Washed with ethanol Dried at 45 °C for 24 h Ground and sieved (mesh-100)	Rough and cracked granule surface Reduce gelatinization parameters Decrease RDS and increase RS	Wu et al. (2020)

(continued)

Table 15.2 (continued)

Starch	Dual treatment	Sequence for treatment method	Outcome	Reference
Corn	Microwave + hydroxypropylation	Starch paste (40% in 20% sodium sulphate solution) pH adjusted to 10.5 Addition of propylene oxide Microwaved at 250, 300, or 350 W for 4, 6, or 8 min, respectively pH adjusted to 6.0–6.5 Filtered by vacuum filtration Drying at 45 °C for 24 h Ground and sieved (mesh-100)	Degree of substitution increases as microwave power increases Highest substitution with 6 min duration at 300 W Increase stability of freeze-thaw Increase hydrophilicity Increase lipophilicity Decrease T_{onset}, T_{peak}, $T_{conclusion}$, and $\Delta H_{gelatinization}$ Increase transparency	Lin et al. (2019)
High-amylose mung bean	Enzyme + microwave	Starch:acetate buffer suspension (1:15) Heat at 100 °C for 10 min Debranched by pullulanase (30 U/g starch) Incubated at 55 °C for 16 h with shaking Deactivation of enzyme (95% ethanol and centrifuged (3000 *g*, 15 min)) Dried at 45 °C for 24 h to moisture of 10–11% Moisture adjusted to 20% Microwave at 350 W for 3 min Retrograded at 25 °C, 4 °C, or −18 °C for 24 h Dried at 45 °C for 24 h	Degree of crystallinity decrease from native but increases as temperature of retrogradation decreases to 4 °C from 25 °C Decrease degree of order Degree of double helix increases as temperature retrogradation to 4 °C from 25 °C Granules destroyed Changes of A-type crystal to B-type crystal and V-type poly morph. Degree of order and degree of double helix higher for microwaved-debranched than only debranched sample Degree of double helix and order increases as retrogradation temperature decreases to 4 °C from 25 °C Decrease RDS and SDS Increase RS	Huong et al. (2021)

(continued)

Table 15.2 (continued)

Starch	Dual treatment	Sequence for treatment method	Outcome	Reference
Potato	Microwave + acetylation	Moisture adjusted to 20% Sealed and microwaved at 600 W for 30 s Mixed with deionized water with stirring for 1 h at 30 °C Addition of acetic anhydride and glacial acetic acid (1:1) at 4, 8, or 12% of starch pH maintained to 8.0–8.5 reaction held for 2 h at 30 °C pH adjusted to 6.5 centrifuged with ethanol solution for 5 min at 1780 *g*	Change crystal type to C Rough surface granules and damaged inner structure Decrease crystallinity Light transmittances decrease but increase as acetic mixture concentration increase Swelling power and solubility increases Decrease in T_{onset} and ΔH Decrease peak viscosity but increase setback, pasting temperature and final viscosity	Zhao et al. (2018)
Rice	Microwave + cold plasma	Moisture content adjusted to 40% Equilibrate for 2 h Microwaved (MW) for 30 s or 90 s at 500 W Treated by cold plasma (CP) for 2, 6, or 10 min	Damage on granule surface Higher molecular weight of amylose Crystallinity decreases Increase solubility for CP_{10min}-MW_{30s} and CP_{10min}-MW_{90s} Lower solubility for other dual treated starch Swelling power decreases Decrease peak viscosity Increase RS	Sun et al. (2022)

(continued)

Table 15.2 (continued)

Starch	Dual treatment	Sequence for treatment method	Outcome	Reference
Taro	Microwave + conventional heating	Moisture adjusted to 25% Equilibrate at 4 °C for 4 days Microwave at 180 W for 5 min Dried at 45 °C for 24 h Moisture adjusted to 25% Treated in hot air oven for 1 h at 110 °C. Dried at 45 °C for 24 h	No changes in amylose content Decrease crystallinity Increase swelling and solubility Decrease syneresis	Deka and Sit (2016)
Taro	Microwave + autoclave	Moisture adjusted to 25% Equilibrate at 4 °C for 4 days Microwave at 180 W for 5 min Dried at 45 °C for 24 h Moisture adjusted to 25% Autoclave for 1 h at 110 °C Dried at 45 °C for 24 h	No change in amylose content Decrease crystallinity No change in swelling but increase solubility Decrease syneresis	Deka and Sit (2016)

15.3.7 Other Effect of Microwave Treatment on Starch

There are other changes were also reported when starch is modified using microwave irradiation treatment. A previous report mentioned the lowering of the consistency coefficient of starch viscosity. This was observed when the power and duration of treatment used were higher (Chen et al., 2021). The report describes that the treatment may have reduced entanglements that occurred naturally between the chains in the starch structure hence allowing higher diffusion rates (Chen et al., 2021). Additionally, the treatment has also increased the flow behavior index for the treated starch which was also observed as the duration of treatment and power of the microwave increased (Chen et al., 2021). In analysis, the elasticity behavior of starch was evaluated by determining the dynamic storage modulus (G′) of the starch (Kumar et al., 2020). The microwave irradiation resulted in a changes in term of elasticity of the modified starch (Chen et al., 2021; Kumar et al., 2020). However, the findings were contradicted with each other as in a study by Kumar et al. (2020), the elasticity was reported to increase while in report by Chen et al. (2021), it was decreased. Chen et al. (2021) describes the reasons behind the change as due to the reduction in the rigidity loss of swollen treated starch granules while enhancing the interaction between the amylose released and the starch granules. Additionally, Kumar et al. (2020) reported that the shear rate of the treated starch decreases as compared to the native counterparts and an increase in apparent viscosity. The viscosity of starch is reflected by the loss modulus (G″) (Chen et al., 2021). The increase in loss of modulus and elasticity with the increase in frequency depended on the microstructural and conformational changes in the starch granules as described by Kumar et al. (2020). However, in another study, apparent viscosity was reported to decrease as its shear rate increased when treated for 15–20 seconds implying it possessed shear-thinning behavior (Xie et al., 2013). In the same study, a treatment duration of up to 15 seconds has an increase in its apparent viscosity. This implies that different duration of treatment can influence the starch's rheological properties.

15.4 Application

There is a limited production of microwave-treated starch. As of now, it is mainly produced on a lab scale. Hence, information on the use of microwave-treated starch is scarce. However, based on many studies which utilized the microwave heat treatment technique, there are many possible fields in which the microwave treated starch can be applied i.e., food industry. Since the microwave treated starches showed lower swelling properties than its native, this suggests its suitability in the formulation of food products such as noodles or pasta. This will reduce the sogginess of the product upon long exposure to soups or sauces which retain the texture and taste of the food. Furthermore, an increase in oil binding capacity can enhance the flavor retention of the food product. Besides that, the treatment which causes a decrease in the paste clarity or transparency of the starch can be used in the preparation of a natural juice-like appearance. An increase in the water and oil binding capacities of microwave treated starch indicates the possibility of the starch to be used as an emulsifier or thickener in food products such as sauces.

The utilization of microwave starch can also be extended in the formulation of health-beneficial food. This focuses on the benefits of the starch in providing low glycemic index values (Li et al., 2018) which will be beneficial for diabetic patients. Other than that, a low glycemic index can also benefit obese-prone individuals by reducing the amount of blood sugar released after food consumption. Apart from that, the low digestibility of the starch also can act as dietary fibers such as resistant starch. This resistant starch can act as a prebiotic substance which will also enhance the health of the individual consuming the food by altering the gut microbiota.

There are many other potential uses of microwave-treated starch. One is in the medicine encapsulation of drugs. Other is in the polymer industry, where microwave modified starch can be used in the production of biodegradable plastics. Additionally, the modified starch may also have a potential as a medium for control released of fertilizers in agriculture sector allowing optimization of fertilizers usage. Furthermore, it can be made as a fruit or vegetable coating for the preservation and maintaining the freshness of the foods. Studies exploring the use of microwave-treated starches are limited and therefore need additional attention.

15.5 Summary

Microwave heat treatment displays potential as an efficient treatment that can improve the physicochemical and functional properties of the starch. This also includes combining other physical or chemical starch modification method with microwave treatment. Improvement in functional food properties is also contributed by microwave treatment. The current literature is still lacking on the utilization of microwave starch application. Besides that, questions on how the outcome for each starch can be different from each other need to be further investigated. The influence of microwave on the average molecular weights of amylose and amylopectin chains requires investigation especially the mechanisms influencing both the increase and decrease of molecular weights. Understanding the structure of each starch from different origin and their isolation technique need further exploration.

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Dr. Shahrul Razid Sarbini is a Professor at the Faculty of Agricultural Science and Forestry, Universiti Putra Malaysia. He is currently the Campus Director of Universiti Putra Malaysia Kampus Bintulu, Sarawak. His field of study covers functional foods, prebiotics, probiotics, and gut microbiota.

Mr. Mohd Alhafiizh Zailani is a lecturer teaching chemistry at the Centre for Pre-University Studies, Universiti Malaysia Sarawak. He is a Ph.D. candidate at Universiti Putra Malaysia majoring in the field of food science. His research interest is the on biologically active substances which include functional food.

Chapter 16
Gamma Irradiation of Starch

Mehvish Habib, Kulsum Jan, Iqra Qureshi, Savita Rani, and Khalid Bashir

16.1 Introduction

In food irradiation, foods are purposefully subjected to a certain dose of radiation. The Irradiation dose (exposure) is measured in an international (SI) unit called the gray (Gy) and is defined as the absorption of 1 Joule of radiation energy per kg of material (Arvanitoyannis, 2010). Gamma Irradiation is a physical and non-thermal technique, and according to the Codex Alimentarius Commission, more than 80 countries are utilizing the food irradiation process commercially (Bashir & Aggarwal, 2016a). The radiation dosage depends on the product's characteristics and the specified radiation goals (Bashir & Aggarwal, 2016b). Depending on the dose scale, food irradiation disrupts many biological processes, such as ripening and sprouting, as well as regulating insect infestation, reducing the amount of spoilage and pathogenic microbes, including *Aspergillus, Salmonella, Escherichia coli*, and several others (Lusk et al., 2014). Numerous advantages of food irradiation have been identified, such as disinfestation in fruits, cereals, and pulses, sprout suppression in potatoes and onions, decreased microbial load as well as sterilization of eggs, meat, spices, and seafood, and most critically, increased food safety as well as shelf life (Arvanitoyannis, 2010). In addition, irradiation effectively inhibits and eliminates food allergies and anti-nutritional factors (Al-Kaisey et al., 2002). The main advantages of radiation therapy are as:

- Fast acting without the addition of a catalyst
- Only minimum sample preparation is required
- It is a non-thermal procedure

M. Habib · K. Jan · I. Qureshi · K. Bashir (✉)
Department of Food Technology, Jamia Hamdard, New Delhi, India

S. Rani
Department of Life Science, Sharda University, Greater Noida, India

V. S. Sharanagat et al. (eds.), *Starch: Advances in Modifications, Technologies and Applications*, https://doi.org/10.1007/978-3-031-35843-2_16

Table 16.1 Food irradiation main potential applications and general dose requirements (Farkas & Mohacsi-Farkas, 2011)

Application	Dose (kGy)	
Sprouting inhibition	0.03–0.12	 The "Radura" logo
Disinfestations of insects	0.2–0.8	
Disinfestations of parasites	0.1–3.0	
Elongation of shelf-life (**radurization**)	0.5–3.0	
Elimination of non-spore forming pathogenic bacteria (**radicidation**)	1.5–7.0	
Reduction of microbial load in dry food ingredients	3.0–2.0	
Production of shelf-stable meat, poultry, and fishery products at room temperature (**radappertization**)	25–60	

- It may be dispersed immediately after treatment and doesn't leave any residue behind
- High penetrability (products may be handled in their final packaging)

As per the regulations, the term "Treated with radiation" or "Treated by irradiation" shall appear on the food label along with the international sign for irradiation, "Radura symbol" (Table 16.1) (Becker, 1983; Diehl, 1985; Pohlman et al., 1994; Farkas, 1998; Sádecká, 2007). The Codex Alimentarius Commission states that three different radiation kinds may be used in food irradiation for commercial purposes: Gamma rays, X-rays, and Accelerated Electrons (Bashir & Aggarwal, 2016a; Verma et al., 2019; Verma et al., 2018). Owing to its great penetrating power and outcomes, gamma irradiation is chosen over X-ray as well as Electron Beam Irradiation (Bashir & Aggarwal, 2016a). Radiation is divided into three categories based on absorbed dosage:

16.2 Gamma Irradiation of Starch

One of the most common storage carbohydrates in human nutrition is starch (Amylum). Depending on the intended end use, starch seems to have certain limitations in its natural state that may be resolved by making some structural changes in the starch polymer. The alteration may be accomplished via physical approaches (irradiation and heat), chemical methods (phosphate treatments, acid/ base treatments, monosodium, vinyl chloride, sodium tripolyphosphate, and phosphoryl-chloride) or biological approaches (cyclo-Malto dextrinase, trans-glucosidase, a and b-amylases, amylo-maltases). The modified starch exhibits better properties, such as increased rheology in terms of visco-elasticity, fluid retention, and superior oil holding capabilities, as well as convenience in food preparation and freezing-thawing stability (Tester et al., 2004; Bashir et al, 2017a, b). Due to its flexibility and adaptability as a dietary fiber, thickening agent, and fat substitute, modified starch has drawn much interest from the food industry over the past few years. Besides that, moderate starch intake has been associated with the protection of illnesses such as diverticulitis, cancer, and coronary heart disease (Asp & Bjorck, 1992; Lopez et al., 2001).

Over the past years, starch modification by gamma irradiation has received significantly more attention. The free radicals produced by gamma radiation results in starch molecular changes and fragmentation. By randomly cleaving the glycosidic chains, starch depolymerization by free radicals gradually decreases the molecular sizes of amylose and amylopectin. To present, all researchers have reported that gamma irradiation treatment lowered the amylose content of A and C-type starches, and the majority have found that gamma irradiation treatment raised the amylose content of B-type starches. Gamma irradiation has been associated with decreases in relative crystallinity in most cases. However, some A-type starches showed an increase at low doses and then a fall at higher doses. Gamma irradiation can elevate the resistant starch fraction, whereas some researchers observed that irradiation treatment could decrease resistant starch content. Radiation processing can promote crosslinking in the starch matrix under oxygen. Graft copolymerization of chemicals onto starch by a simultaneous irradiation technique can also be achieved to produce a biodegradable film or superabsorbent hydrogel (Xiangli Kong, 2018; Bashir et al., 2021; Bashir & Aggarwal, 2016b; Gani et al., 2012, 2013).

16.3 Mechanism of Starch Modification by Gamma Irradiation

Food’s response to gamma radiation in terms of its physicochemical and functional characteristics depends on

- **Irradiation dose:** The number of structural changes in the material will increase with the amount of applied irradiation dosage
- **Moisture content**: Since water serves as a vehicle for the production and movement of free radicals, irradiation results in irradiation-induced effects, which are more pronounced in environments with greater water content
- **Temperature:** Foods must be exposed to radiation at lower temperatures to prevent the development of off-flavors, while greater temperatures favor the secondary influences of irradiation
- **Atmospheric conditions**: The effects of irradiation are amplified by atmospheric oxygen because it encourages the creation of free radicals

Gamma irradiation may either de-polymerize or polymerize the monomeric units of starch based on the irradiation dosage, moisture content, and climatic conditions. Simple sugars, dextrins, and simplified acids are produced via depolymerization, while denser and more compact structures are produced through polymerization

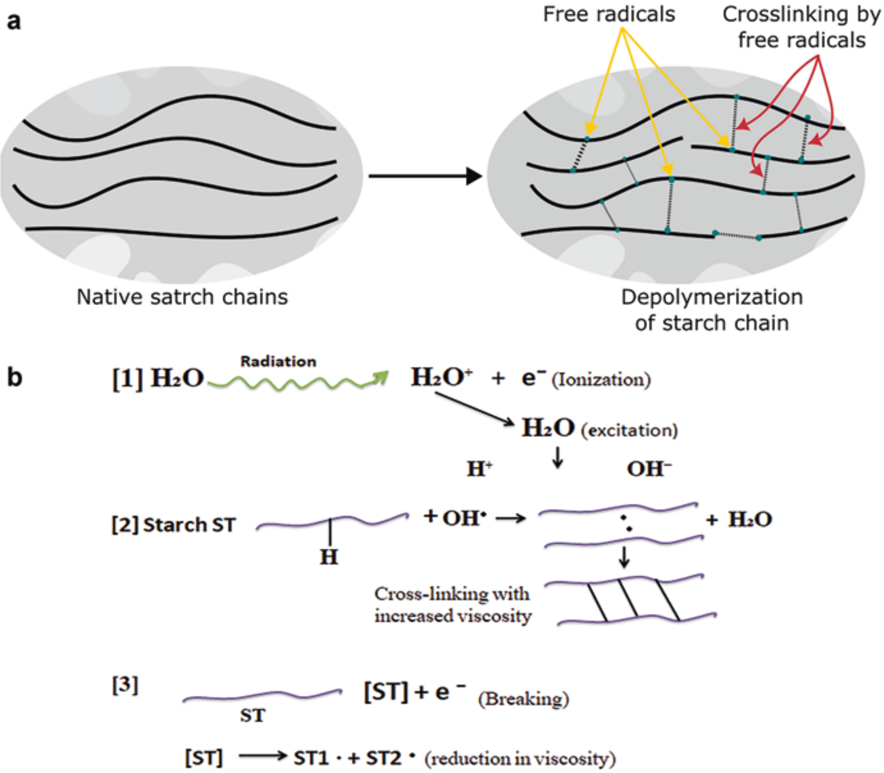

Fig. 16.1 Irradiation impacts starch polymers outlining probable degradation and crosslinking of starch chains (**a**) without water and (**b**) in water (Bhat & Karim, 2008; Bashir et al., 2021)

(Fig. 16.1) (Bashir et al., 2021). The water molecules in the meal are affected when starch is exposed to gamma rays. High-energy electrons and short-lived free radicals (H and OH) are formed from water molecules. These free radicals and high-energy electrons modify starch by fragmenting or crosslinking its molecules and causing other chemical modifications. The process of crosslinking benefits from the presence of oxygen. Gamma rays may also bring on direct breakage of glycosidic linkages at the chain ends. Native starch has hydrogen bonding between the starch granules. In contrast, irradiation breaks these bonds, reducing starch viscosity (Bashir & Aggarwal, 2016a). When starch is cooked, the polymeric chains break apart, resulting in a viscosity and consistency index drop. Gamma irradiation has a substantial impact on the crystalline structure of starch. Irradiation substantially influences starch properties, as it might induce changes in properties like dissolution rate, gelatinization, gelation, retrogradation tendency, rheological properties, digestibility, amylose-amylopectin ratio, elastic modulus, mechanical strength, and viscosity. The moisture level in the food product is connected with the gamma irradiation dosage needed for starch modification; meals with greater moisture need lower dosages for alteration. According to surface morphology reviews, the granule structure is unaffected by even modest doses of ionizing radiation (Bashir & Aggarwal, 2016a; Gani et al., 2013).

16.4 Impact of Gamma Irradiation on Properties of Starch

Gamma irradiation aims to improve the starch's useful properties, such as gelation property, emulsion capacity, foaming capacity, solubility index, swelling index, and water and oil absorption capacity. Numerous research has demonstrated how gamma irradiation improves the general properties and how the properties of goods made using gamma-irradiated starch are significantly enhanced.

16.4.1 Physical Structure of Starch Granules

The amylopectin A and B chains form double helices that assemble into crystalline arrays. Wide-angle X-ray diffraction (WAXD) analysis may differentiate between the two types (A and B type) of crystal arrangements (Perez & Bertoft, 2010). The C-type is a mixture of A and B polymorphs. Gamma Irradiation has been ascribed to change the polymorph and crystallinity of starch granules to various extents (Table 16.2). In most of the reports, gamma irradiation did not alter the polymorph type of the starch (Bao et al., 2005; Chung & Liu, 2009, 2010; Gani et al., 2012, 2013; Kong et al., 2009; Liu et al., 2012; Lu et al., 2012; Singh et al., 2011; Sofi et al., 2013; Wani et al., 2014), even at 500 kGy (Liu et al., 2012).

The wide-angle X-ray diffractograms can be used to determine the crystallinity of granules. At higher doses (>2 kGy), the percentage of the crystallites in starch granules has been reported to decrease (Bao et al., 2005; Chung & Liu, 2010; Ciesla et al., 1991a, b; Gani et al., 2012, 2013; Liu et al., 2012; Lu et al., 2012; Singh et al., 2011; Sofi et al., 2013; Wani et al., 2014). Chung and Liu (2010) reported bean starch (C-type) to be less susceptible than potato starch (B-type). The extent of decrease in the degree of crystallinity by irradiation appeared to be influenced by the type of polymorph, as the crystals of A-type polymorph are more compact than that of B-type, which is also more hydrated, and thus more susceptible to irradiation (Chung & Liu, 2010; Perez & Bertoft, 2010). Indeed, higher moisture content rendered starch more susceptible to molecular degradation by ϒ-irradiation (Kamal et al., 2007). The disruption of the crystalline part in the granules has been confirmed by small-angle X-ray diffraction analysis (Ciesla et al., 1991a, b). Irradiation doses of 10–30 kGy have been associated with destructed of some long-range ordered structures, as reflected by the decreased intensity of small-angle reflection related to 100 Å spacing in potato starch (Ciesla et al., 1991a, b).

At lower doses (<2 kGy), one study showed that the percentage of crystallinity in rice starch increased at 1 kGy (Bao et al., 2005), while others showed that it decreased at 0.1 kGy in potato starch (Lu et al., 2012) and at 0.5 kGy in potato

Table 16.2 Influence of ϒ-irradiation on the physical structure of starch granules

Starch source	Irradiation and sample parameters	Techniques of characterization	Major findings	References
Lotus stem	5–20 kGy, 2 kGy/h, moisture 12%	SEM, WAXD	Irradation caused damage to some granules, polymorph pattern unaffected with decreased relative crystallinity	Gani et al. (2013)
Potato	10–30 kGy, 1.58 kGy/h, dried sample, moisture unknown	WAXD	Irradation decreased the relative crystallinity	Ciesla et al. (1991a)
Potato	10–30 kGy, 1.58 kGy/h, dried sample, moisture unknown	SAXS	Irradiation destructed long-range ordering as reflected by the decreased intensity of small angle reflexion related to 100° spacing	Ciesla et al. (1991a)
Broad bean, Indian horse chestnut	5–15 kGy, 83Gy/min	SEM, FT-IR, WAXD	Irradiation caused fissures on the granule surface without affecting the size and shape; the intensity of 1018 cm^{-1} (FT-IR) increased, suggesting an increase of the amorphous part of the starch; polymorph pattern unaffected with decreased relative crystallinity	Wani et al. (2014); Sofi et al. (2013)
Kidney bean	5–20 kGy, 2 kGy/h	WAXD	Degree of crystallinity decreased and the polymorph pattern unaffected	Gani et al. (2012)
Potato and white bean	Upto 50 kGy, 2 kGy/h	SEM, PLM, WAXD	Some granules were destroyed, especially at 50 kGy, degree of crystallinity decreased and the polymorph pattern unaffected	Chung and Liu (2010)

starch (Singh et al., 2011). Thus, it appears that the effect of ϒ-irradiation at lower doses on the degree of crystallinity in starch needs more studies. A lower dose rate (0.4–2 kGy/h) of irradiation resulted in a higher relative crystallinity of corn starch, suggesting possible re-arrangement and re-formation of double-helical structures that pack into crystallites (Chung & Liu, 2009). When starch is exposed to gamma radiation, the intensity peaks drop, changing the relative crystallinity of the exposed starch. This change can be attributed to the destruction in the ordered structure (amylopectin) and disorder of the double helices of the starch granule (Fig. 16.2) (Hussain et al., 2014; Bashir et al., 2017a; Dar et al., 2018; Verma et al., 2019; Bashir & Aggarwal, 2017, 2019).

Fourier transform infrared spectroscopy (FT-IR), which has been used to probe the changes in glycosidic bonds and the crystalline order in the granules (Chung & Liu, 2009, 2010; Ezekiel et al., 2007; Sofi et al., 2013; Wani et al., 2014), revealed

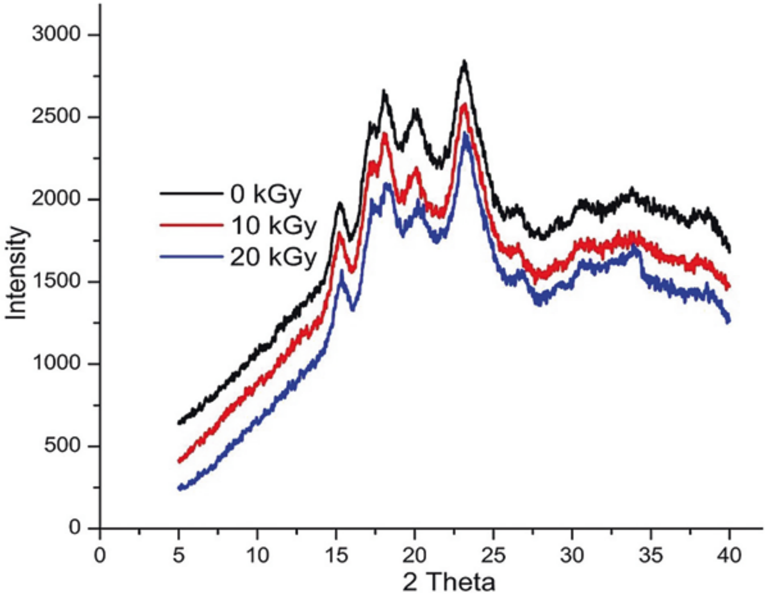

Fig. 16.2 X ray diffraction of native and irradiated pearl millet starch (Jan et al., 2021)

that the degree of order at the granule surface decreased as the radiation dose increased (Chung & Liu, 2009, 2010), results that agreed well with those from WAXD.

16.4.2 Structural and Morphological Changes

Morphological changes of starches from diverse sources as affected by gamma irradiation have been examined by scanning electron (SEM) (Abu et al., 2006; Chung & Liu, 2010; Esteves et al., 1997; Ezekiel et al., 2007; Gani et al., 2013; Rayas-Duarte & Rupnow, 1993; Singh et al., 2011; Sofi et al., 2013; Wani et al., 2014) and light (Chung & Liu, 2010; Liu et al., 2012) microscopy. The exposure of isolated starch granules to gamma irradiation at lower doses does not impact any significant change; however, at higher doses (>5 kGy), irradiation resulted in fissures on the granule surface and further granule damage (Sofi et al., 2013; Wani et al., 2014). The specific dose for causing observable damage on the surface appeared dependent on the starch source (Chung & Liu, 2010). For example, bean starch (C-type polymorph) was more susceptible as compared to potato starch (B-type polymorph) when subjected to the same dose of irradiation (Chung & Liu, 2010). It would be interesting to test more samples to verify if the polymorph type of starch is a dominant factor in determining susceptibility to surface damage in this aspect. The size of small granules increased when the starch was extracted from an irradiated crop (e.g., potato tubers irradiated at 0.1 and 0.5 kGy) after storage (Ezekiel et al., 2007). This suggested that other un-investigated factors in the potato tubers affected the granular morphology since the dose used was relatively low.

Polarized light microscopy provides information on the anisotropic order and its granular organization. The Maltese cross in some starch granules is unclear when the dose increased to a certain degree (50 kGy), suggesting the disruption of the crystallite order (Chung & Liu, 2009). The center of the granules was more susceptible to loss of structural organization (Chung & Liu, 2009), suggesting the more organized nature of the periphery (Baker et al., 2001). As starch is a larger molecule than the other components of the food matrix, gamma radiation is very likely to cause a structural breakdown. Irradiation does not change the starch granule native structure. Studies have shown that irradiation doses up to 20 kGy do not result in any significant fissures on the starch granules' surface (Fig. 16.3).

16.4.3 Swelling and Solubility Index

Gamma Irradiation at various doses and dose rates on starches from diverse botanical origins decreases the granule swelling while increasing the water solubility and amylose leaching of native starch granules (Table 16.3) (Chung & Liu, 2010; Chung et al., 2010a, b; Gani et al., 2013; Lee et al., 2003; Singh et al., 2011; Sofi et al., 2013; Wani et al., 2014). Correlation analysis demonstrates that irradiation dose (0–500 kGy) is positively related to the water solubility of maize starch at 30 °C and 90 °C (Liu et al., 2012). This could be attributed to the partial breakdown of starch granules upon irradiation and the production of lower molecular weight fractions with higher water solubility (Chung & Liu, 2009; Wani et al., 2014). It would be interesting to study the structure of the leached materials during swelling as affected

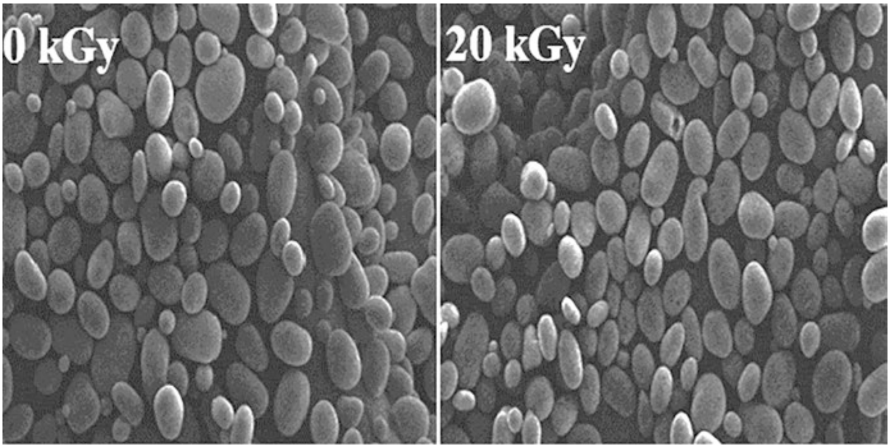

Fig. 16.3 Scanning electron micrographs of cowpea starch (Verma et al., 2019)

Table 16.3 Influence of gamma-irradiation on swelling and solubility of starch granules

Starch type	Irradiation parameters	Swelling	Solubility	References
Kidney bean	5–20 KGy, 2 kGy/h	Decreased	Increased	Gani et al. (2012)
Wheat, corn, potato, rice	5–20 kGy, 7 kGy/h	Decreased	Increased	Lee et al. (2003)
Crosslinked waxy and native waxy maize starches	10–100 kGy, 10 kGy/h	Decreased for native waxy and increased for crosslinked starch	Increased	Chung et al. (2010a, b)
Broad bean/Indian horse chestnut	5–15 kGy/83 Gy/min	Decreased	Increased	Sofi et al. (2013); Wani et al. (2014)
Rice	0.5–1.5 × 10^6 rad	Decreased	Increased	El Saadany et al. (1974a)

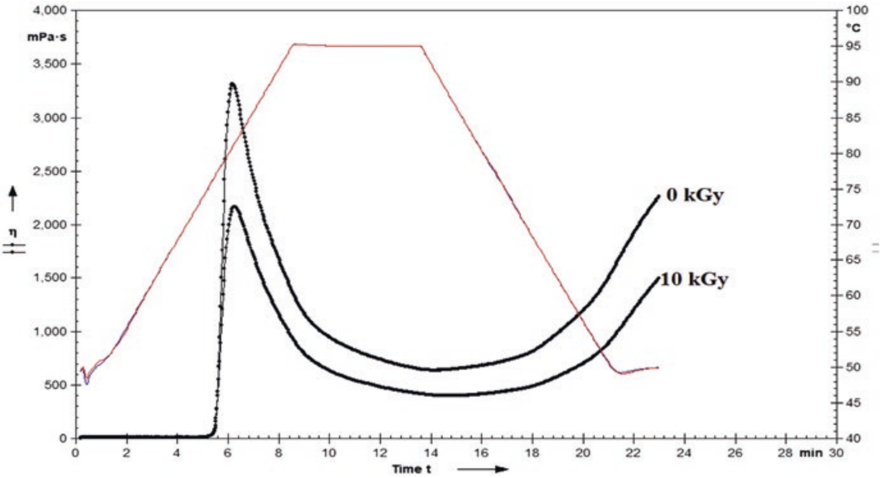

Fig. 16.4 Pasting properties of unirradiated and irradiated Buckwheat starch (Verma et al., 2018)

by irradiation. Swelling of crosslinked waxy maize starch increases upon gamma irradiation up to 100 kGy (Chung et al., 2010a, b).

16.4.4 *Rheological Properties*

The most measured rheological parameter of irradiated starch is the pasting property, that is, the development of viscosity of a starch and water mixture under a programmed heating-cooling cycle and a constant shearing force. The rheological properties of food materials are governed by starch, protein, and additives (hydrocolloids). Gamma irradiation results in the structural breakdown of the starch, protein, and food hydrocolloids, which causes a decrease in the pasting properties (peak viscosity, trough viscosity, breakdown viscosity, and final viscosity), consistency index, and flow behavior index (Fig. 16.4). Irrespective of the starch source, most of the reports demonstrate that gamma irradiation decreases the viscosity of starch during pasting (Abu et al., 2006; Chung & Liu, 2009, 2010; Chung et al., 2010a, b; Gani et al., 2012, 2013; El Saadany et al., 1974; Ezekiel et al., 2007; Liu et al., 2012; Lu et al., 2012; Kong et al., 2009; Singh et al., 2011; Sofi et al., 2013; Wani et al., 2014) (Table 16.4). Employing a wide range of doses (0–500 kGy), correlation analysis showed that dose was negatively related to pasting time (PT), peak viscosity (PV), trough viscosity (TV), final viscosity (FV), and setback (SB) (Liu et al., 2012). This is also reflected in decreased swelling capacity and increased water solubility and amylose leaching, as discussed above, as well as the decreased intrinsic and apparent viscosity (Kamal et al., 2007; El Saadany et al., 1974a, b). As discussed in the structure section, the decrease in pasting viscosity could be attributed to damaged granules and the scission of starch chains by free radicals. Irradiation conditions greatly affect the pasting behavior of starch. For example, the breakdown in viscosity during the pasting of maize starch increased first upon irradiation up to 10 kGy before decreasing with doses of up to 500 kGy (Liu et al., 2012). PV relates more to the granular structure and swelling properties, and TV more to the molecular structure of starch chains and water solubility. Thus, the changes in BD reflect the combined outcome of irradiation on the granular and molecular structure of starch.

Table 16.4 Effect of gamma irradiation on rheological properties of starch

Starch source	Irradiation parameters	Rheological parameters	Major findings	References
Broad bean/ Indian horse chestnut	5–15 kGy, 83Gy/min	Pasting	Viscosity decreased	Sofi et al. (2013); Wani et al. (2014)
Lotus stem	5–20 kGy, 2 kGy/h, moisture 12%	Pasting	Viscosity decreased	Gani et al. (2013)
Potato	0.1 kGy	Pasting	Pasting time and viscosity decreased	Lu et al. (2012)
Kidney bean	5–20 kGy, 2 kGy/h	Pasting	Viscosity decreased	Gani et al. (2012)
Corn	5–100 kGy, 3.2 kGy/h	Intrinsic viscosity	Intrinsic viscosity dropped drastically initially before levelling off towards higher radiation doses	Kamal et al. (2007)
Rice	0.5–1.5 × 10^6 rad	Pasting, viscosity measured by diverse methods	Viscosity decreased	El Saadany et al. (1974a)
Sweet potato	5–10 kGy	Intrinsic and apparent viscosity	Viscosity decreased	El Saadany et al. (1974b)

Starch type and composition also play an important role in the pasting properties of starch as affected by irradiation. For example, starches from two amaranth cultivars had different susceptibility to the effects of irradiation (2–10 kGy) on pasting (Kong et al., 2009). This could be attributed to different compositions and organizations of crystalline and amorphous regions in the granules. Thus, it appears that some aspects (if not all) of the pasting of starch can be controlled by selecting radiation conditions and starch type to achieve desired properties.

Dynamic oscillatory analysis has been used to measure the rheological properties of irradiated starch (Kong et al., 2009; Singh et al., 2011). G' (storage modulus) and G'' (loss modulus) of amaranth and potato starch gels during temperature and frequency sweeps were decreased by irradiation (up to 10 kGy) (Kong et al., 2009; Singh et al., 2011), reflecting weaker gels from irradiated starch. The gelation of starch pastes at this stage is dominated by the re-association of amylose released during granule swelling and swollen as fillers in the amylose network (Brunnschweiler et al., 2005). The dynamic rheological results, thus, might be due to disrupted granules and cleaved molecules of the starch imparted by radiation (Bashir et al., 2017a, b; Dar et al., 2018; Kumar et al., 2017; Verma et al., 2018).

16.4.5 Thermal Properties

The influence of gamma irradiation on gelatinization parameters depends on the factors such as the type and moisture content of starch and irradiation conditions (dose and dose rate). Gamma Irradiation greatly alters the gelatinization properties of starch as determined by DSC (Table 16.5). However, correlation analysis has demonstrated a negative correlation between irradiation dose (0–500 kGy) and transition temperatures (T_o, T_p, $T_{c,}$ and ΔH) of maize starch (Liu et al., 2012). Several studies have been conducted to study the impact of irradiation on the transition properties of the starches from different sources. Potato starch irradiated at 446 kGy had drastically reduced gelatinization parameters. It was also reported that gelatinization temperatures and ΔH decreased when the dose was greater than 50 kGy (Liu et al., 2012). The decrease in gelatinization parameters can be attributed to the disruption of some crystalline domains of granules, as discussed above (Chung & Liu, 2009). T_o and T_p of potato starch increased and ΔH decreased when starch was irradiated at 20 kGy (Ciesla & Eliasson, 2002). Starch extracted from irradiated cowpea paste and flour (dose up to 50 kGy) had increased Tp (Abu et al., 2006), which suggests a possible crosslink between starch chains. Other studies suggested that the increase in gelatinization parameters was due to the increase in the metastable crystalline phase (Rayas-Duarte & Rupnow, 1993) and molecular realignment of starch chains within both amorphous and crystalline regions (Chung & Liu, 2009). Despite some contradictory results from different reports on the gelatinization properties of starch as affected by irradiation, the molecular basis for the increase/little effect/decrease in the parameters is still to be better understood, especially when the dose is less than 20 kGy. Several authors have reported a significant

Table 16.5 Influence of gamma irradiation on gelatinization parameters of starch

Starch type	Irradiation parameters	T_0	T_P	T_C	$T_{C-}T_0$	ΔH	Reference
Maize	1–500 kGy	Decreased	Decreased	Decreased		Decreased	Liu et al. (2012)
Corn	2–50 kGy, 0.40, 0.67, and 2 kGy/h, moisture 9%	Decreased	Decreased	Decreased	Increased	Decreased or increased depending on irradiation conditions	Chung and Liu (2009)
Amaranth	2–10 kGy, 1 kGy/h	Little[a]	Little	Little	Little	Little	Kong et al. (2009)
Dry beans	2.5–20 kGy		Increased			Increased	Rayas-Duarte and Rupnow (1993)
Crosslinked waxy and native waxy maize starches	10–100 kGy, 10 kGy/h	Decreased	Decreased	Decreased	Increased	Decreased	Chung et al. (2010a, b)

increase in the transition temperature by gamma irradiation (Abu et al., 2006; Ciesla & Eliasson, 2002; Singh et al., 2011), while several others have demonstrated a significant decrease (Chung et al., 2010a, b; Liu et al., 2012; Bao et al., 2005; Chung & Liu, 2009, 2010; Liu et al., 2012).

16.4.6 Digestibility

The digestibility of foods determines how well the gastrointestinal tract absorbs and digests macronutrients (Welch, 2011). A food's composition, molecular integration of its components, and the presence of naturally occurring anti-nutritional components all affect how easily it can be digested. The food becomes more digestible because of the modifications in the macromolecules' structural integrity (breakdown/fragmentation), primarily starch and protein, caused by gamma irradiation. As proteins' hydrogen and disulfide bonds break down, more peptide bonds become exposed to enzymes and destabilize their structure. According to reports, gamma irradiation causes resistant starch to develop, decreasing the starch's digestibility. The development of beta bonds, carboxyl groups, and crosslinking, which are resistant to enzyme activities, is responsible for the decreased digestibility (Sudheesh et al., 2019; Bashir et al., 2021).

The effect of gamma irradiation (1, 2, and 5 kGy) on the digestibility of rice starches was investigated by Polesi et al. (2018). They reported a modest reduction in starch digestibility at 2 kGy, but samples treated with a 5 kGy dose showed an increase in the in vitro digestibility values. The process of fragmentation and crosslinking, respectively, were attributed to the increase or decrease in the digestibility levels. The corresponding consequence is seen with a specific dose depending on the predominant process (fragmentation or crosslinking). The impact of gamma irradiation (0.5, 1, 2.5, 5, and 10 kGy) on the digestibility of *Caryota urens* starch was investigated by Sudheesh et al. (2019). According to their observations, the formation of simple sugars, depolymerization of starch chains, surface fissures, reduction in amylose, and relative crystallinity all contributed to an increase in the digestibility of starch when the dose was increased. Yoon et al. (2010) examined how gamma irradiation (5, 10, and 20 kGy) affected maize starch's ability to be digested. Once the dose was increased, they observed a notable increase in the quantity of starch that was easily digested. The total percentage of the resistant starch was found to increase with irradiation dose, which was attributed to converting some fraction of slowly digestible starch into resistant starch. Similar results have been reported by Chung et al. (2010a, b).

Hassan et al. (2018) investigated the effects of gamma irradiation (0.5, 1, 1.5, and 2 kGy) on the properties of sesame protein. They observed a notable rise in protein digestibility when the irradiation dose increased. Osman et al. (2014) investigated how Sudanese faba beans' nutritional value was affected by gamma irradiation (0.5 and 1 kGy). They reported that gamma irradiation had no effect on the proximate composition or mineral content. However, irradiation allowed the tannin and phytic acid content of the beans to drop significantly. The reduction in phytate level can be attributed to the phytate's conversion to inositol or the irradiation-induced breaking of the phytate ring. Subsequent research revealed that samples exposed to radiation had increased protein digestibility. Protein denaturation, protein inhibitor activity, and inactivation of anti-nutritional factors were all attributed to improved protein digestibility (tannin and phytate). They also reported that cooking resulted in a further reduction of the anti-nutritional content. The impact of gamma irradiation (15, 30, and 45 kGy) on the protein digestibility of canola seed protein was investigated by Ebrahimi et al. (2009). Higher doses were found to decrease phytic acid and improve protein digestibility. Brazilian beans were subjected to gamma irradiation at doses of 0.5, 1, 2.5, 5 and 10 kGy in a study by Villavicencio et al. (2000). According to their observations, irradiation significantly reduced the phytate and tannin concentrations of the beans while gradually increasing the total phenolic content at higher dosages. Farag (1986) investigated the effects of gamma irradiation on trypsin inhibitor and hemagglutinin, two anti-nutritional components in soybeans. The samples treated with 10 kGy showed a significant reduction in trypsin inhibitor and lectins of 55% and 50%, respectively. Similar results were reported by Lima et al. (2019) and Brigide and Canniatti-Brazaca (2006). When creating diets for diabetics and other conditions connected to their condition, the variable quality of starch's digestibility has become quite important. The source, chemical makeup, and content of starch all significantly impacted how digestible it was. Numerous studies have shown that irradiation increases the in vitro digestibility of starch (Bravo et al., 1998; Rombo et al., 2001). Following is some research on how gamma irradiation affects starch digestibility. Several studies have been conducted that show that gamma irradiation has a significant positive characteristic of the products crafted with gamma-irradiated starch.

Chung and Liu (2009) conducted a study on the influences of gamma irradiation. They reported that a 50 kGy dose raised the content of Readily Digestible Starch (RDS) but decreased at a 10 kGy dose. The content of Resistant Starch (RS) was first reduced up to 2 kGy before starting to rise to 50 kGy. They also discovered that swell-ability and carboxyl content was decreased with lower irradiation doses. Chung et al. (2010a, b) investigated the waxy maize starches gamma irradiated at 10, 20, 40, and 100 kGy for their in vitro digestibility and pasting abilities. They showed that the RS content was down by around 20% compared to the original characteristics. The RDS, however, significantly increased. They also concluded that the granule interior had greater alpha-amylase activity than the exterior. Yoon et al. (2010) examined the impact of gamma-radiation (5, 10, 15, and 20 kGy) doses on maize starch's in vitro digestibility. They observed that while the starches' mean molecular weight decreased, the quickly digested and resistant starch proportions rose. Waxy corn starch was discovered to be more radiation sensitive than ordinary corn starch. They concluded that chain degradation and structural alteration are both indicated by the rise in resistant starch concentration. The summary of the effect of gamma irradiation on starch is presented in Table 16.6.

Table 16.6 Summary of the effect of gamma irradiation on starch

Starch	Dose	Major findings	References
Pigeon pea	1 Mrad	The gelatinization temperature reduced, the maltose content gradually rose with time, and the irradiation starch's a-amylase sensitivity increased.	Nene et al. (1975)
Corn	1 & 5 kGy.	Comparative viscosities were lower than a reference sample that had undergone a 30 min treatment at a high temperature of 140 °C.	Kertez et al. (1959)
Maize		The depolymerization of the starch macromolecules had occurred, and the severity had grown with rising dosage levels. Starch solubility, capacity for swelling, dough stability, and amylogram peak viscosity all rise when the dose amount is raised.	Saini (1968)
Wheat flour and potato	20 kGy	The temperatures at which the starch gelatinized were considerably different between the two. The crystalline structures of the amylopectin were destroyed by irradiation-induced amylopectin structure deformation, which was the cause of the variations in gelatinization temperatures	Ciesla et al. (1999)

16.5 Case Studies

The usage of plastic materials has decreased in recent years due to growing concerns about environmental pollution, and interest has grown in developing substitute packaging materials that are environmentally benign, pollutant-free, and biodegradable. Starch has undergone substantial research to create polymeric packaging materials based on starch. However, compared to plastics, the mechanical qualities are poorer. However, these restrictions have been reduced by developing more recent technologies like gamma irradiation. The influences of gamma and EBI (0, 10, 30, 50, 70, 90, and 110 kGy) on hydrogels made of PVA and starch were investigated by Zhai et al. (2002). They found that the irradiation caused PVA and starch to crosslink. Gamma irradiation (0, 10, 20, and 30 kGy) was employed by Jo et al. (2005) to create the biodegradable film. Pectin and gelatin were the raw ingredients used. They discovered that a film with the best tensile strength was formed at an irradiation dosage of 10 kGy. Gamma irradiation was utilized by Al-Assaf et al. (2007) to modify the Acacia Senegal film structure in a controlled manner (gum Arabic). Acetylene was also employed as a mediating gas for the methodical alteration. They started with 6.2 kGy and then compared it to 10.5 and 26.5 kGy, which are greater dosages. Hydrogels started to develop at higher dosages. They concluded that irradiation might be utilized to create films with the necessary properties. Kim et al. (2008) used “locust bean gum” (0, 0.75, and 1.5%, w/v), PVA, glycerol, and sucrose to study how gamma irradiation affected the growth of maize starch-based film. The dosages of radiation applied to the samples were 0, 3, 6, 12, and 24 kGy. The outcomes showed that a smooth, intact, and yellowish-colored coating had developed. According to the findings, a 1.5% (w/v) locust bean gum irradiated with 3 kGy resulted in 85% greater elongation when evaluated to non-irradiated samples in terms of tensile strength. It was established that adding 0.75% locust bean gum and irradiating it at 6 kGy was optimum for a starch-based film. They observed that irradiation might be a useful technique for improving the mechanical qualities of films made of starch.

Severino et al. (2015) evaluated on inoculated green bean samples the antibacterial activity of modified chitosan-based coatings containing nanoemulsion of essential oils (EOs), gamma irradiation, and modified atmosphere packaging (MAP), alone or in combinations, against Escherichia coli O157:H7 and Salmonella Typhimurium. Firstly, four different nanoemulsions, made of carvacrol, mandarin, bergamot, and lemon Eos, respectively, were compared in terms of minimum inhibitory concentration (MIC) against the two bacteria evaluated in vitro using the microbroth dilution method. The most efficient antibacterial agent was found to be carvacrol nanoemulsion, which was chosen to be combined with modified chitosan (MC) to produce a bioactive coating. Secondly, after coating deposition and MAP, the radiosensitivity of E. coli and S. Typhimurium to gamma irradiation was assessed on inoculated green beans. Findings revealed that the radiosensitization of E. coli and S. Typhimurium enhanced significantly by 1.32-fold and 1.30-fold, respectively, when MC-based coating containing carvacrol nanoemulsion was used without MAP. Surprisingly, using bioactive coating under MAP had a synergistic impact, increasing radiosensitivity for *E. coli* and *S. Typhimurium* by 1.80-fold and 1.89-fold, respectively. Thirdly, the antibacterial effects of the antimicrobial coating, gamma irradiation, MAP alone, and their combinations were evaluated against these two bacteria during 13-day storage of green beans at 4 °C. Bioactive coating deposition or gamma irradiation treatment resulted effective in controlling the growth of the two bacteria during the entire shelf-life. Moreover, it was also found that the combined treatment of antimicrobial coating, gamma irradiation, and MAP caused the reduction of microbial population to undetectable levels during the whole storage period for E. coli and from day 7 to the end of storage for S. Typhimurium. The data may interest food manufacturers seeking to guarantee food safety with a lengthy shelf life.

Jo et al. (2005) investigated and examined the effects of gamma irradiation (0, 10, 20, and 30 kGy) on the expansion of "biodegradable films" from gelatin and pectin. They discovered that samples exposed to gamma radiation (10 kGy) created films with greater qualities than those treated in any other way. Pietranera and Narvaiz (2001) employed gamma irradiation to treat the starches in maize, cassava, agar-agar, and kappa-carrageenan (2 kGy). They discovered a considerable reduction in the gel strength and starch viscosity (made from carrageenan and agar). Zhai et al. (2002) examined the influences of gamma as well as EBI on hydrogels made of starch & PVA: "Polyvinyl Alcohol" (0, 10, 30, 50, 70, 90, and 110 kGy). They observed that PVA and starch formed crosslinks as a result of irradiation.

Al-Assaf et al. (2007) utilized the gamma irradiation approach to regulate and alter the Acacia Senegal film structure (gum arabic). They further employed acetylene as a "mediating gas" for the systematic alteration. They started with a dose of 6.2 kGy and then contrasted that with greater doses of 10.5 and 26.5 kGy. More hydrogels are produced at higher doses. They got to the idea that films with desirable qualities may be produced using irradiation. Kim et al. (2008) investigated how gamma irradiation affected the production of a corn-starch-based film made with locust bean gum (0, 0.75, and 1.5%, w/v), glycerol, PVA, and sucrose. The samples underwent radiation treatments at dosages of 0, 3, 6, 12, and 24 kGy. The outcomes showed the expansion of a smooth, uniform, yellowish coating. Compared to non-irradiated samples, a 1.5% (w/v) locust bean gum's tensile strength showed 85% greater elongation after exposure to 3 kGy of radiation. Singh et al. (2011) studied the impacts of gamma irradiation (0.01, 0.05, 0.1 and 0.5 kGy) on the gel texture, rheological, thermal, granule morphology, and structural properties of starch obtained from potatoes. They discovered the destruction of the crystalline structure and the emergence of carboxyl content (0.09–0.11%). The gelatinization parameters (To, Tp, and Tc) improved while the viscosity (final, breakdown, trough, and peak) dropped. Additionally, they discovered that as the irradiation dosage grew, the gel's cohesiveness increased, and its hardness reduced. They concluded that irradiation might be utilized as an effective and quicker approach for modifying starch as opposed to enzymatic and chemical alterations after observing how the effects of irradiation on textural properties (adhesiveness, chewiness, and gumminess) and retrogradation varied with variety.

Liu et al. (2012) investigated the alteration of corn starch structure as well as physio-chemical characteristics. The amount of radiation applied to the maize starch powder ranged from 0, 1, 2, 5, 10, 20, 50, 100, 200, and 500 kGy. They discovered that solubility rose when the dose was raised, but the viscosities (breakdown, trough, peak, and final), enthalpy, and gelatinization temperature considerably decreased. While the starch granule surface showed no visible indications of degradation, the crystallinity was observed to have decreased somewhat. They concluded that radiation had a positive correlation with solubility and a negative correlation with pasting characteristics, relative crystallinity, and thermal properties. The impact of gamma radiation (0, 1, 2, 5, 10, 20, 50, 100, 200 and 500 kGy) on corn starch's functional, physicochemical, and structural characteristics was examined (Liu et al., 2012). They observed that raising the dose resulted in decreased peak, trough, breakdown, and final viscosities as well as decreased gelatinization temperature and enthalpy while simultaneously boosting solubility. The starch granules' surface showed no physical indications of degradation, and the crystallinity was determined to have been considerably decreased. Irradiation was favorably correlated with solubility and adversely correlated with pasting characteristics, relative crystallinity, and thermal properties. For maize starch irradiation at 3, 5, 10, 20, and 50 kGy, Bettaïeb et al. (2014) found equivalent findings. Additionally, they said that the starch's crystallinity remained unaffected up to 50 kGy since there was no alteration in XRD.

Wani et al. (2014) reviewed the impact of gamma radiation on the starch derived from ("Aesculus Indica") Indian horse chestnut. Starch was given doses ranging from 0 to 5, 10, and 15 kGy. The findings revealed a tendency toward increased freeze-thaw, solubility (0.15–0.535 g/g), water holding capacity (0.94–1.0 g/g), and carboxyl content (0–0.06%), whereas a trend toward reducing peak viscosity, syneresis (3.47–0.64%) when held for 120 hours, and setback viscosity. In both native and irradiated samples, XRD revealed a typical pattern. They discovered that the underutilized Indian horse chestnut plant might be employed to make starch. Non-irradiated starch may be utilized in foods that need refrigeration due to its high viscosity. The impact of gamma irradiation on the functional, physicochemical, and thermal properties of chickpea starch was investigated in recent research. The findings indicate that the pasting properties reduced significantly ($p \leq 0.05$) in a dose-dependent way for peak, final, setback, and trough viscosities as well as pasting temperature. Irradiation led to improvements in syneresis, solubility, and swelling. The ability to absorb water and oil both considerably improved with dose, from 1.17 to 4.36 and 1.34 to 2.18 g/g of starch, respectively. Temperatures for gelatinization To, Tp, and T c were dramatically reduced. The typical C-type pattern of the starches was seen by XRD: "X-Ray Diffraction" (Bashir et al., 2017b).

Dar et al. (2018) examined the impact of gamma irradiation (5, 10, 15 and 20 kGy) on the morphology of starch granules and the physiological, antioxidant, and chemical characteristics of oat and buckwheat starch. They discovered that although the antioxidant characteristics of the starch greatly improved, the content, apparent amylose, overall crystallinity, and pasting ability of the starch decreased when the radiation dosage was raised. Similar findings were obtained for potato starch by Verma et al. (2018) and brown rice starch by Kumar et al. (2017). Kithul

(*Caryota urense*) starch was exposed to gamma irradiation at different doses (0.5 kGy, 1 kGy, 2.5 kGy, 5 kGy, 10 kGy). Its impact on physicochemical, morphological, rheological properties and *in vitro* digestibility of kithul starch was studied by Sudheesh et al. (2019). Irradiation decreased the pH, swelling index, amylose, and moisture content of kithul starch and increased the carboxyl content, acidity, and solubility. Free radical-mediated damage developed cracks and fissures on the granular surface of starch. Fourier transforms infrared (FT-IR) showed a marked reduction in the hydroxyl (OH) groups after irradiation. Irradiation did not affect the crystalline pattern of native starch (Type-A pattern), but it decreased the relative crystallinity of kithul starch. Irradiation increased the light transmittance and freeze-thaw stability but reduced the syneresis. *In vitro* digestibility of kithul starch improved after irradiation, and resistant starch followed the decreasing trend. The lower value of storage modulus and loss modulus of irradiated kithul starches indicated its weak gel formation.

Starches from lotus seeds, both native and gamma-irradiated, were evaluated for their physical-chemical, thermal, pasting, and morphological characteristics. The amylose content of starches decreased with increasing gamma radiation doses of 5, 10, 15, and 20 kGy. The bimodal size distribution of the starch granules in lotus seeds ranged from tiny to large. There were no appreciable changes in shape or size at low irradiation doses (5, 10 kGy), according to observation under a scanning electron microscope (SEM). However, minor variations were noticed during the treatment of 15 kGy and 20 kGy, including roughness. The pasting qualities of the starch (peak, trough, final, and setback viscosities) were significantly reduced by irradiation. For native starch, first and second peaks were detected at To, Tp, and Tc of 137.44, 138.29 and 143.21 °C, as well as 169.51, 169.72, and 177.73 °C, respectively. The temperatures for gelatinization decreased after irradiation. While the relative crystallinity of the irradiated starch decreased in a dose-dependent manner compared to the native lotus seed starch, the diffraction pattern of the irradiated starch remained unchanged (Gani et al., 2013). Lam et al. (2021) aim to evaluate the effect of gamma irradiation and pyrolysis on rice starch's physicochemical and structural properties. While in the single mode, pyrolysis makes a significant contribution 21.74 g/100 g of the indigestible fraction (IDF) content, irradiation at doses of 10, 30, and 50 kGy gave IDF values of 4.47, 6.63, and 9.89 g/100 g, respectively; in the combined mode (when the irradiation and pyrolysis were applied successively), IDF increased sharply, reaching 43.72, 47.95, and 52 g/100 g. The structure and functional group characteristics of rice starch were maintained after irradiation, pyrolysis, as well as their combined mode, according to XRD patterns and FTIR spectra. This suggests a potential technique for irradiation treatment in producing resistant dextrin by acidic catalyst-free pyrolysis for both food and non-food applications. According to the results, gamma irradiation significantly raised the indigestible fraction (IDF) content to a high level. IDF and water solubility in treated starches were affected synergistically by the combined treatment of irradiation and pyrolysis. This implies a new potential for food radiation treatment applications in manufacturing resistant dextrin at high content by acidic catalyst-free pyrolysis. Irradiation efficiently accelerated the pyrolysis reaction as an acidic catalytic agent.

16.6 Conclusion

Gamma irradiation has shown the potential to be a safe and cost-effective alternative to chemical and microbiological modification of starches. Future studies must determine the best ways to combine ionizing radiations to achieve the functional qualities of modified starch and effective crosslinking. Additional guidelines must be established for administering the dose, and methods must be developed to determine whether the food has been exposed to radiation. It is clear from the study that gamma irradiation, which modifies starch, is a burgeoning industry in the field of food technology.

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Chapter 17
Enzymatic Modification of Starch

Ajit Kumar Singh, Anit Kumar, Kirtiraj K. Gaikwad, and Youn Suk Lee

17.1 Introduction

Starch and its derivatives are utilized as key ingredients in a diverse range of industries. These include the food processing industry (thickeners, gelling agents, and encapsulating agents), the packaging industry (biopolymer films and coatings, adhesive and binding, paper making, and corrugating), as well as the chemical, pharmaceutical, and textile industries. However, most starches in their natural state have a limited range of direct applications due to their sensitivity to changes in temperature, pH, and shear stresses. As a result, it has limitations that hinder its widespread adoption in commercial processes. These limitations include low shear resistance, thermal and shear stress instability, and a high tendency for retrogradation (Wang et al., 2020). Therefore, various modification techniques are employed to alter the characteristics of native starch to attain desired physicochemical and functional properties as well as to match the technical and commercial standards (Fig. 17.1).

In general, the modification of starches has been widely categorized into four categories: physical, chemical, enzymatic, and genetic modification through altering chain length, branch point creation, debranching, and disproportionation

A. K. Singh · Y. S. Lee (✉)
Department of Packaging and Logistics, Yonsei University, Wonju, Gangwon-do, South Korea
e-mail: leeyouns@yonsei.ac.kr

A. Kumar
Department of Food Technology, School of Chemical Technology, Harcourt Butler Technical University, Kanpur, Uttar Pradesh, India

K. K. Gaikwad
Department of Paper Technology, Indian Institute of Technology Roorkee, Roorkee, Uttarakhand, India
e-mail: kirtiraj.gaikwad@pt.iitr.ac.in

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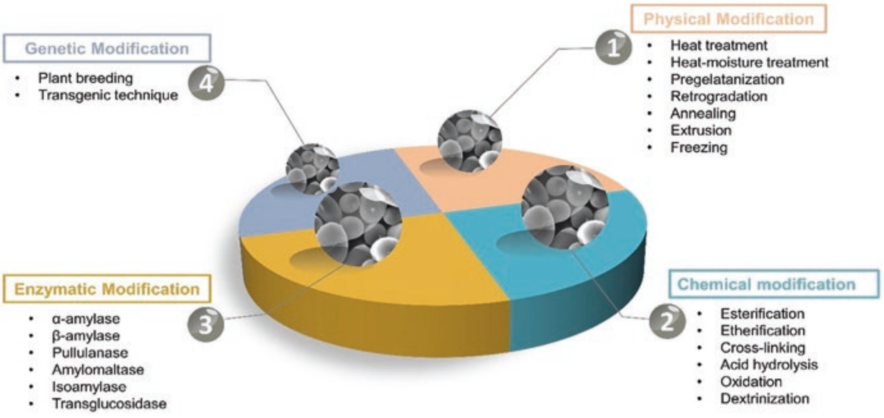

Fig. 17.1 Methods for modification of starch

(Neelam et al., 2012; Park et al., 2008). Physical modifications by thermal treatments, heat-moisture interventions, freezing and thawing, annealing, ultrasonic, and ultra-high-pressure treatments are simple and economical. In contrast, chemical approaches include adding functional groups to the starch molecules through oxidation, etherification, esterification, cross-linking, and acid treatment. On the other hand, the genetic modification of starch targets the enzymes involved in the manufacture and breakdown of starch in plants and uses transgenic technology carried out by plant breeding or biotechnology to introduce genes for new enzymes. Enzymatic modifications have been adopted in recent decades to create a starch with an improved structure, partially replacing chemical and physical methods for preparing modified starch. Enzymes are safer and healthier than chemical methods and support clean-label ingredients for both the environment and food consumers. In addition to having a clean label, enzyme-modified starches also provide a multitude of other benefits, such as improved purification, uniformity, affordability, stability, absence of undesirable compounds, and essential functional characteristics.

In this context, the modification of starches has sparked the development of novel processing techniques and consumer preferences to improve upon the limitations of native starches and expand the range of starch's use in industrial settings. Considering the scope and demand for the numerous functional and added-value features offered by these alterations, starch modification trends are expected to flourish further depending on demand for various sectors. This chapter covers an in-depth discussion of the enzymatic modification of starch, the different enzymes involved, and the resulting changes in starch characteristics like morphology, crystallinity, gelatinization, and rheological properties. In addition, the significance and application of enzymatic modification of starch production used for food products, packaging, and other purposes are briefly explored to highlight the improved properties of this modification.

17.2 Fundamentals of Enzymatic Modification

Traditionally, the purpose of producing modified starches was to overcome the technological limitations imposed by using native starches. The inherent limitations of native starches, including their insolubility in cold water, lack of viscosity, poor thermal, shear, and acid stability, capacity to thicken after cooking, retrogradation tendency, loss of ordered structure after gelatinization, and resulting syneresis in starchy systems, are intended to be overcome by various modification methods to increase their range of applications. However, the modification of starch causes an improvement in the characteristics of the amylose and amylopectin polymers while minimizing the negative attributes, making starch an advantageous polymer in a number of food and non-food industries (Calvin, 2016). For example, in the food industry, modified starch is used to substitute fat, encapsulate bioactive ingredients, prepare edible food coatings, preserve gel strength and consistency, control retrogradation, stabilize emulsification, improve freeze-thaw stability, and texturize food products. Modified starch, on the other hand, is also a significant key ingredient in various non-food industries, including the development of packaging materials in paper, adhesive, and bioplastics production, as well as the pharmaceutical, cosmetic, and textile sectors. Generally, starch is modified using physical and chemical methods that are simple, affordable, and simple to modify; however, in the context of increasing safety, consumers prefer more advanced, clean, and environmentally friendly approaches, looking for another alternative modification technique. Since enzymes are safer and more advantageous for the environment and the consumer than chemical methods, enzymatic modifications have now become extensively used in recent years, partially replacing the chemical methods of producing modified starch (Park et al., 2018).

While applying chemical modification procedures, such as those that use hypochlorite, phosphates, acetates, and acids, it has been observed that there may be an adverse effect on the environment due to the production of a significant volume of effluents during the process. Furthermore, due to the rising demand for clean-label ingredients in the food and pharmaceutical industries, several applications for these modified starches may be limited by the likely presence of trace elements in the finished product. In general, physical and enzymatic methods of starch modification are recognized as environmentally friendly since they provide “Clean Label ingredients” that are devoid of artificial and synthetic constituents (Bangar et al., 2022). In enzymatic modification, hydrolyzing enzymes are employed to process starch, which has a wide range of advantages, including a minimal by-product, targeted and effective hydrolysis products, a better yield, advanced process control, and finished products with distinctive features. The starch-modifying enzymes are derived from various plant sources and microorganisms, resulting in an enzymatic process that requires less energy and generates a higher yield. The commonly used enzymes for starch modification are transferases, debranching enzymes, and endo- and exo-amylases. In this context, the ultimate focus of enzymatic modification, as opposed to other types of modification, is to develop a starch with a new structure that allows for the modification of the molecular mass, branch chain length dispersion, and amylose/amylopectin ratio through enzyme reactions when the enzymes are reacting with starch granules (Park et al., 2018). Although enzymatic modification is beneficial and safe, the high cost of the enzyme and the longer reaction times result in high manufacturing costs for such modified starches, limiting their commercial application. In this context, a combination of physical and enzymatic physical modification, also known as dual-modification techniques, can address this cost issue for manufacturing modified starch with crucial characteristics (Adewale et al., 2022).

17.3 Mechanism and Action

Since modifications fundamentally refer to structural changes made to the starch molecule due to various functional, physical, and processing conditions. As a result, these modifications may positively or negatively influence the structure and functionality of starch molecules. In order to enhance the structural and physiological properties of these starches and get favorable results in various combinations, the structure needs to be further modified. Moreover, compared to chemical modification, which has been observed to minimize the tendency of starch to retrograde and improve dispersion, enzymatic treatment of starch may yield a more streamlined and desirable structure able to produce binding interactions to a larger degree (Conde, 2017). Enzymatic modification, thus, offers to produce desired changes in the starch structure by creating holes and pits in the starch granules. This enables water molecules to pass through the porous structure and aid in the gelatinization of the fluid, assist fluid permeation, or slow down the release of the fluid that is encased for predictable and regulated actions. During enzymatic modification, the starch molecule normally disintegrates and is rearranged, and the finished products depend on the types of enzymes applied. Designing a starch with a modified structure that allows the molar mass, branch chain length distribution, and amylose/amylopectin ratio to be transformed by enzymatic reactions when the enzymes interact with gelatinized starch is one method of modifying starch through enzymatic means (Park et al., 2018). For various food and non-food uses, these approaches often result in starches with modified physicochemical qualities and modified structural attributes.

Further, enzymatic modification can be employed on starch granules using enzymatic hydrolysis that can occur in certain parts of native starch granules. In such cases, while crystalline lamellae are more resistant to enzymatic degradation, less well-organized amorphous regions are more vulnerable to enzymatic attack (Park et al., 2018; Wang et al., 1995). Furthermore, since the adsorption capacity of porous starch granules is lower than that of inorganic adsorbents with higher specific surface areas, some starches, particularly cereal starch granules, have been found to contain confined pores that extend from the exterior to the interior of the granules (Guo et al., 2021). These pores are sites for immediate enzyme attack. Possible approaches in which enzymes can affect the starch granule exist. Pinholes, sponge-like erosion, multiple medium-sized holes, unique loci driving to single holes in individual granules, and surface erosion are the five forms of enzyme attack that have been observed (Lacerda et al., 2018). For instance, the effects of sequential enzyme modifications on sweet potato starch granules using transglucosidase in combination with maltogenic α-amylase and β-amylase exhibited an irregular truncated structure as shown in Fig. 17.2a and with increasing transglucosidase treatment time from 2 to 12 h, the pore size increases (appears like pinholes first) and then destroyed granules with exposed interior and granule fragments (sponge-like erosion) (Guo et al., 2019c). In general, enzymes can digest channels from specific places on the surface toward the center of the granule (endo-corrosion) or erode the entire granule surface or parts of it (exo-corrosion). In this context, Lacerda et al. (2018) investigated the effects of α-amylase, amyloglucosidase, and their combination on waxy rice starch and morphology study as shown in SEM micrograph Fig. 17.2b revealed shallow to deep pores and later appearance of internal canals and even exo-corrosion as treatment time was increased from 3 to 12 h. The amorphous region is relatively exposed and accessible at the surface of the granules, which causes such a phenomenon (Chen & Zhang, 2012). Some enzymes have been employed in this context to create porous starches, including glucoamylase, pullulanase, α-amylase, β-amylase, isoamylase, and cyclodextrin-glycosyltransferase (Guo et al., 2021).

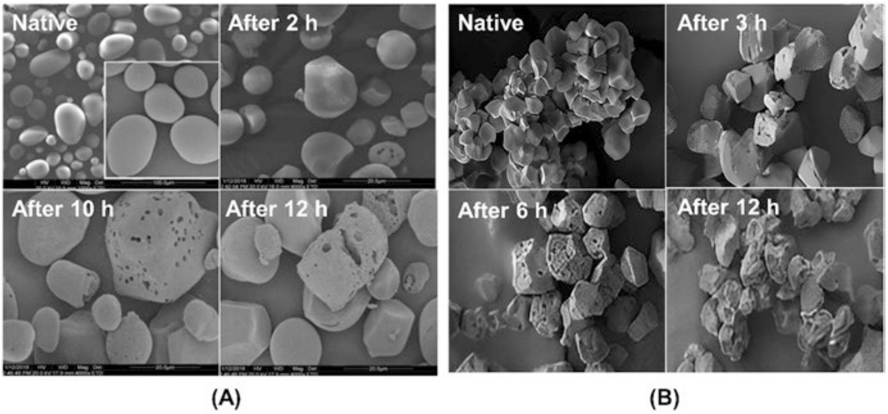

Fig. 17.2 SEM image of enzymatically modified starch: (**a**) native and modified sweet potato starch after treatment with transglucosidase in combination with maltogenic α-amylase and β-amylase glucoamylase for 2 h, 10 h, and 12 h; (**b**) native and modified sweet potato starch after treatment with amylase in combination with amyloglucosidase for 3 h, 6 h, and 12 h. (Figures (**a**) and (**b**) are adapted with permission from Guo et al. (2019c) and Lacerda et al. (2018), respectively. Copyright 2022, Elsevier, and 2023, John Wiley and Sons, respectively)

17.4 Starch Modifying Enzymes

In enzymatic modification, enzymes (hydratases) are utilized to expose starch, stimulating the creation of highly functional derivatives. The main role of enzymatic modification, which is primarily used to prepare glucose syrup or high fructose corn syrup, is starch depolymerization into oligosaccharides or starch transformation by transferring glycosidic linkages and residues that support in breaking down the α-1,4 bond between two glucose units (Amaraweera et al., 2021). In recent years, numerous enzymes have been identified and applied to modify starches. Figure 17.3 illustrates a list of the enzymes employed in the enzymatic modification of starch.

The complicated structure of the starch polymer necessitates the usage of endoamylases and exoamylases in combination to either depolymerize the starch into oligosaccharides and smaller sugars or to alter the starch by transferring oligoglucosidic bonds and residues and forming new bonds (debranching enzymes and glycosyl-transferases) (Hii et al., 2012). Generally, endoamylases, exoamylases, debranching enzymes, and transferases are the four major categories into which the effective enzymes on starch can be divided that eliminate chain branches in amylopectin. The functional characteristics of the four major classes of starch-converting enzymes that have been characterized so far are summarized in Table 17.1.

Among the four different kinds of starch-converting enzymes, endoamylases and exoamylases, which are amylases, can hydrolyze starch. The manner the glycosidic link is cleaved determines how these enzymes are categorized. Endoamylases have the aptitude to break down the, α,1-4 glycosidic linkages found in the internal (endo-) region of the amylose or amylopectin chain (El-Fallal et al., 2012). The well-known endoamylase enzyme α-amylase (EC 3.2.1.1) hydrolyzes α-1,4 linkages in the interior of starch polymers randomly, resulting in the creation of linear

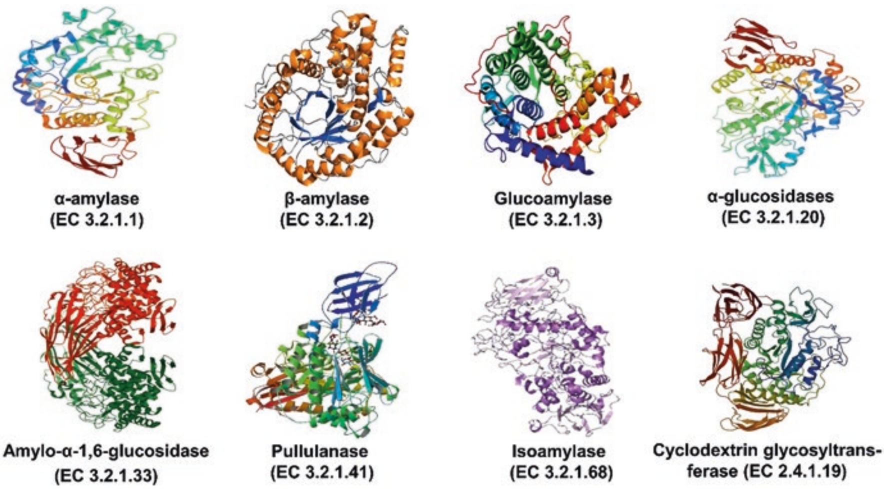

Fig. 17.3 Key enzymes involved in the enzymatic modification of starch

Table 17.1 Major types of starch-converting enzymes and their functional characteristics

Types of starch-converting enzymes	Example(s)	Functional characteristics	References
Endoamylases	α-amylase (EC 3.2.1.1)	Promotes the production of linear and branched oligosaccharides with different chain lengths by catalyzing the hydrolysis of internal α-1,4-glycosidic bonds	Park et al. (2018)
Exoamylases	β-amylase (EC 3.2.1.2) Glucoamylase (EC 3.2.1.3) α-glucosidases (EC 3.2.1.20)	Hydrolyze the α-1, 4 or α-1, 6 glycosidic linkages from the non-reducing end, resulting in successively shorter end products	Gopinath et al. (2017)
Debranching enzymes	Amylo-α-1,6-glucosidase (EC 3.2.1.33) Pullulanase (EC 3.2.1.41) Isoamylase (EC 3.2.1.68)	Catalyze the hydrolysis of the α-1,6-glucosidic linkages in glycogen and/or amylopectin	Hii et al. (2012)
Transferases	Cyclodextrin glycosyltransferase (EC 2.4.1.19) Amylomaltase (EC 2.4.1.25)	Create a new glucosidic link by cleaving a α-1,4 glucosidic bond on the donor molecule and transferring a part of the donor to the glucosidic acceptor	Hii et al. (2012)

and branched oligosaccharides, or α-limit dextrins. In low molecular weight products like glucose, maltose, and maltotriose units, the well-known endoamylase enzyme α-amylase (EC 3.2.1.1) hydrolyzes α-1,4 linkages in the inner of starch to produce linear and branched oligosaccharides, or α-limit dextrins. Although α-amylase is present in a wide range of plants, animals, and microbes, fungal and bacterial sources predominate in manufacturing sectors (Souza & Magalhães, 2010). The second class of enzymes, known as exoamylases, either selectively cleave α,1-4 glycosidic bonds, like β-amylase (EC 3.2.1.2), or both, α,1-4 and α,1-6 glycosidic bonds, like amyloglucosidase or glucoamylase (EC 3.2.1.3) and α-glucosidase (EC 3.2.1.20) (El-Fallal et al., 2012). Another major group of starch-converting enzymes is the debranching enzymes that exclusively catalyze the hydrolysis of α-1,6-glucosidic bonds in amylopectin and/or glycogen with the help of enzymes such as amylo-α-1,6-glucosidase (EC 3.2.1.33), pullulanase (EC 3.2.1.41), and isoamylase (EC 3.2.1.68). Debranching enzymes, one of the four major types of starch-converting enzymes, are extensively employed in industry and are further categorized into two major groups: direct and indirect. These enzymes break down amylopectin exclusively, allowing longer, linear starch molecules to finally (Hii et al., 2012).

The transferases comprise the fourth type of starch-converting enzymes. They cleave an α,1-4 glycosidic link of the donor molecule and relocate a part of the donor to a glycosidic acceptor while forming a new glycosidic bond. Cyclodextrin glycosyltransferase (EC 2.4.1.19) and amylomaltase (EC 2.4.1.25) form a new α-1,4 glucosidic link, whereas branching enzyme (EC 2.4.1.18) forms a new α-1, 6 glucosidic bonds. Through intramolecular transglycosylation, cyclodextrins are created from starch by cyclodextrin glycosyltransferases (CGTases), a significant enzyme in manufacturing. Depending on the type of acceptor molecule present during the final stages of the reaction, this enzyme also functions as a hydrolase or a transglycosylase (Leemhuis et al., 2010). Furthermore, in terms of the type of enzyme reaction, amylomaltases (EC 2.4.1.25) and CGTases are extremely similar. The main differentiation is that CGTases produce a cyclic product while amylomaltases perform a transglycosylation reaction that results in a linear product (El-Fallal et al., 2012). Other enzymes are crucially significant in modifying various starches, and dual or compound enzyme modifications of numerous starches are more frequently occurring than single enzyme modifications (Bangar et al., 2022).

17.5 Characteristic Changes in Enzymatically Modified Starch

Considering the advantages of eco-friendly practices and the increasing need for clean-label ingredients for use in various food and non-food industries, enzymatic modification of starch has become a spectacular approach to transforming the desired functional attributes. Depending on the specific functional and reactionary changes in starch that are required for a certain application, the choice of enzymes and treatment method is determined. As discussed in the previous section, enzymatic modification using a diverse range of enzymes has been explored in recent years on starch from a variety of sources to influence structural and functional properties such as solubility, crystallinity, swelling capacity, surface characteristics, viscosity, pasting, gelatinization, and binding properties, as well as thermal and freeze-thaw consistency. In nature, enzymes act as homogenous organic catalysts, and the concept of intermediate compound formation describes their mechanism. According to the mechanism for forming intermediate compounds, the enzyme attaches to the substrate (starch) and initiates chemisorption, which entails rupturing old bonds and forming new ones to create a starch-enzyme complex (Bangar et al., 2022). As a result, the instability of the new bonds and the ease with which they can be converted into product-enzyme complexes cause the enzyme to remove from the surface and diffuse away from the modified starch.

In recent years, the starch from major sources, including rice, oats, maize, potatoes, cassava, wheat, and sweet potatoes, has been modified using various enzymes, leading to an improvement in structural and functional characteristics (Guo et al., 2019b, c; Li et al., 2020; Shah et al., 2018; Shang et al., 2018). In general, the effect of enzyme modification on structural and functional properties such as solubility, crystallinity, swelling capacity, surface characteristics, viscosity, pasting, gelatinization, and binding properties is dependent on the plant source of the starches (cereal, tuber, legume, fruit, and vegetable), type of starches (regular, high-amylose, and waxy), and type of enzymes (branched or debranched). Amylose content, for example, is associated with starch solubility, whereas amylopectin is primarily responsible for swelling ability. When heated in water, starch granules swell and rapidly expand and stretch until granules get rigid and thickened (Bangar et al., 2022). In this context, swelling and solubility indices indicate the extent of interaction for both starch and water under specific heat conditions. The swelling index is the unit weight of water absorbed per gram of dry starch at a given temperature. The solubility index indicates the capacity of amylose molecules to liberate from starch granules by the proportion of starch granules that dissolve when heated to a specific temperature (Li et al., 2020). Furthermore, enzymatic modification of starches alters rheological properties such as viscosity reduction, increased starch elastic behavior, and digestibility. The changes in properties occur primarily as a result of the creation of pore spaces and fissures in starch granules, which results in a highly porous structure that allows water molecules to enter and aid in gelatinization, liquid ingestion, or slowing the release of encased fluid (Bangar et al., 2022; Purcell et al., 2014). For example, Li et al. (2020) studied the modification of rice starch using a combination of the autoclaving and sequential triple enzyme with β-amylase, transglucosidase, and pullulanase noticed that the modified starch used to have a higher swelling index, a higher solubility index, and a lower apparent viscosity.

Another characteristic change essential in starch modification is crystallinity, as starch is semi-crystalline in structure and insoluble in water. The crystalline region comprises double helices of amylopectin, while the amorphous region comprises amylose chains and branched segments of amylopectin (Dome et al., 2020). Furthermore, the ratio of amylose and amylopectin influences the starch structure in terms of crystallinity, granule size, and the chemical nature and arrangement of starch within the granule. Enzymatic modification of starch alters crystallinity by adjusting the amylose/amylopectin ratio, chain number and length, density, and branch points. For example, potato starch granules modified with branching enzyme and transglucosidase resulted in a significant reduction in crystallinity due to changes in amylopectin external chain length, chain number per amylopectin cluster, branch density, and the percentage of branch points (Guo et al., 2019a). Table 17.2 outlines the different characteristics and changes in starch from various sources during the enzymatic modification of starches.

Because of the different sizes, shapes, particle size distributions, amylose concentrations, and crystal structures of starch granules, the enzymatic modification of such granules is greatly influenced by different factors. Enzymes enhance the structure of starches by changing the length of the starch chain and the development of branch points, which results in debranching action, phosphate substitution, and disproportionation. The simultaneous effects of enzymatic treatment with α-amylase and amyloglucosidase on rice starch, increased mesopore and macropore fractionation, and created starch granules that can withstand higher operating temperatures and serve as thermoresistant adsorbents in comparison to native starches (Lacerda

Table 17.2 Characteristics changes during enzymatic modification of starches

Starch source	Enzymes used	Characteristics changes	References
Rice	α-amylase/Amyloglucosidase	Production of porous starch by enzymatic hydrolysis Increased mesopores and macropores fractionation Porous starch granules that can withstand higher operating temperatures and act as a thermoresistant adsorbent than native starches	Lacerda et al. (2018)
Wheat	α-amylase/Branching enzyme/ Glucoamylase	A substantial decrease in amylose content and long-side chains Significant increase in pore size and specific surface area Enhancement in the adsorption capacities of modified wheat starch	Guo et al. (2021)
Maize	Cyclodextrinase/Cyclodextrin glucosyltransferase	Significant improvement in the content of malto-oligosaccharides (MOSs) by dual-enzyme treatment Intense depolymerization effect on starch structure Reduction of the starch digestibility by sequential treatment of selected enzymes	Ji et al. (2021)
Potato	4,6-α-glucosyltransferase	Introduction of short branches and increased the ratio of short- to long-branches Effectively reduction in viscoelastic properties	Li et al. (2021)
Cassava	Amylomaltase	A significant decrease in molecular size and a more branched structure with a denser core Controlling the reaction time and the substrate constituent results in modified starch with extended chains and products with a cyclo-structure	Boonna et al. (2019)
Pea	Maltogenic amylase/ Pullulanase	Lower molecular weight values and a higher amylose content than native starch Lower digestibility and ideal crystalline structure formation	Liu et al. (2020)

(continued)

Table 17.2 (continued)

Starch source	Enzymes used	Characteristics changes	References
Sweet potato	Maltogenic α-amylase/β-amylase/Transglucosidase	Transformation of crystalline types, as well as increased branch density Reduced relative crystallinity, viscosity, gelatinization temperature, and melting enthalpy, accompanied by increased solubility	Guo et al. (2019c)
Tapioca	α-amylase/Amyloglucosidase	Substantial improvement in specific surface area and pore volume Effective at entrapment of bioactive compounds and flavoring agents	Prompiputtanapon et al. (2020)
Banana	Amylopullulanase or Pullulanase type II	Annealing of amylose chains to form compact structural forms Decrease in water solubility index and increase in amylose content and crystallinity index	Das et al. (2022)
Fox nut	Amylopullulanase or Pullulanase type II	Decreased water-holding capacity, oil-holding capacity, and swelling power, with enhanced cohesiveness, springiness, and gumminess when compared to native starch Increased crystallinity and a rise in degradation temperature	Biswas et al. (2020)
Red kidney beans	Pullulanase/Autoclaving	An increase in water absorption and solubility indexes, as well as a decrease in swelling power Increase in crystallinity and decrease in viscosity, indicating starch molecule rupture	Reddy et al. (2013)
Waxy corn	1,4-α-glucan branching enzyme	Enhancement in the number of α-1,6 branch points and reduction in the average chain length Reduced peak viscosity and setback value of starch, resulting in improved paste stability	Ren et al. (2017)

et al., 2018). Similarly a novel triple enzyme combination (α-amylase, branching enzyme, and glucoamylase) was employed to modify wheat starch by reducing amylose contents, shortening the length of branch chains, and increasing the degree of branchin enabled a noticeable increase in specific surface area and pore size (Guo et al., 2021). In this case, α-amylase hydrolysed the (1→4) linkages of amylose and amylopectin, which reduced chain lengths and enhances the number of short-side chains, followed by branching enzyme to achieve a higher degree of starch hydrolysis. In the enzymatic modification of starch, the third enzyme, glucoamylase, enabled effective hydrolysis of the new glucans with more non-reducing ends. Enzymatic modification with various enzymes such as cyclodextrinase, cyclodextrin glucosyltransferase, 4,6-α-glucosyltransferase, amylomaltase, maltogenic amylase, pullulanase, β-amylase, transglucosidase, amylopullulanase, and 1,4-α-glucan branching enzyme are influencing the property of native starches while also providing environmentally friendly and consumer safe solutions. Their use on various starches, including maize, potato, cassava, pea, tapioca, banana, sweet potato, and red kidney beans, resulted in significant changes in characteristics, as shown in Table 17.2.

17.6 Application of Enzyme-Modified Starches

As a natural material, starch performs a distinct role and is used in a wide range of commercial applications. Among natural materials, starch is one of the least expensive polysaccharides used in various food products. Starch-containing food products are considered major sources of calories and meet the basic need of diet and energy required by all sections of society due to their availability, accessibility, and affordability. Therefore, the physicochemical attributes of natural starch are modified further to enhance functional characteristics for various applications. As previously discussed, enzymatic modification of native starches in the food industry is beneficial and safe for overcoming various inherent problems, such as an inability to endure high shear stress, increased retrogradation, poor water solubility, paste clarity, increased mechanical strength, and limited freeze-thaw stability (Woo et al., 2021). The main benefit of using enzymatic modification over chemical modification is the clarity of enzymatic reactions, better yields, lesser by-products, and product safety. With the help of specific enzymes, modified starch produces linear short chains and branches that could demonstrate improved functional properties for developing the desired product. Because of its multidimensional functional characteristics in food products such as thickening agents, emulsifiers, swelling, encapsulants, gelling agents, binders, and fat substitutes, starch plays a vital role in the quality and nutritional benefits of several food products in the food processing sector such as bakery, beverages, syrups, and ice cream. Furthermore, starch has emerged as a sustainable, low-cost, and abundant polymer capable of developing environmentally friendly food packaging alternatives, owing to its exceptional

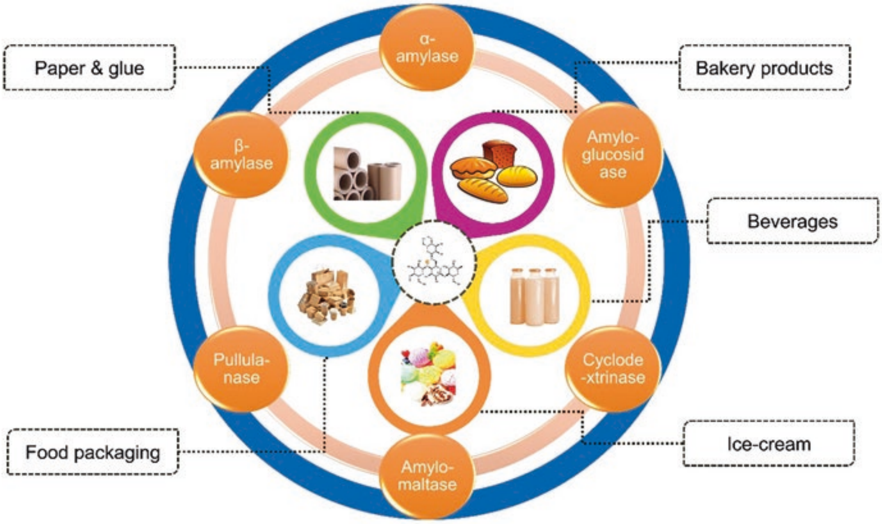

Fig. 17.4 Application of enzymatically modified starch in food and related industry

degradability and reproducibility in terms of addressing environmental issues and reducing petroleum dependency on plastic materials (Liu et al., 2022). Figure 17.4 depicts the major applications of modified starch through enzymatic modification in various food and related industries.

17.6.1 Bakery Products

Starch is a major ingredient in the bakery industry, providing structure and texture to baked goods such as bread, biscuits, crackers, cookies, cakes, pastries, and pies. In order to improve their quality and functionality, native starches are modified in various processing conditions, such as thickening agents, water-binding properties, emulsion stabilizers, and gelling agents in bread and other baked products. Enzymatic modification of starch occurs in situ during the production of bakery products. Native wheat flour contains a lot of β-amylase, which is mostly inactive and easily inactivated by heat prior to starch gelatinization. In this context, an investigation on starch functionality and digestibility on white wheat bread produced using maltogenic amylase and amylomaltase suggests a significant improvement in bread characteristics, starch retrogradation, and digestibility (Korompokis et al., 2021). The effective combination of enzymatic starch modification and the adaptable application of identified enzymes improves bread's nutritional and organoleptic properties. Here, enzymatic modification by maltogenic amylase assisted in the reduction of bread crumb firming rate and starch digestibility, whereas modification by amylomaltase resulted in the lowering of bread crumb firming rate. Furthermore, bread firming is primarily caused by changes in the starch fraction and the emergence of a continuous, rigid, crystalline starch network, which results in increased stiffness and shorter shelf life. This is due to amylose crystallization during cooling immediately after baking, whereas amylopectin recrystallizes over a longer period by retrogradation (Goesaert et al., 2009). Enzymatic treatment with various amylases has been shown to reduce the rate of bread firming by partially degrading the structure of amylose and amylopectin, resulting in changes in crystallization. In a similar study, the impact of α-amylase during the storage of straight-dough wheat bread resulted in extensive degradation of the starch network during bread storage, resulting in partial structure collapse and poor crumb resilience (Bosmans et al., 2013). The most significant effect of α-amylase enzymatic modification was improved bread volume, which resulted in smaller changes in crumb firmness and resilience (Table 17.3).

17.6.2 Beverages

Modified starch plays a crucial part in maintaining the texture and structure of food and beverages. Starch serves numerous functions in beverages, including thickening ingredients, providing stability, retaining moisture, offering freeze-thaw stability, stabilizing ingredients, enhancing flavor, preventing separation, and replacing fats. Increasing customer interest in minimal processing and the availability of products containing clean and simple ingredients has further resulted in advancements in the use of clean and fresh additives and production techniques for the food industry, such as enzymatic modification of starches. (Lopez-Ochoa et al., 2022; Singh et al., 2021). The influence of enzymatic modification (pullulanase and amyloglucosidase) on the pasting and thermal properties of cassava starch and the quality attributes of fermented dairy beverages, for example, resulted in a substantial increase in the overall acceptability of the beverages when compared to the control sample (Lopez-Ochoa et al., 2022). In this context, pullulanase and amyloglucosidase enzymes modify starch properties, making them a promising alternative to chemically modified starches by increasing viscosity and lowering syneresis in beverages. Therefore, such starch can be widely used to produce starch-based beverages due to its availability, affordability, and applicability.

17.6.3 Ice Cream and Frozen Dessert

Ice cream and frozen desserts are considered ready-to-eat meals or convenience foods that can be consumed anywhere and anytime. Generally, ice cream and most frozen desserts contain fat, milk solids-not-fat (the primary source of protein), water, sweeteners, emulsifiers, stabilizers, and flavors (Kale et al., 2020). However, rising consumer health concerns and demand for low-fat ice cream lead to the

Table 17.3 Effect of enzymatic starch modification on the product quality

Enzyme(s)	Starch sources	Applications	Desired outcomes	References
Maltogenic amylase/ Amylomaltase	Wheat starch	Bread	Reduced starch digestibility and bread crumb firming rate, resulting in improved bread nutritional and organoleptic properties	Korompokis et al. (2021)
α-amylase	Wheat starch	Straight-dough	Enhanced bread volume with less change in crumb firmness and resilience	Bosmans et al. (2013)
Pullulanase/Amyloglucosidase	Cassava starch	Beverage	Improved pasting and thermal stability resulting in increased viscosity and reduced syneresis	Lopez-Ochoa et al. (2022)
α-amylase	Taro starch	Ice cream	Enhanced stability and organoleptic properties of ice cream and resulted in improved foam stability, viscosity, and overrun	More et al. (2017)
α-amylase	Potato starch	Yogurt	Production of low-fat yogurt with improved physiochemical, texture, and sensory attributes	Ahmad et al. (2022)
4-α-glucanotransferase	Rice starch	Mayonnaise	Reduced-fat mayonnaise that mimics full-fat mayonnaise with gum in terms of rheological characteristics and appearance	Mun et al. (2009)
α-amylase	Wheat starch	Extruded noodles	Extruded noodles with a well-developed porous structure using optimal α-amylase concentration.	Li et al. (2019)
α-amylase	Corn starch	Active packaging films	Development of porous corn starch films with improved adsorption capabilities and slower polyphenol release than films prepared with unmodified corn starch.	Miao et al. (2021)
Pullulanase	Sago starch	Edible films	Creation of edible film with enhanced physical and mechanical properties as well as antimicrobial activity	Maizura et al. (2007)
Pullulanase/β-amylase	Corn starch	Paper surface sizing	Starch treated with pullulanase and β-amylasee as surface-sizing agents yield stronger paper due to optimal viscosity and molecular mass distribution	Wang et al. (2022)

adoption of starch in maintaining the stability and organoleptic properties of ice cream and frozen desserts. In this context, More et al. (2017) observed taro starch enzymatically modified with α-amylase to be a beneficial stabilizer in ice cream with effective foam stability, viscosity, and overrun in one of their studies. Enzymatically modified starch used as a stabilizer not only reduces costs but may also result in a better texture and lower melting point because it has been shown to inhibit freeze-thawing very well. In a similar study, ice cream manufactured by replacing 20% of the fat with enzyme-modified sweet potato starch was considered beneficial in reducing fat content in the formulation of low-calorie ice cream without affecting the physiochemical properties (Kale et al., 2020). As a result, modified starches with distinct functionality, nutritional, and other properties have stimulated interest in ice cream and frozen dessert formulations in recent years. Considering the benefits of enzymatically modified starch over other forms of modification in terms of food safety and green environmental approach, such starches are expected to be employed for other purposes as well.

17.6.4 Miscellaneous Food Products

Apart from bakery products, beverages, and ice cream, the incorporation of enzymatically modified starches has been widely explored in various food products over the past several years. This comprises low-fat yogurts, spreads, extruded products, and baby foods to enhance the sensory, physicochemical, and textural characteristics of the products in which they are included. In addition to serving as a significant source of energy and micronutrients like minerals and vitamins, yogurt also has probiotic, nutraceutical, and therapeutic benefits due to its distinctive characteristics (Sandoval-Castilla et al., 2004). Yogurt with minimal or even no fat is currently in demand from consumers who are concerned about their health. However, the lowering in fat content significantly impacts the sensory and organoleptic features. Enzymatically modified starch has thus been recognized as a clean-label ingredient for producing low-fat yogurt with a creamy, smooth texture and consistency. In this regard, the physicochemical, texture, microstructure, color, antioxidant, and sensory qualities of yogurt were found to be improved in a study pertaining to the production of low-fat yogurt by substituting 25% whole milk powder with potato starch modified with α-amylase (Ahmad et al., 2022).

Another widespread use for modified starch is as a water-soluble gelling agent to stabilize high-shear emulsions like mayonnaise and salad dressings. In order to provide a smooth and creamy and the required mouth feel for overall acceptability, enzymatically modified starches are often used as a fat substitute. For instance, the modified reduced fat mayonnaise demonstrated shear thinning behavior and yield stress when the mayonnaise fat was partially replaced with the 4-α-glucanotransferase-modified rice starch paste at levels up to 50% in combination with xanthan gum (Mun et al., 2009). These findings indicate that enzymatic modification of starch has the potential to produce reduced-fat mayonnaise with rheological properties and visual characteristics comparable to those of full-fat mayonnaise with gum. Similar to other food products, starch noodles can have their quality enhanced by altering the way that starches interact throughout the production of starch dough, slicing, heating, freezing, thawing, and drying. For example, altering native granular starches influences how they retrograde, gelatinize, and form a paste. Concerning this, Li et al. (2019) explored the development of extruded noodles incorporating thermostable and mesophilic α-amylase employing different combinations of enzymatic modification and food extrusion parameters. By employing an enzymatic modification strategy, mesophilic α-amylase at a concentration of 1.6% was revealed to be the most viable way of creating extruded noodles with a well-developed porous structure. Since the rheological properties, cohesiveness, and elastic behavior of native starches are limited when they are processed, the food processing industry are generally preferring clean-label starches with enhanced functional attributes. In this context, the key advantage of enzymatic modification is that the resulting starch is regarded to be a safe, natural resource and ingredient that can be marketed as clean-label starch.

17.6.5 Food Packaging

Considering biopolymers derived from sustainable sources have substantial advantages in packaging applications, innovations and research in eco-friendly food packaging materials are currently in a period of rapid advancement. With the concept of beneficial food-packaging interactions, active packaging is a novel packaging technology that can be used to develop packaging systems that protect foods dynamically and ensure quality (Singh et al., 2022). Starch, a renewable, inexpensive, and ubiquitous polymer, can create eco-friendly packaging solutions and has an exceptional degradation rate and reproducibility in terms of addressing environmental threats and minimizing the use of fossil-based plastic materials. In this context, Miao et al. (2021) employed porous starch formed by enzymatic modification of corn starch to create novel active starch films as slow-release active packaging materials. Compared to films prepared from unmodified corn starch, the porous starch exhibited improved tea polyphenol adsorption capacity and slower polyphenol release. The prolonged slow release of polyphenols from the films is a desirable characteristic for continuing the protection provided by the active film to the food under investigation. Thus, enzymatically modified porous starch offers a clean, safe, and effective means to interact with food products while delaying the release of active ingredients. Another approach that has been used for a long time in food packaging is edible films and coatings. Overall, starch is the most potential renewable and sustainable material for edible films/coatings due to its easy availability, low price, better extraction rate, nutritional value, easy fabrication, biodegradability, biocompatibility, and edibility with functional properties (Shah et al., 2016). For instance, the edible film with alginate and lemongrass oil was developed using sago starch that had been enzymatically modified using pullulanase. This film showed enhanced antibacterial activity as well as significant mechanical properties (Maizura et al., 2007). Enzymatically modified starch is a clean-label ingredient in such formulations where antimicrobials and edible starch films are used to inhibit the growth of microorganisms in food. This may have a substantial impact on food safety and shelf-life extension.

17.6.6 Paper and Glue

Another significant use of modified starch that is extensively used in the paper and glue sector is the production of starch pastes for coating and surface sizing of paper as well as increasing the bonding ability of glues to serve as an excellent adhesive property. Starch modification is deemed an efficient strategy to control the high viscosity of the native starch slurry, which limits it from being used in surface sizing in a practical approach. In contrast to chemical modification, enzymatic modification provides a preferable strategy to improve the rheological properties of starch slurry, particularly in food and pharmaceutical products. In the paper manufacturing industry, the alpha-amylase enzyme is generally employed in developing modified starch-based surface sizing to produce standard grades of paper (Bajpai, 2018). However, various enzymes with different sites and modes of action from those of α-amylase, such as β-amylase, pullulanase, and glucoamylase, have been investigated in recent years. In this regard, pullulanase and β-amylase, are applied for the enzymatic modification of corn starch, resulting in an excellent surface sizing agent to enhance paper quality (Wang et al., 2022). Owing to their optimal viscosity and molecular mass distribution, pullulanase and β-amylase functioned more effectively as surface-sizing agents than α-amylase in this investigation and provided the paper with a higher degree of strength.

17.7 Conclusion

With rising food safety and environmental concerns, the issues identified with starch modification in recent years have encompassed the modification of starch that is simultaneously healthy for consumers and benign to the environment. In this case, the primary benefit of enzymatic modification appears to be that they are safe and clean label starches, implying that they can be classified as ingredients rather than additives and do not need to be identified on food labels as modified food starch or food starch-modified, allowing them to be legitimate as natural ingredients. Thus, the relevance of enzymatically modified starches in resolving the limitations of native starches and increasing the effectiveness of starch for commercial processes cannot be taken for granted. When compared to chemical or genetic modification, they are significantly safer and do not require the inclusion of chemicals or biological agents.

This chapter discussed the enzymatic modification of starches as an emerging novel processing technology and a market trend that is partially replacing chemical and physical methods for a wide range of applications in the food, packaging, pharmaceutical, textile, and paper industries, among others. Endoamylases, exoamylases, debranching enzymes, and transferases have been outlined as starch-modifying enzymes that reduce retrogradation, paste gelling tendencies, and gel syneresis to enhance paste clarity and reflectiveness, gel formation, optical transparency, and dimensional stability, all of which are typically required in practical production. Furthermore, understanding the mechanism of action that occurs during enzymatic modification to transform the molar mass, branch chain length distribution, and amylose/amylopectin ratio influences the design and selection of appropriate starch and is necessary to comprehend for efficient implementation. The increasing use of enzymes such as α-amylase, pullulanase, 4,6-α-glucosyltransferase, and amylopullulanase in starch modification for various food and non-food industries demonstrates the potential and growing demand for clean-label starches in the starch modification industry. Considering the increasing demand for enzymes in starch modification and the limited availability of viable enzyme sources, there is a need to focus on newly developed sources of safe and sustainable enzymes derived from plants and microbes.

Since its inception in the late nineteenth century, starch modification has advanced dramatically. In the near future, the novel advancement of enzymatically modified starches should be considered a promising approach to investigate since it has the potential to supplant the current method of starch treatment by using physical, chemical, and genetic modification.

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Chapter 18
Fermentation of Starch

Samson A. Oyeyinka, Marina Corrado, Brittany Hazard, and Bukola A. Onarinde

18.1 Introduction

Fermentation is an ancient food processing method that has been applied in the biotransformation of raw agricultural produce to develop products for food, livestock and pharmaceutical industries. It is a traditional process used in the production of fermented foods and alcoholic beverages that has been an important part of the human diet for thousands of years (Tamang et al., 2020). Fermented food products have unique characteristics such as extended shelf-life, enhanced economic value, reduced antinutrients, increased nutritional value and improved sensory properties that has made them acceptable to many over the years. Furthermore, fermentation has been used to detoxify and create variety, product diversification and improved digestibility, for example in root and tuber crops (Ramesh et al., 2009). A wide variety of fermented food products exist including cheeses, wine, meat, tempeh, sauerkraut, yoghurt and fermented starch called *polvilhoazedo*. Although fermentation has been widely used in several applications including food processing, it has also been used to modify the functional and physicochemical properties of starch.

S. A. Oyeyinka (✉)
Quadram Institute Bioscience, Norwich, UK

National Centre for Food Manufacturing, College of Science, University of Lincoln, Holbeach, UK
e-mail: soyeyinka@lincoln.ac.uk

M. Corrado · B. Hazard
Quadram Institute Bioscience, Norwich, UK

B. A. Onarinde
National Centre for Food Manufacturing, College of Science, University of Lincoln, Holbeach, UK

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Starch fermentation may be achieved either by fermenting starch isolated from the plant material with microorganisms and their enzymes (Demiate et al., 1999; Nakamura & Park, 1975; Westby & Cereda, 1994; Zhao et al., 2019; Mestres & Rouau, 1997) or by fermenting the plant material e.g. roots and tuber crops before starch extraction (Oyeyinka et al., 2020; Elkhalifa et al., 2004; Jyothi et al., 2005). Whatever is the extraction method, it is important to note that fermented starches usually have entirely different properties from the native starch. These changes, obviously would depend on the length of fermentation, which may vary from 24 to 200 h as well as the type of end product desired (Nakamura et al., 1997). An early study by Nakamura and Park (1975) reportedly used fermentation to impact cassava starch with a unique sour flavour and to also improve its physicochemical properties. The authors found that fermented starch was more soluble and showed higher swelling ability in water compared to non-fermented cassava starch. The biotransformation process during fermentation produces organic acids such as lactic acid, and these acids produce changes in the molecular weight and surface morphology of starch granules (Garcia et al., 2016). Native starches have poor resistance to extreme processing conditions such as high temperature and shear that limits their use in the industry. Hence, starch modification is necessary to overcome these limitations and enhance their industrial application.

Fermentation is regarded a physical method of starch modification, since it does not involve the addition of chemical agents (Garcia et al., 2016). However, it could also be regarded as an enzymatic modification method since it involves the use of enzymes. It may therefore be ideal to regard starch fermentation as a physico-enzymatic modification method. Whatever classification is adopted, both methods are environmentally friendly and are being encouraged for starch modification because they do not involve the use of harmful chemicals (Oyeyinka et al., 2020). One major product of industrial significance made from fermented starch is sour starch, also known as *polvilho azedo*, an important product in Brazil (Díaz et al., 2018). It is a product obtained from the natural fermentation of freshly extracted cassava starch for approximately 30–40 days (Cereda, 1987; Gomes et al., 2005), though the period of fermentation may vary and explains the variations in the levels of acid produced, which influences the properties of products made from the fermented starch (Gomes et al., 2005). Sour starch has good baking expansion characteristics that could be explored in bakery applications (Mestres et al., 2000; Marcon et al., 2007, 2009), for example, in the production of biscuits and cheese-bread (Camargo et al., 1988).

Besides starch modification, fermentation has been used to produce ethanol (Nichols et al., 2005; Moshi et al., 2015; Strąk-Graczyk & Balcerek, 2020) and saccharify starch for various industrial use (Anuradha et al., 1999; Matsumoto et al., 2011). Lu et al. (2005) studied the influence of fermentation on the physical properties of rice flour and the rheological characteristics of rice noodles and found that the fermentation process changed the amorphous region of rice starch granule as well as the chemical components, resulting in better quality rice noodles made from fermented rice flour. Both bacteria and fungi (yeast) are involved in the fermentation process and this process could be natural or artificially induced, involving the use of enzymes that are inoculated into a sterile starch environment. The modification of starch through fermentation is a low-cost technology that presents a unique opportunity to change the properties of starch with less impact on the environment. This chapter discusses the effect of fermentation on starch composition, structure, functional and physicochemical properties.

18.2 Modification Mechanism and Methodology

The fermentation process can proceed in several ways depending on the type of organism involved and the pathway utilised in the fermentation process. Each of these fermentation types follows different pathways and uses a different type of organism and enzyme system to generate the desired product. Starch fermentation may involve different strains of bacteria or yeast, resulting in different intermediate or end products. For example, while most bacteria would produce lactic acid as the final product, ethanol is the end product when yeast are involved in the fermentation process. Conventionally, ethanol is produced via two steps. The first step involves the depolymerisation of starch into glucose by α-amylase and glucoamylase, while the second step involves fermentation of glucose by yeast (*Saccharomyces cerevisiae*) to ethanol (Cripwell et al., 2020). In some instances, *Saccharomyces cerevisiae* which has been genetically engineered may depolymerise the starch and also ferment the resulting sugars to ethanol (Cripwell et al., 2019; Chandel et al., 2018). For lactic acid production, lactic acid bacteria (LAB) are the major group of bacteria involved and these organisms are well-known to ferment carbohydrate-rich foods including starch. The main function of LAB in fermented foods is to ferment carbohydrates into lactic acid that can quickly acidify the food to enhance the shelf-life and microbial safety of the products (Hu et al., 2022). This category of bacteria can either be homo-fermentative or hetero-fermentative. As with ethanol production, starch hydrolysing enzymes (α-amylase and glucoamylase) depolymerises starch firstly into reducing sugars but rather than ethanol production, the reducing sugars are transformed into lactic acid by LAB (Datta & Henry, 2006). The metabolic pathways for the fermentation of carbohydrate by LAB is through the Embden-Meyerhof-Parnas (EMP) pathway where the sugars are converted into pyruvic acid and subsequently reduced to lactic acid as the only product, when homo-fermentative LAB are involved (Bintsis, 2018). On the other hand, hetero-fermentative LAB follow the phosphoketolase pathway (PKP) to an intermediate product (glucose-6-phosphate) for the formation of pyruvic acid, which then produces lactic acid and additional metabolites such as acetic acid carbon dioxide and ethanol (Hu et al., 2022).

Different types of LAB such as *Pedicoccusspp* (Pintado et al., 1999), *Enterococcus faecium* (Shibata et al., 2007), *Lactobacillus manihotivorans* (Pintado et al., 1999; Ohkouchi & Inoue, 2006), *Lactobacillus fermentum* (Sanni et al., 2002; Santoyo et al., 2003), *Lactobacillus amylovorus* (Zhang & Cheryan, 1991), *Lactobacillus*

Table 18.1 Fermentation type, conditions and microorganisms isolated from fermented starch

Starch source	Type of fermentation	Incubation time and temperature	Microorganisms isolated from the starch	References
Canary seed	Natural	0–45 days (room temperature)	*Aerobic mesophilic bacteria; Moulds, Yeasts*	Batista et al. (2020)
Cassava	Inoculum containing *Lactobacilli*, *Streptococci*, *Corynebacteria* and yeast cells	0–72 h (room temperature)	NR	Moorthy et al. (1993)
Cassava	Natural	0–72 h (room temperature)	NR	Oyeyinka et al. (2020)
Cassava	Natural	0–90 days	NR	Alonso-Gomez et al. (2016)
Corn	Natural	0–21 days (26-30 °C)	NR	Yuan et al. (2008)
Rice	Rice leaven	0–60 days (30 °C)	NR	Zhang et al. (2016)
Sweet potato	Inoculum from previously fermented cassava	0–72 h (30 °C)	Microbial counts on nutrient, Streptococcus, MRS and yeast extract glucose agars all increased	Jyothi et al. (2005)
Sweet potato	Natural	0–12 months (room temperature)	*Acetobacter lovaniensis*, *Bacillus velezensis*, *Bacillus cereus*, and *Gluconacetobacter liquefaciens*,	Ye et al. (2019)
Wheat	Natural	0–72 h (30 °C)	*Saccharomyces cerevisiae* and *Lactobacillus plantarum*	Fan et al. (2021)
Wheat	Natural	0–96 h (30 °C)	Lactic acid bacteria, mainly *Lactobacillus plantarum* and yeasts, mainly *Saccharomyces cerevisiae*	Zhao et al. (2019)

NR Not Reported

amylophilus (Naveena et al., 2005; Altaf et al., 2006), *Lactobacillus plantarum* (Thomsen et al., 2007), *Streptococcus bovis* (Narita et al., 2004) and *Leuconostoc strains (*Thomsen et al., 2007*)* have been used in the production of lactic acid from starch. Besides LAB, fungi such as *Rhizopus oryzae* and *Rhizopus arrhizus* have been reportedly used in the fermentation of starch (potato) for lactic production (Huang et al., 2005). The fermentation of starch has been mainly achieved by natural process rather than the use of inoculum (Table 18.1). Only very limited studies reported the isolation of the microflora present in fermented starch (Zhao et al., 2019; Jyothi et al., 2005; Batista et al., 2020; Ye et al., 2019; Fan et al., 2021). In general, microbial counts of yeast and bacteria reportedly increased with increase in fermentation period, up to about 48 h in wheat starch, and thereafter showed a decline (Zhao et al., 2019). *Lactobacillus plantarum* and *Saccharomyces cerevisiae* were isolated form wheat that was naturally fermented for up to 96 h (Zhao et al., 2019; Fan et al., 2021), while Ye et al. (2019) reported the presence of *Acetobacter lovaniensis*, *Bacillus velezensis*, *Bacillus cereus*, and *Gluconacetobacter liquefaciens* in sweet potato starch naturally fermented for a period of 12 months. Variation in the type of microflora may depend on the source of starch, period of fermentation as well as possible contamination during the fermentation process. Lactic acid production has wide applications in food, leather, pharmaceutical, textile and other chemical industries including biodegradable plastics (John et al., 2007, 2009). Approximately 85% of lactic acid production is used in food and food-related applications, while non-food uses account for about 15% (John et al., 2009). Lactic acid has been used for decades as an acidulant in food and beverage sector and has also found application as a flavouring agent in different products such as bread and bakery products, jellies, mayonnaise, and processed eggs, often in synergy with other acidulants (John et al., 2007; Datta et al., 1995). The degree of changes in starch structure, functional and physicochemical properties will depend on factors such as the length of fermentation, type of microorganisms involved including the type of enzymes, fermentation conditions as well as the composition of the starting starch material. For instance, Zhao et al. (2019) found that the molecular weight and amylose content of wheat starch decreased with an increase in fermentation days. The starch extracted from fermented cassava root also showed a reduction in amylose content and exhibited changes in morphology such as micro-holes, rough surfaces and clumping of starch granules, with an increase in the period of fermentation (Oyeyinka et al., 2020).

18.3 Effect of Fermentation on Starch Structure and Composition

The molecular structure of starch may vary with the botanical source, genetic background or varietal differences as well as type of modification. The starch granules are made up of amylose and amylopectin, with the former consisting of α-(1,4)-linked D-glucose residues, while the latter has additional α-(1,6)-glucosidic linkages, resulting in branching within the starch molecule. Details of starch structure and composition from native starches are abundant in the literatures and are not the focus of this chapter. This section will discuss starch composition, morphology, crystallinity pattern and chain length distribution of starch molecules as influenced by fermentation.

18.3.1 Starch Composition

Starch is composed of mainly amylose and amylopectin but may contain other non-starch components such as lipids, proteins and phosphate. The ratio of amylose and amylopectin as well as their molecular structure are the predominant factors that influence starch functional and physicochemical properties. However, other non-starch components can also significantly influence the functionality of starch. For example, the substantially high swelling of potato starch granules is associated with the presence of high phosphate monoester content in potato starch (Jane et al., 1999). The presence of lipids in starch has also been found to limit hydration and swelling during pasting and gelatinisation (Oyeyinka et al., 2017).

The impact of fermentation on starch composition has been contradictory in the literature (Zhao et al., 2019; Oyeyinka et al., 2020; Jyothi et al., 2005; Camargo et al., 1988; Fan et al., 2021; Moorthy et al., 1993; Martinez & Quiroga, 1988). The amylose content of starch significantly reduced (Zhao et al., 2019; Oyeyinka et al., 2020; Jyothi et al., 2005; Moorthy et al., 1993) or did not change significantly after fermentation (Camargo et al., 1988; Martinez & Quiroga, 1988). Jyothi et al. (2005) further reported a significant reduction in the percentage of starch levels in starch isolated from fermented potato tubers. The variation in the effect of fermentation on amylose content as reported in the literature may be due to the variation in method of fermentation. For example, a study that reported no significant change in amylose content, fermented the starch with mineral acid (HCl), organic acid (Acetic acid) and enzyme hydrolysis (α-amylase) (Camargo et al., 1988), while the studies that reported a reduction in amylose content fermented the starch by natural fermentation (Zhao et al., 2019; Oyeyinka et al., 2020; Jyothi et al., 2005; Moorthy et al., 1993). Reduction in amylose content can be attributed to the activities of hydrolytic enzymes such as α and β-amylase, which presumably degrade amylose and amylopectin during the fermentation process (Zhao et al., 2019; Oyeyinka et al., 2020; Fan et al., 2021). Amylose leaching during soaking and fermentation of plant materials may also contribute to the reduction in the amylose content of starch (Oyeyinka et al., 2020). In terms of non-starch components, Camargo et al. (1988) found that non-starch component did not substantially change after fermentation. Other reports on fermented starch did not document the impact of fermentation on non-starch components, suggesting the need to analyse these components in future studies.

18.3.2 Granule Morphology

The morphology of starch granules is mostly examined using a scanning electron microscope (SEM) but can also be determined using a light microscope (LM). Granule shape and size are important structural properties that can impact functional and physicochemical properties. Native starches are usually smooth with no fissures and indentations on the granule surface (Alonso-Gomez et al., 2016; Sukhija

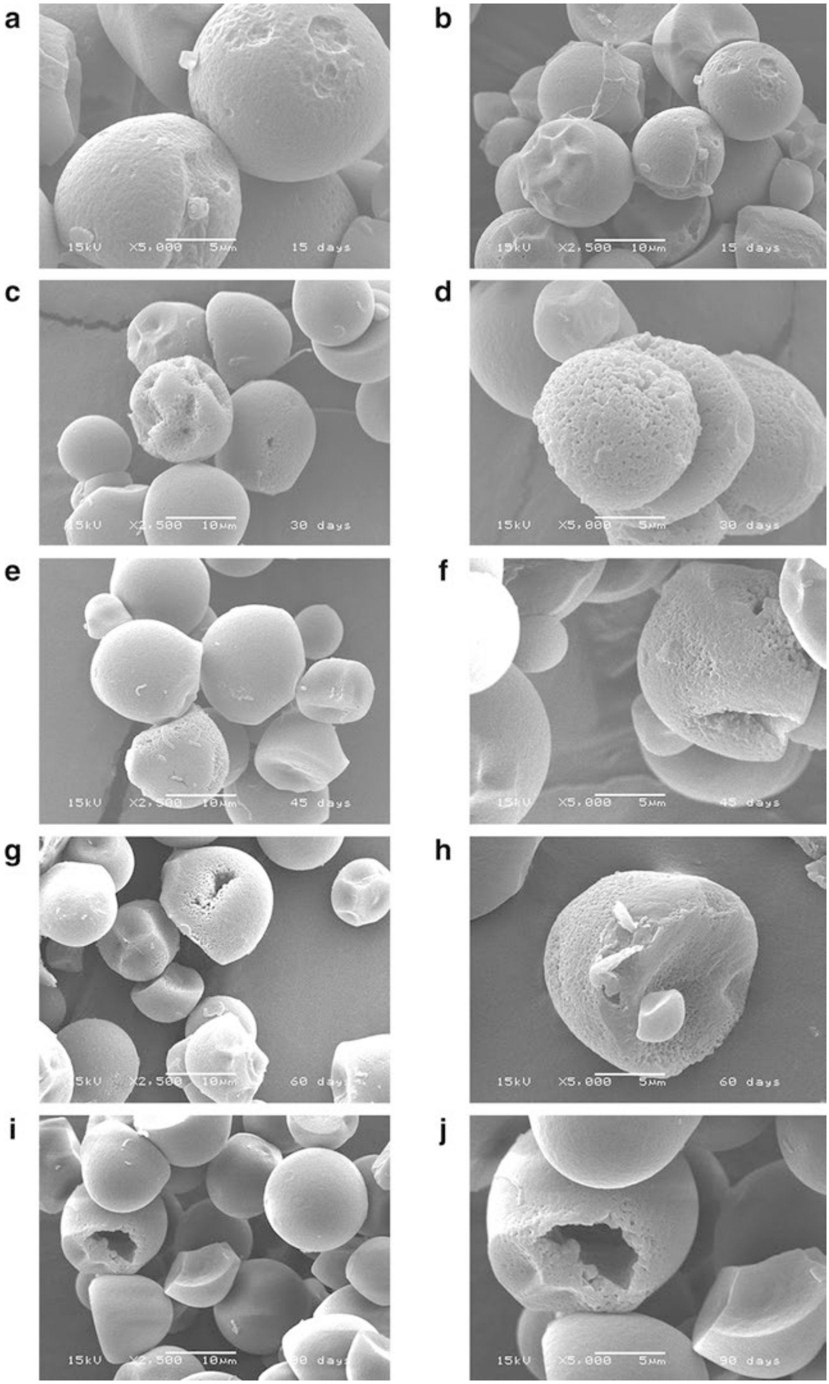

Fig. 18.1 (**a**)–(**j**) The SEM images of fermented cassava starch as a function of the steeping time for 15, 30, 45, 60, 75, and 90 days. (Alonso-Gomez et al., 2016)

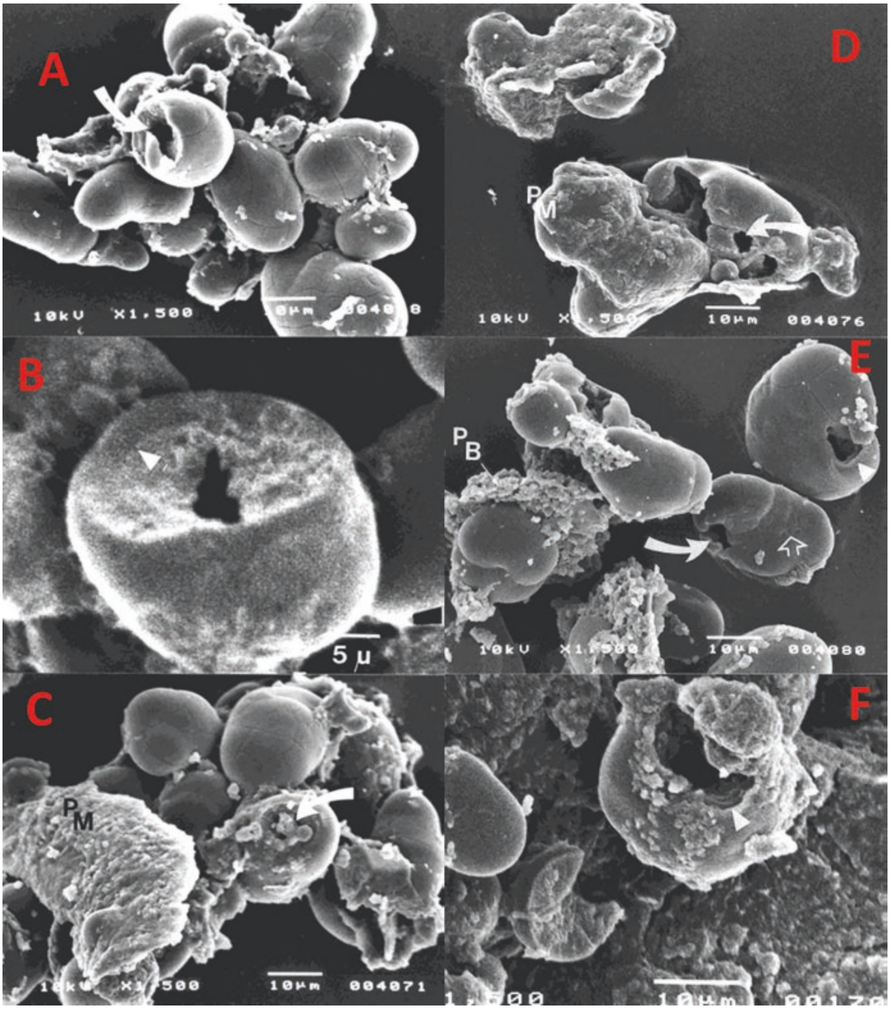

Fig. 18.2 Morphology of lentil flour fermented for 96 h at different flour concentration and temperature (Sotomayor et al., 1999). *PM* protein matrix, arrows and arrow heads indicate internal cavities and lamellae, respectively. (**a**): Flour concentration of 79 g/L at 38 °C; (**b**): Flour concentration of 79 g/L at 38 °C. (**c**): Flour concentration of 79 g/L at 42 °C; (**d**): Flour concentration of 221 g/L at 28 °C. (**e**): Flour concentration of 221 g/L at 42 °C; (**f**): Flour concentration of 221 g/L at 42 °C

et al., 2016). The effect of fermentation on granule morphology generally depends on the length of fermentation time, with varied result reported by different authors (Zhao et al., 2019; Oyeyinka et al., 2020; Alonso-Gomez et al., 2016). Using SEM, Oyeyinka et al. (2020) reported that starch isolated from fermented (24–76 h) cassava root clumped together and showed partly broken granules with fissures or micro holes. According to these authors, the degree of starch granule distortion

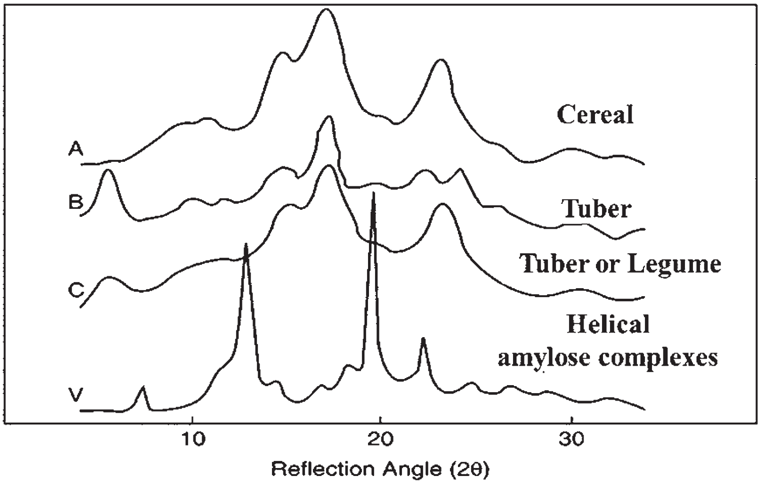

Fig. 18.3 X-ray diffraction patterns for different starch types. (Cui, 2005)

increased with fermentation time (Oyeyinka et al., 2020). Alonso-Gomez et al. (2016) also reported the presence of micro-holes on cassava starch surface fermented for 15 days, with exposed inner parts of starch after fermentation for up to 90 days (Fig. 18.1), further confirming the influence of the length of fermentation on changes in granule morphology. Starch granule distortions such as a cavity (Fig. 18.2) in the middle of lentil starch (Sotomayor et al., 1999), as well as indentations and pitting on the surface of sour cassava starch has also been reported (Cardenas & De Buckle, 1980).

18.3.3 Crystallinity Pattern

Starch crystalline pattern is largely dependent on crystalline lamellae arrangement within the amylopectin molecule (Jenkins & Donald, 1995). X-ray diffractometer (XRD) studies on starch revealed two major types of polymorphs (A and B) which are differentiated based on the double helices packing and level of hydration (Imberty & Perez, 1988). A third classification, the C-polymorph is considered a mixture of the A and B-polymorphs (Pérez et al., 2011). Although variation has been reported among the crystalline patterns for starches of the same botanical origin, the A-type pattern is generally found in cereal starches, the B-type is associated with root and tuber starches, while the C-type is generally found in legume starches (Fig. 18.3). Fermentation generally did not change the crystalline A-type of cassava starch (Alonso-Gomez et al., 2016), wheat starch (Zhao et al., 2019; Fan et al., 2021), C-type for potato starch (Ye et al., 2019) or the A + V hybrid crystalline structure of rice starch (Tu et al., 2021). However, variations have been observed on

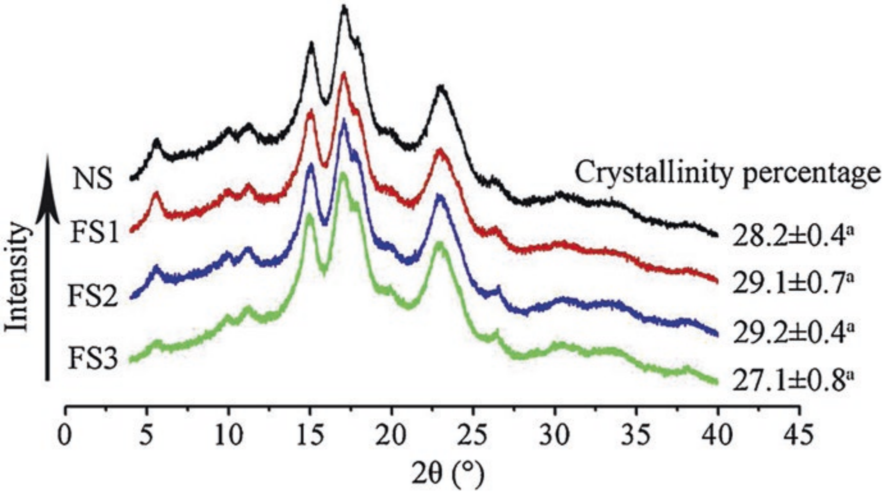

Fig. 18.4 X-ray diffraction patterns of native and fermented potato starch. (Ye et al., 2019)

the relative crystallinity (RC) of fermented starch. For instance, some authors found that the RC of fermented wheat starch significantly increased (Zhao et al., 2019; Fan et al., 2021), others reported a decrease for fermented rice starch (Tu et al., 2021), while the RC did not change for fermented sweet potato starch (Ye et al., 2019). Fan et al. (2021) found significantly higher RC for fermented wheat starch (approx. 39%) compared to unfermented starch (approx. 36%). Zhao et al. (2019) also reported that the RC increased by approximately 6, 8, 12 and 14% when wheat starch was fermented for 24, 48, 72 and 96 h indicating the fermenting organisms including their hydrolytic enzymes degraded the amorphous region of the starch granules. On the contrary, the RC rather decreased for rice starch co-fermented with varying concentrations (1, 3, 5, 7%, starch basis) of a mixture of yeast and *Lactobacillus* strains at a ratio of 3 to 1, respectively (Tu et al., 2021). The authors found an increase in the RC of fermented rice starch at higher levels of microbial strains (>7%), which was attributed to the assembly of starch molecules according to the changes of starch lamellar. Ye et al. (2019), however, found no significant changes in the RC of potato starch fermented for a period of 3, 8 and 12 months compared with the native starch (Fig. 18.4). It is puzzling that despite fermentation for longer periods, there were no changes in the RC reported for potato starch. The variations observed in the literature with regards to changes in RC suggest starch source as well as the type of microorganisms and concentrations of the cultures, may influence the structure of starches during fermentation. Hence, future studies need to compare the behaviour of starches from the different botanical origins, especially pulses and other non-conventional sources.

18.3.4 Chain Length Distribution

Starch functional and physicochemical properties are mostly influenced by the ratio of amylose to amylopectin as well as the chain length distribution of the amylopectin component of the starch (Jane et al., 1999; Huang et al., 2007). Fermentation of starch reportedly reduced the average molecular weight of starch (Zhao et al., 2019; Ye et al., 2019) and modified the amylopectin chain-length distribution patterns (Ye et al., 2019). The reduction in the molecular weight of wheat starch presumably resulted from the degradation of the long-chain component of amylopectin into short and intermediate amylopectin during natural fermentation (Zhao et al., 2019). The amylopectin chain length profiles of starch are classified into short-chain (DP 6–12)-A, medium-chain (DP13–24)-B_1 and long-chain [(DP 25–36)-B_2 and (DP > 36)-B_3] (Hanashiro et al., 1996). Ye et al. (2019) studied the impact of fermentation on the chain length distribution of sweet potato starch and found the average chain length of fermented starch (10.7–19.7) were generally lower than the native starch. Furthermore, there was a substantial increase in short A chains (DP 6–12) with a corresponding decrease in longer amylopectin chains (B_2 and B_3). de la Concha et al. (2018) similarly reported that longer amylopectin chains in the amorphous region are susceptible to acid and enzyme hydrolysis resulting in higher proportions of short-chain amylopectin. The modification of starch granules during fermentation has been found to depend on factors such as the botanical origin of the starch (Benavent-Gil & Rosell, 2017; Li et al., 2017), type and species of microorganism (Lu et al., 2005; Kou et al., 2017; Lu et al., 2008), the activity of enzymes (Benavent-Gil & Rosell, 2017) and the presence of other substances (Li et al., 2015, 2017).

18.4 Effect of Fermentation on Starch Functional and Physicochemical Properties

Functional and physicochemical properties of starch are important properties that dictate the use of starch in the industry. Although the use of these two terms have been very misleading in the literature, both properties are affected by starch structure and composition. The functional properties of starch are those properties that dictate their role or function in food systems, for example, water absorption, oil absorption, swelling power, solubility, paste clarity and texture. On the other hand, physicochemical properties of starch are those intrinsic physical and chemical properties of starch that determines the eating, cooking, and processing qualities of starch. Examples of physicochemical properties are the pasting and gelatinisation properties of starch. In this section, the impact of fermentation on the functional and physicochemical properties of starch from different botanical sources has been discussed.

18.4.1 Water and Oil Absorption Capacities

The ability of food materials to absorb water depends on the presence of carbohydrates including starch, proteins, and fibre. Hydroxyl groups in starch have a strong affinity for water molecules and may form hydrogen bonding with water molecules. The size of starch granules, amylose content and the presence of non-starch components like lipids may also influence the ability of starch granules to absorb water. Oyeyinka et al. (2020) reported that the ability of fermented starch to absorb water increased with the increasing length of fermentation. According to their report, the hydrolytic action of the enzymes may have produced sugars that are known to act as humectants. Julianti et al. (2011) studied the physicochemical and functional properties of starch from four cassava varieties subjected to natural fermentation by soaking in tap water for 16 days or distilled water for 16 h at 30 °C or soaking in 1% lactic acid solution for 16 h at 30 °C. The fermented starches were further dried using two drying methods, sun and oven drying. Starch soaked in tap water for 16 days reportedly showed greater ability to absorb water compared to those soaked in distilled water and lactic acid solution for 16 h (Julianti et al., 2011). The authors did not give any plausible reason for the variation in the reported values, however, the variation in the water absorption capacities could be due to the differences in experimental conditions. The study should have subjected the starches to similar fermentation time to avoid introducing variations into the experiment. In terms of oil absorption capacity (OAC), starch fermented for 24 h reportedly showed a significant reduction in OAC compared to the unfermented starch. However, there was an increase in the OAC of the fermented starch thereafter (Oyeyinka et al., 2020). The reason for the initial decrease and subsequent increase is not very clear, suggesting the need for more studies on the water and oil absorption capacities of fermented starch.

18.4.2 Swelling Power and Solubility

Fermentation has been shown to generally reduce the swelling ability of starch (Oyeyinka et al., 2020; Jyothi et al., 2005; Fan et al., 2021; Moorthy et al., 1993; Yuan et al., 2008) but increased starch solubility (Nakamura & Park, 1975; Jyothi et al., 2005; Liu & Shen, 2007). Starch swelling properties involve interaction between the amorphous and crystalline regions of starch and it is influenced by several factors including ratio of amylose to amylopectin content, granule size, the molecular structure of amylose and amylopectin, the magnitude of interactions between amorphous and crystalline regions and botanical origin (Oyeyinka & Oyeyinka, 2018; Hoover et al., 2010). Starches with low amylose content would generally have higher swelling power since the swelling behaviour of starch has been linked primarily to the property of its amylopectin (Tester & Morrison, 1990). As discussed earlier (Sect. 18.3.1), fermentation generally reduced the amylose

Table 18.2 Pasting and gelatinisation temperatures of fermented starches

Starch source	PV (cP)	BD (cP)	FV (cP)	SV (cP)	PT (°C)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	Length of fermentation	References
Canary seed	1149	−29.6	1684	505	78	NR	NR	NR	NR	NR	Batista et al. (2020)
*Cassava	3014	2718	458	164	69	61.7	69.4	71.9	3.3	NR	Gomes et al. (2005)
*Cassava	400–580	NR	NR	NR	NR	69.1–72.3	71.9–77.6	75.3–82.2	1.4–2.0	0–72 h	Moorthy et al. (1993)
Cassava	5210–5930	1350–3030	3450–5620	603–1760	74–75	66.0–71.0	72.0–80.0	76.0–82.0	2.8–4.0	0–72 h	Oyeyinka et al. (2020)
Cassava	NR	NR	NR	NR	NR	57.9–60.7	63.5–67.0	72.2–73.5	1.8–3.5	0–90 days	Alonso-Gomez et al. (2016)
Corn	1620–1960	290–500	1550–2100	170–780	76–81	NR	NR	NR	NR	0–21 days	Yuan et al. (2008)
Rice	NR	NR	NR	NR	NR	43.5–70.4	72.2–81.7	79.6–88.2	3.3–18.0	0–60 days	Zhang et al. (2016)
*Sweet potato	998–3696	486–1698	NR	NR	80–82	75.4–81.5	NR	88.1–95.9	8.9–16.8	0–48 h	Jyothi et al. (2005)
Sweet potato	2246–5346	1384–2326	1237–3737	375–885	78.2–79.6	66.6–68.7	73.9–75.0	81.9–83.9	10.7–12.3	0–12 months	Ye et al. (2019)
Wheat	2775–2836	633–641	3117–3202	975–1007	NR	54.5–54.9	61.6–62.1	67.6–68.0	7.1–7.4	0–72 h	Fan et al. (2021)
Wheat	2023–2762	439–555	2314–3336	821–962	NR	NR	NR	NR	NR	0–96 h	Zhao et al. (2019)

PV Peak viscosity, *BD* Breakdownviscosity, *FV* Final viscosity, *SV* Setback viscosity, *PT* Pasting temperature, *NR* Not reported
*Reported in RVU but converted to cP by multiplying by 12 (cP = 12 × RVU) for uniformity. * Authors reported values in Brandender Unit (BU)

content of starch and therefore, reduction in swelling power of fermented starches may not be associated with the amylose content. According to Oyeyinka et al. (2020), the reduction in swelling power of fermented starch is presumably due to depolymerization of starch into sugars, that are not capable of swelling compared to the unfermented starch. Previous research reported that higher proportions of long chains amylopectin are known to contribute to increased starch swelling (Sasaki & Matsuki, 1998; Charles et al., 2005). Therefore, the reduction in swelling power of starch after fermentation confirms the formation of smaller chains (DP 6–12) amylopectin from longer amylopectin chains (B_2 and B_3) as found by earlier authors (Ye et al., 2019). Since most of the studies on the swelling and solubility of fermented starches focused on roots and tuber starches, future research should explore starch from pulses and cereals, especially because cereal starches contain endogenous lipids which may also impact starch swelling and solubility.

18.4.3 Pasting

The pasting properties of starch are usually measured using a rapid viscoanalyser (RVA) or Brabender viscoamylograph (BVA) and these properties are related to the cooking quality of grains and flour materials. Pasting refers to changes in the starch properties upon further heating after gelatinisation has occurred (Hoover et al., 2010). These changes include further swelling and leaching of polysaccharides from the starch granule interior, accompanied by an increase in viscosity (Hoover et al., 2010; Tester & Morrison, 1990). Differences have been observed in the effect of fermentation on the pasting properties of starch (Table 18.2). For example, the peak viscosity, trough viscosity, final viscosity, and setback decreased significantly during fermentation of wheat starch (Nakamura & Park, 1975; Zhao et al., 2019; Ye et al., 2019; Fan et al., 2021) but only peak and breakdown viscosities decreased after fermentation of cassava starch (Oyeyinka et al., 2020) or sweet potato starch (Jyothi et al., 2005). Furthermore, the pasting temperature of the fermented starch increased for both sweet potato and cassava starches (Oyeyinka et al., 2020; Jyothi et al., 2005; Ye et al., 2019). The pasting properties of starch are mainly influenced by the molecular structure of amylose and amylopectin rather than their ratio (Jane et al., 1999; Huang et al., 2007). Changes in the pasting properties of starch could be associated with modification of the molecular structure of the various starch granules in the respective studies described above. Zhao et al. (2019) associated the decrease in starch viscosity to reduction in molecular weight of starch after fermentation. Other authors attributed the reduction in starch viscosity after fermentation to the increased solubility of fermented starch in hot water during pasting (Moorthy et al., 1993) as well as differences in the associative forces among the starch granules (Jyothi et al., 2005). The changes in the molecular structure of fermented starches and the impact of these changes on the physicochemical properties of starch from different botanical origin needs further investigation.

18.4.4 Gelatinisation

The thermal properties including onset gelatinisation temperature (T_o), peak gelatinisation temperature (T_p), conclusion gelatinisation temperature (T_c) and enthalpy of gelatinisation (ΔH) of fermented starch from different starch sources measured using a differential scanning calorimeter (DSC) are shown in Table 18.2. Fermentation generally increased the transition temperatures of cassava (Oyeyinka et al., 2020; Moorthy et al., 1993) and sweet potato starches (Jyothi et al., 2005; Ye et al., 2019) but reduced their enthalpies. The increase in gelatinisation temperatures could be associated with the modification in starch structure such as an increase in greater proportions of short-chain amylopectin chains as well as the reduction in amylose contents of fermented starches. Earlier researchers noted that the amylose content of starch may influence its gelatinisation temperature (Stevens & Elton, 1971). Moorthy et al. (1993) attributed the increase in gelatinisation temperatures of fermented cassava starch to the presence of fibre, which presumably inhibited the ingression of water into the starch granule interior, hence, delaying the gelatinisation process. As previously noted, fermentation of starch results in changes in the molecular structure of amylopectin chain length and these changes may explain the increase in gelatinisation temperatures. Noda et al. (1996) found that the chain length distribution of amylopectin explains the differences in the gelatinisation properties of starches. Besides the amylose content and changes in the molecular structure of amylopectin, the presence of sugars during fermentation and organic acids in the fermented starch may also influence the gelatinisation temperatures of fermented starch. The addition of sugars and other polyols to starch-water systems have been reported to increase starch gelatinisation temperatures (Perry & Donald, 2002; Kohyama & Nishinari, 1991; Ahmad & Williams, 1999). Ahmad and Williams (1999), for example, found that the gelatinisation temperature of sago starch in the presence of sugars increased in the order sucrose > maltose > glucose > fructose > ribose. Only a few studies reported the sugar and acid contents of fermented starches and it may be difficult to assess the exact impact of these components on starch gelatinisation behaviour. Cardenas and De Buckle (1980) found that the gelatinisation temperature of cassava starch did not change significantly after fermentation for about 30 days. These authors found lactic acid, acetic acid and butyric acid in varying amounts in the fermented starches, with the former present in the largest quantity. It seems that the length of fermentation may also play a significant role in the gelatinisation behaviour of fermented starches and this needs to be factored into future research design. Optimisation techniques may also be required to determine the optimum conditions that would produce starch with desirable characteristics.

18.4.5 In Vitro *Digestibility*

Starch digestibility has attracted much interest in recent times because of its contribution to colon health, especially the slowly digestible starch (SDS) and resistant starch (RS) fractions. Nutritionally, starch can be classified into three types, rapidly digestible starch (RDS), SDS and RS depending on the rate and extent of digestion and absorption in the colon (Hoover et al., 2010). The RDS is the proportion of starch hydrolysed within 20 min, SDS is the fraction hydrolysed between 20 and 120 min, while RS is not hydrolysed within 120 min of consumption (Englyst et al., 1992). The RS fraction is known to be fermented by microorganisms in the large intestine to form short-chain fatty acids, which contributes significantly to intestinal health (Hoover et al., 2010; Englyst et al., 1992). For example, RS serves as prebiotics for the growth of gut microbiota and has been reported to lower cholesterol and blood sugar levels including increased absorption of minerals (Hoover et al., 2010), while SDS has the potential to stabilise glucose metabolism, manage diabetes and provide satiety (Lehmann & Robin, 2007). Studies on the digestibility of fermented starches revealed differences in the levels of RDS, SDS and RS when compared to the native starch (Zhang et al., 2016; Tu et al., 2021). After fermentation of normal rice, Tu et al. (2021) reported higher amounts of SDS and RDS but a lower amount of RS compared with starch extracted from rice that was not fermented native starch. Although the RS decreased after fermentation, the days of fermentation did not significantly influence the RS content, indicating that the decrease in RS may be due to the increase in the proportion of SDS and RDS, respectively. According to Tu et al. (2021), the insignificant changes in RS after fermentation confirms the inability of α-amylase to hydrolyse starch despite the fermentation process. This result agrees with the findings of Zhang et al. (2016), where changes in RS did not follow a particular trend but a significant increase in the SDS and a decrease in RDS of waxy rice starch during fermentation (6–60 days) was reported. In another study, Fan et al. (2021) reported that fermentation delayed the hydrolysis of wheat starch, which was further reduced in the presence of a hydrocolloid (konjac gum). Previous research indicated that several factors such as starch composition (amylose to amylopectin ratio), degree of crystallinity, type of crystalline polymorphic form, the molecular structure of amylopectin, and granule size affect starch digestibility (Hoover et al., 2010; Hallström et al., 2011; Kaur et al., 2010). The starch source, fermentation conditions (time, microbial strains and concentrations) as well as changes in the molecular structure of the crystalline domains of starch may also play a significant role in the digestibility patterns of fermented starch.

18.4.6 Texture of Starch Gel

During cooking of starch in water, gelatinisation occurs and the gelatinized starch on cooling thickens to form a gel. The process of gel formation in starch is a complex one that involves the re-arrangement and re-alignment of starch molecules (amylose and amylopectin), leading to retrogradation. The gel formation process is thought to result from the formation of junction zones through molecular associations between amylose-amylose, amylose-amylopectin and amylopectin-amylopectin chains. Starch retrogradation is an important physicochemical process that is undesirable in certain foods, for example, baked goods due to its impact on staling and poor acceptability of foods such as bread. However, it may be desirable for some type of starchy products such as breakfast cereals, Chinese rice vermicelli, dehydrated mashed potatoes and parboiled rice because of the improvement in mechanical, structural, and sensory properties (Wang et al., 2015; Karim et al., 2000). Hardness, also referred to as firmness, is one of the most important textural properties of starch gel. In general, the firmness of gel from fermented starch has been reported to increase with the period of fermentation (Ye et al., 2019; Yuan et al., 2008). The firmness of sweet potato starch gel reportedly increased by approximately 29%, 189% and 386% when the starch was fermented for 3, 8 and 12 months, respectively (Ye et al., 2019). Yuan et al. (2008) also reported higher gel firmness for fermented corn starch than gel from the unfermented starch. Yuan et al. (2008) attributed the increase in gel firmness to the breakdown of short chains of amylopectin in the amorphous regions, resulting into a higher ratio of long-to-short chains in amylopectin. Longer amylopectin chains have been associated with higher gel strength in starches (Chung et al., 1998; Won et al., 2000). However, the firmness of sweet potato starch negatively correlated with longer amylopectin chains, indicating that there could be other factors influencing the gel strength of fermented starches. This seems plausible since fermentation using starter cultures did not significantly change the gel firmness of cassava starch, but reduced that of rice and potato starch gels with the latter displaying higher reductions in gel hardness (Chinsamran et al., 2005). Other factors that may explain the variation in gel firmness are fermentation conditions which includes time, use of starter cultures, starch source as well as starch composition before the start of fermentation.

18.5 Current and Potential Applications of Fermented Starch

There is a growing demand for starches with novel functionality that could serve various industrial uses. Fermentation is an age-long traditional processing technology that has been widely used to improve the nutritional value of foods but has also been applied in modifying macromolecules, especially starch. Fermented starch may be used as a functional ingredient in other foods or consumed directly as found in some regions of the world. For instance, sour starch obtained during fermentation has greater applications in the baking industry. Mestres et al. (2000) compared the expansion ability of fermented maize flour and cassava starch during baking and found that cassava starch fermented at 20 °C for 15 days, with subsequent sun-drying showed higher expansion ability and therefore greater baking potentials than maize flour. Marcon et al. (2009) reported that sour cassava starch cheese bread and roscas (Spanish and Portuguese bread dish eaten in Mexico, South America) are on the market and these products are characterised with a firm texture. Mesa et al. (2019) investigated the application of sour cassava starch and two chemically modified starches on the quality and textural properties of gluten-free (GF) cheese bread made from frozen GF cheese dough. The authors reported higher hardness and number of crumb pores in bread with sour cassava starches compared to those with chemically modified starches. In another baking study by Camargo et al. (1988), fermented starch reportedly produced highly expanded biscuits with a low specific volume compared to native cassava starch that gave a hard and dense texture with no expanded structure. The studies reported above are indications of the potential of fermented starch in the baking industry which, though are currently widely used in South America, may be explored in other developing nations of the world where fermented foods are consumed, for example in Africa and Asia.

Besides the baking application, fermented cassava starch has also been explored in the production of ethanol (Nichols et al., 2005; Moshi et al., 2015; Strąk-Graczyk & Balcerek, 2020; Abouzied & Reddy, 1987; Büttner et al., 1992), lactic acid (Matsumoto et al., 2011; Linko & Javanainen, 1996) and sugars (Sebayang et al., 2017; Nehete et al., 1992; Gaouar et al., 1998; Saha & Zeikus, 1989). Fermented starch may have future applications in the production of syrups such as high fructose corn syrup (HFCS) since there seem to be pressure on corn for other uses. HFCS is a liquid alternative sweetener to sucrose that is made from corn starch but could also be made from other starch sources when modified. An earlier study by Etejere and Bhat (1985) reported that extracted starch is cooked and consumed with soups as a traditional meal in some tribes in Africa. Future applications of fermented starches may also be introduced to such communities to create variety in their diet. Another potential application of fermented starch is in the preparation of sour custard which may be relished by people who are regular consumers of *ogi*, a fermented maize gruel. Custard is mainly produced from starch with the addition of colouring and flavouring additives and is reportedly consumed as a breakfast or weaning food in developing nations of the world (Ogundele et al., 2020). There has been an attempt to enhance the sourness of custard so it serves as an alternative to *ogi* (Salami et al., 2019). According to these authors, the preparation of *ogi* is time-consuming and custard could serve as a good alternative to *ogi* but the sour taste, typical of the *ogi* is lacking in custard. Salami et al. (2019) reportedly used lime, soursop and tamarind to impact sour taste to custard made from corn starch and found that soured custard samples were more dispersible in water, showed better swelling power and significantly higher peak and final viscosities than the corn flour sample. Therefore, the use of fermented starch in custard preparation may be a future application that needs to be researched. Studies such as consumer acceptability of the custard sample as well as textural, pasting and *in vitro* digestibility of the custard sample are worthy of future consideration. Furthermore, the application of fermented starch as thickening agents in soups, gravies and other food products may also be investigated in future research.

Other promising future applications of fermented starch is in the production of sugar alcohols, also known as polyols. Polyols are neither alcohols nor sugars but rather a group of low-digestible carbohydrates which can serve as alternatives to sucrose for various industrial use (Ghosh & Sudha, 2012). They are currently used as sweeteners in the production of low-calorie foods and may include erythritol, isomalt, lactitol, maltitol, mannitol, sorbitol, xylitol, and hydrogenated starch hydrolysates (Ibrahim, 2016). According to these authors, they naturally occur in many fruits and vegetables, but may also be produced from monosaccharides, disaccharides, and polysaccharides. The polysaccharide-derived polyols, i.e., hydrogenated starch hydrolysates (HSH) are important food ingredients because of their sweetness, low cariogenic potential, and useful functional properties (Modderman, 1993). For example, they can be blended with other natural and artificial sweeteners and used to enhance bulkiness, texture, and viscosity of food (Ibrahim, 2016). Furthermore, they have potentials in the development of low-glycaemic foods since they are slowly absorbed from the small intestine into blood circulation when compared to natural sugar glucose or sucrose (Modderman, 1993). Furthermore, their significance in the pharmaceutical industry such as their anti-dental plaque effect or drug activity related to their glass-forming structures in freeze state has further promoted research around their properties and applications (Niu et al., 2017; Shankar et al., 2013). HSH are produced by the hydrogenation of partially depolymerized starch from corn, potato, or wheat. However, several other starch sources may also be explored in the future to produce HSH. Therefore, future research should be expanded to explore other starch sources including the non-conventional sources like pulse starches to produce polysaccharide-derived polyols for food and non-food applications.

18.6 Conclusion

Starch fermentation leads to a composite functional ingredient made of partially digested starch polymers, reducing sugars, and several other metabolites including organic acids and volatile compounds, depending on the species involved in the fermentation. Fermentation is usually associated with acidification of the substrate, which contributes to the altered viscosity of fermented starch pastes. However, during fermentation, enzymatic modification of starch polymers also takes place. In this chapter we have summarised the possible uses of the products of complete or partial starch fermentation as functional ingredient. This shows that starch fermentation has potential to be used as a processing technique to develop functional ingredients.

Fermentation can be used as a means of biological conversion utilising bacteria, yeast and often archaea species to ferment starch thanks to starch degrading enzymes. It should be considered that often other commensal species may coexist in fermentation populations despite not being able to degrade starch. These species cross-feed on intermediate metabolites produced by generalist species. Whether competitive and cooperative interactions between species take place in starch fermentation and the effect of these interactions on starch and reducing sugars obtained requires further investigation. These microbial population used for fermentation is highly dependent on the environment, the starting material used (tubers, cereals, etc.) the handling techniques used in pre- and post-harvest and the starch isolation method, if isolated before fermentation. The use of naturally fermented starch finds its roots in the tradition of Latin America. Originally, natural fermentation was used to detoxify and preserve starchy roots, like cassava, however starch fermentation can produce a product with improved viscoelastic texture, compared to the raw material. It is typically a spontaneous process that has been exploited for preservation but also transformation and enhancement of sensory characteristics and nutritional content of foods. Because of the spontaneous nature of the process, standardisation is required to utilise the different fermentation techniques described in this chapter for industrial applications. Fermentation variables like temperature, pH, ionic strength, level of oxygenation have a strong influence on cellular yield. Batch fermentation, starter culture selection and standardisation of the metabolites produced are also key factors to consider translating this ancient technique into a modern industrial processing method.

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Chapter 19
Effect of Germination on Starch

Ciro Baruchs Muñoz-Llandes, Cristina Martínez-Villaluenga, Heidi María Palma-Rodríguez, Alma Delia Román-Gutiérrez, Javier Castro-Rosas, and Fabiola Araceli Guzmán-Ortiz

19.1 Introduction

Currently, germination has been proposed as a method to modify native starches as an alternative to chemically modified starches, and multiple changes in their properties have been found (Oliveira et al., 2022). Germination is a complex metabolic process in which hydrolytic enzymes deeply modify macromolecules as starch.

C. B. Muñoz-Llandes
Instituto de Ciencias Agropecuarias, Universidad Autónoma del Estado de Hidalgo, Rancho Universitario, Tulancingo de Bravo, Hidalgo, Mexico

Área Académica de Química, Universidad Autónoma del Estado de Hidalgo, Carretera Pachuca- Tulancingo, Ciudad del Conocimiento, Mineral de la Reforma, Hidalgo, Mexico

C. Martínez-Villaluenga
Department of Characterization, Quality and Safety (DCCS), Institute of Food Science, Technology and Nutrition (ICTAN), Spanish National Research Council (CSIC), Madrid, Spain

H. M. Palma-Rodríguez
Instituto de Ciencias Agropecuarias, Universidad Autónoma del Estado de Hidalgo, Rancho Universitario, Tulancingo de Bravo, Hidalgo, Mexico

A. D. Román-Gutiérrez · J. Castro-Rosas
Área Académica de Química, Universidad Autónoma del Estado de Hidalgo, Carretera Pachuca- Tulancingo, Ciudad del Conocimiento, Mineral de la Reforma, Hidalgo, Mexico

F. A. Guzmán-Ortiz (✉)
CONAHCYT- Universidad Autónoma del Estado de Hidalgo, Carretera Pachuca- Tulancingo, Ciudad del Conocimiento, Mineral de la Reforma, Hidalgo, Mexico

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The alterations of the structural, physicochemical, and functional properties of starch after germination can affect the application of starches in industries. It has been reported that germination can reduce the relative crystallinity of starch, elevate gelatinization temperature, and slightly reduce or increase peak viscosity (Xing et al., 2021; Gutiérrez-Osnaya et al., 2020; Li et al., 2017) depending on a number of factors as germination time and temperature, starch composition, crop, and variety (Wang et al., 2020). It is important to identify the most relevant parameters of the germination process that modify starch properties to establish specific conditions that allow for alternative uses and implement a simple and economic method as germination. The functionality of the starch of seed subjected to a germination process has different characteristics from a native starch. During germination enzymes such as amylases, proteases, lipases, phytases, β-glucanases are activated, which are responsible for multiple changes in the structure of the seeds. Starch is one of the main macromolecules affected. When the starch structure is modified due to enzyme hydrolysis, the foaming capacity increases and the size for the molecule is reduced, which are desirable characteristics in the baking industry. The swelling capacity is also decreased and the solubility index increases, which could be caused by a high content of amylose released from the germinated starch, which acts as a swelling inhibitor (AL-Ansi et al., 2021). When the starch is hydrolyzed, water is also allowed to enter the starch granule more easily, which allows it to increase its solubility (Su et al., 2020). Other properties such as the water and oil absorption capacity are also modified because of the changes in starch and protein by germination. Therefore, analyzing the characteristics, structural, morphological, and physicochemical changes of starch granules due to germination is of interest to establish its functionality and diversify and increase its possible application in the food and non-food industry. Therefore, this chapter compiles key information regarding the effect of germination conditions on the composition and physicochemical and structural properties of starch granules from different grains and seeds.

19.2 Germination

The germination process starts when the dry seed containing necessary genetic information absorbs water under optimal time and temperature conditions, which promote the expansion and elongation of the embryo emerging from the seed (Hermann et al., 2007). Germination involves the restoration of the basal metabolism of the seed; key metabolic changes take place before and after the emergence of the radicle.

At the beginning of the germination process, the reserve nutrients are in the form of storage, and it is the key to obtain low molecular-weight molecules such as sugars and essential amino acids, improving their bioavailability. They can still be used during embryo development in germination (Bewley, 2001), a process during which the digestibility of nutrients is improved (Chinma et al., 2021; Muñoz-Llandes et al., 2019).

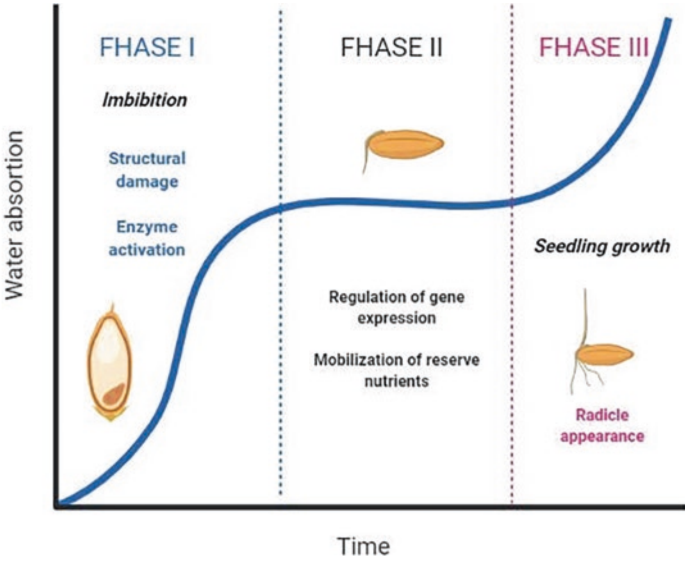

Fig. 19.1 Different phases of germination in the time

The germination process is mainly divided in three stages (Fig. 19.1). Stage I, imbibition, corresponds to water absorption in the seed through the micropyle, a pore on the seed coat (Castillo et al., 2020). The seed cells suffer structural damage due to enzymatic activation, mostly at membrane level, which hydrolyzes structural components of the cell wall. This leads to leaching of low molecular-weight solutes, and then the restoration and restricted leakage of solutes (Bewley et al., 2012). In this stage, the activity of the glycolysis and pentose–phosphate pathways are reestablished, along with the activation of the enzymes involved in the Krebs cycle. Some organelles or structural fractions are repaired while others are hydrolyzed and synthesized, as the mitochondrial membrane of the cells that store lipids. In the embryo, the ribosomal population and mRNA are reduced after rehydration to create polysomes and, later, the proteins necessary for cell structure and metabolic machinery are synthesized (Ballinas et al., 2019). In gramineous plants, there is head and endosperm resistance against the embryo. Then, there is an enzymatic degradation of certain areas of these structures before their emergence. For instance, that of homogalacturonans in the cell wall of the seed, which are esterified by the enzyme pectin methylesterase. This affects the porosity and elasticity of the cell wall, promoting water adsorption (Müller et al., 2013).

Once the seed has absorbed enough water, Stage II starts with the reactivation of different types of metabolism catalyzed by enzymes and gene expression. This triggers the growth and emergence of the radicle, leading to the development of the seedling (Han & Yang, 2015). This step involves a greater energy expenditure, obtained through the mobilization of stored compounds, such as carbohydrates, lipids, and proteins, allowing for the survival of the seed until the seedling can carry out photosynthesis (Yu et al., 2014). Finally, in Stage III, the radicle emerges through the seed coat (Nonogaki et al., 2010). During the germination of caryopses (cereal grains), the scutellum is involved in the transference of phytohormones gibberellins secreted by the embryo, which indicate thatthe aleurone layer to start or accelerate the synthesis and secrete hydrolytic enzymes. The scutellum then transports hydrolytic products from the endosperm to the embryo (Sreenivasulu et al., 2006). The germination of the seed is regulated by a complex network signaling and regulating gene expression (Han & Yang, 2015). Essentially, this process disturbs the nutritional and physicochemical properties of grains. The time, temperature, type of grain and/or seed, variety, and amylose/amylopectin content are some of the factors that determine the degree of starch modification during germination (Noda et al., 2004; Guzmán-Ortiz et al., 2019). Starch is stored and modified cyclically during seed germination due to the complex enzymatic activity involved, affecting the structural and morphological modification of starch granules.

19.3 Enzymatic Activation

During germination, the major stored carbohydrates are modified by hydrolytic enzymes as α-amylase, β-amylase, and α-glucosidase. Firstly, α-amylase catalyzes the hydrolysis of α-1,4 glycosidic bonds, producing sugars as maltose and glucose, necessary for seedling development. The activity of the enzyme increases considerably during the first days of germination, and it depends on the conditions of the process, mostly time and temperature (Table 19.1). However, it has been reported that, in cereals as wheat, this enzyme also synthesizes *de novo* during the germination process (Krapf et al., 2019; Olaerts et al., 2017; Baranzelli et al., 2018). The enzyme α-amylase plays a key role in starch hydrolysis, generating hydrolysis products that disturb the osmotic balance of the membrane cells; in turn, this leads to a greater water absorption and, eventually, promotes radicle elongation and growth (Bewley, 2001). The stability of this enzyme depends on the acid pH of the endosperm promoting starch mobilization and other physiological processes, such as peptide transport, phytate solubilization, and secretion of hydrolytic enzymes in charge of expanding the cell wall (Macnicol & Jacobsen, 1992).

It is known that α-amylase acts upon starch molecules by breaking the α-1,4 glycosidic bonds between glucose residues of the non-reducing end, creating glucose and maltose from amylose and dextrins from amylopectin (Muslin et al., 2002). Maltose simultaneously turns into glucose after the action of enzyme α-glucosidase. Other enzymes, as dextrinase, hydrolyze the α-1,6 bonds of dextrins to create short chains that will later be hydrolyzed by amylases and create glucose (Stanley et al., 2011). These sugars obtained from starch degradation are absorbed in the scutellum and turn into saccharose. They are carried by the phloem to the embryonic axis where they feed the embryo of the growing seedling through glycolysis and the Krebs cycle (Han & Yang, 2015).

Table 19.1 Effect of germination conditions on α-amylase activity in different seeds

Raw material	α-amylase activity in ungerminated seed	α-amylase activity during germination	Soaking conditions	Germination time	Relative humidity	Temperature of germination	Reference
Waxy brown rice	0.04 U/g	0.35 U/g approx.	N/R	2 days	80%	25 °C	Wang et al. (2020)
		3.19 U/g approx.				30 °C	
		3.19 U/g				35 °C	
Yellow corn	–	6 CU/g	Distilled water (1:3) p/v 14 h, 23 °C	1 day	95–96%	15 °C	Helland et al. (2002)
		7.7 CU/g approx.		2 days			
		8.1 CU/g approx.		3 days			
		8.3 CU/g approx.		4 days			
		12 CU/g approx.		5 days			
		16 CU/g approx.		6 days			
		19 CU/g approx.		7 days			
Rough rice (*Oryza sativa* L.)	1.42 U/g	21.30 U/g	Distilled water 24 h	1 day	90–95%	28–30 °C	Moongngarm (2011)
		45.36 U/g		2 days			
		54.59 U/g		3 days			
		64.46 U/g		4 days			
Barley variety Pokko		290 U/g	Distilled water 5 h	5 days	36%	15 °C	Autio et al. (2001)
		430 U/g	Distilled water 7 h		46%		
		680 U/g	Distilled water 7 h		56%		
Barley variety Kustaa	–	180 U/g	Distilled water 5 h		36%		
		260 U/g	Distilled water 7 h		46%		
		440 U/g	Distilled water 7 h		56%		
Barley variety Arve	–	310 U/g	Distilled water 5 h		36%		
		390 U/g	Distilled water 7 h		46%		
		590 U/g	Distilled water 7 h		56%		

(continued)

Table 19.1 (continued)

Raw material	α-amylase activity in ungerminated seed	α-amylase activity during germination	Soaking conditions	Germination time	Relative humidity	Temperature of germination	Reference
Oats variety Meeri	0.65 U/g	2.75 U/g	Distilled water (1:6) p/v, 4 h, 20 °C	4 days	–	12 °C	Aparicio-García et al. (2020)
		1.57 U/g		2.5 days		14 °C	
		10.81 U/g		6.5 days			
		0.81 U/g		1 day		16 °C	
		4.07 U/g		4 days			
		25.12 U/g		9 days			
		1.47 U/g		2.5 days		18 °C	
		19 U/g		6.5 days			
		5.39 U/g		4 days		20 °C	
Oats variety Barra	0.16 U/g	1.33 U/g	Distilled water (1:6) p/v, 4 h, 20 °C	4 days	–	12 °C	Aparicio-García et al. (2020)
		0.92 U/g		2.5 days		14 °C	
		30.14 U/g		6.5 days			
		0.26 U/g		1 day		16 °C	
		20.97 U/g		4 days			
		1.76 U/g		9 days			
		6.75 U/g		2.5 days		18 °C	
		3.35 U/g		6.5 days			
		14.92 U/g		4 days		20 °C	
Wheat (*Triticum aestivum* L.)	12.62 U/g	30.88 U/g	–	1 day	80%	15–20 ° C	Baranzelli et al. (2018)
		35.93 U/g		2 days			
		39.52 U/g		3 days			
Barley variety *Commander*	2 U/g	4 U/g	Distilled water, 24 h	1 day	–	–	Quek et al. (2019)
		23 U/g		2 days			
		28 U/g		3 days			
		27 U/g		4 days			

Raw material	α-amylase activity in ungerminated seed	α-amylase activity during germination	Soaking conditions	Germination time	Relative humidity	Temperature of germination	Reference
Barley variety Morales	3 U/g	6 U/g	Distilled water, 24 h	1 day	–	–	Quek et al. (2019)
		12 U/g		2 days			
		13 U/g		3 days			
		15 U/g		4 days			
Waxy brown rice	0.054 U/g	0.099 U/g	Distilled water, 12 h, 4 °C	12 h	80%	30 °C	Liu et al. (2022)
		0.483 U/g		1 day			
		0.727 U/g		1.5 days			
		1.180 U/g		2 days			

Unites per gram of sample, UC/g: Ceralpha Units per gram of sample

It has been proven that, in cereal grains, mRNA transcription of α-amylase is activated when glucose is depleted and is suppressed when this sugar is available (Han & Yang, 2015). Reports indicate that the transcription levels of this hydrolase in rice increase in aleurone cells two days after imbibition. Gibberellic acid (GA) is synthesized in the scutellar tissue and makes the transcription factor GAMYB bind to the GA-responsive element as an α-amylase expression promoter (Lee et al., 2014). Then, the enzyme starts its activity and produces hydrolysis in starch.

In seeds of cereals like barley, it has been reported that α-amylase is the most abundant hydrolase produced in aleurone tissue during germination (Jacobsen & Beach, 1985). In wheat, it has been reported that the activity of α-amylase reaches its peak activity around 7–8 days into germination and disappears after 12 days (Zhao & Ma, 2018). In legumes, the behavior of α-amylase has also been evaluated during germination. An increase in α-amylase of up to 200% has been reported in mung bean after 24 h of germination (Rahman et al., 2007). The increase in enzymatic activity leads to a decrease in total starch content and elevated free sugars, although the latter are not always increased. This is because the germ to cover the metabolic requirements for seedling growth can use a fraction. The percentage reduction of the total starch content may vary depending on the germination time. A prolonged germination time favors a greater reduction, after six days of germination and 25 °C in legumes such as green pea, lentil, and mung bean has been reported to reduce 34, 37.06 y 43.38% respectively of total starch (Świeca & Gawlik-Dziki, 2015), while four days after germination the decrease is less than 11.75, 29 and 15% in dolichos, jack bean, and mucuna, respectively (Benítez et al., 2013). However, the type of seed also influences the degree of starch hydrolysis. In lentil (*Lens culinaris* Merr.) and yellow pea (*Pisum sativum* L.); a decrease of 14.77 and 6.55%, respectively, has been reported after 6 days of germination at 25 °C (Xu et al., 2019).

Several factors, such as the composition of the starch granule (amylose/amylopectin), can affect the efficiency of the enzymatic activity. Amylose is quicker and easy to hydrolyze when compared against amylopectin, which has a structure with more complex branching that prevents a fast hydrolyzation (Zheng et al., 2006; Guzmán-Ortiz et al., 2019). A low amylose content (12.5%) in rice germinated for five days shows lower amylase activity versus rice with regular amylose content (20.2%). This proves there is more starch hydrolysis in varieties where the amylose content is superior (Kalita et al., 2017).

Grain humidity is another major factor in enzymatic activation. Some authors have reported that a soaking stage before germination allows triggering hydrolase activation (Table 19.1). When the humidity content is increased the enzymatic activity change (Autio et al., 2001). The soaking process for 24 h, as a step prior to germination, favors the increase in α-amylase activity until 15-fold. If the soaking time is prolonged for a period of 48 h, the enzyme activity can increase up to 43.5 times. This behavior has been studied in rice (Table 19.1) (Moongngarm, 2011). This is likely related to water activity, since a minimum

Table 19.2 Effect of germination on the starch composition of different seeds

Starch source	Native starch Total starch (%)	 Amylose (%)	 Amylopectin (%)	Sprouted starch Total starch (%)	 Amylose (%)	 Amylopectin (%)	Reference
Waxy rice	76	1.16	74.84[a]	N/R	4.88	N/R	Wang et al. (2020)
Quinoa	Mengli variety (MQ) Quinoa gray color						Xing et al. (2021)
	58.18	3.57	54.61[a]	50.50	3.61	46.89[a]	
	Zhongli (ZQ) variety White Quinoa						
	60.40	17.37	43.03[a]	42.07	6.95	35.12[a]	
	Yunnan variety (YQ) Quinoa red color						
	63.12	4.44	58.68[a]	45.51	3.61	41.9[a]	
Chinese rice	Zhengxian variety						Wu et al. (2013)
	78.66	22.01	56.65	42.19	11.32	30.86	
	Nanjing variety						
	77.44	15.10	62.34	41.71	7.32	34.37	
	Yannuo variety						
	76.34	3.97	72.37	43.64	2.24	41	
Rice (*Oryza sativa* L.)	79.89	17.39	62.5[a]	77.11	12.13	64.98	Xu et al. (2012)
Barley	45.7	22.81	22.89[a]	31.67	26.63	5.04	AL-Ansi et al. (2021)
Bambara peanut	43.53	22.61	20.92	42.4	22.48	19.92	Chinma et al. (2021)

[a] Values determined by difference of total starch content with amylose content

of 0.2 is required to activate hydrolases (Larson, 1968; Guzmán-Ortiz et al., 2019). However, when seeds are hydrated in excess water during soaking, they undergo an anaerobiosis process that creates lesions in cell membranes when it is extended for an excessive amount of time, leading to an inability to germinate (Hegarty, 1978).

Temperature also plays an essential role in enzymatic activation. Different germination temperatures (25, 30, and 35 °C) generated different effect on activity levels of α-amylase in waxy brown rice and a significant increase when temperatures are higher (Table 19.1) (Wang et al., 2020). Same effect was found in Meeri and Barra germinated oats (Aparicio-García et al., 2020).

As stated before, enzymes are activated during germination and modify the physicochemical properties of macromolecules as starch. Therefore, it is important to know the alterations in starch granules by germination, so that they can be a relevant ingredient in the food industry.

19.4 Changes in Starch During Germination

19.4.1 Chemical Composition

The amylose/amylopectin ratio can be modified during germination. The proportion of amylose can be significantly reduced versus that of amylopectin since it is more susceptible to hydrolysis by enzymatic action (Kalita et al., 2017). In some rice varieties the total starch, amylose and amylopectin content decreases after germination (Table 19.2) (Wu et al., 2013; Pinkaew et al., 2017).

In addition to the enzymatic activity, the content of amylose and amylopectin can be affected by the amount of phenolic compounds. During germination, phenolic compounds increase significantly (Ferreira et al., 2019), this can cause amylose to interact with small phenolic compound molecules through covalent bonds and create left-handed helical inclusion complexes called V-amylose (Obiro et al., 2012). However, the complex V-amylose, also is possible to form complex with other compounds present in grains and seed such as fatty acids and hydrophobic organic polymers. Amylose-V complexes can affect the physicochemical properties of starch, such as rheology, retrogradation and digestibility, generating resistant starches (Nimz et al., 2004). Still, these interactions depend on the chemical structure of the starch and the type and concentration of the phenolic compound. It could be hypothesized that during germination there is a greater formation of Amylose-V complexes, since germination favors the increase of phenolic compounds (Zhu, 2015). The degree of solubility also plays an important role in the interactions of polyphenols with starch (Han et al., 2020). These interactions and modifications in starch composition can also affect the physicochemical, rheological, and nutritional properties of starch.

On the other hand, the protein content in isolated starch decreases slightly with germination time (Liu et al., 2022). Values of 0.39% have been reported in ungerminated starch and of 0.41, 0.36, 0.37, and 0.35% at 12, 24, 36, and 48 h of germination at 30 °C in waxy brown rice. Variation in temperature does not significantly influence protein content (Wang et al., 2020). Lipid content has been reported as constant throughout the germination time (Al-Ansi et al., 2021). Values of 0.15% in native rice starch and 0.13, 0.16, 0.13, and 0.14% have been found in germination after 12, 24, 36, and 48 h, respectively (Liu et al., 2022). The lipid content in barley starch has also been constant. Additionally, it has been found that the ash content increases along with germination. In germinated rice starch for 12 and 48 h, a high ash content has been reported compared to ungerminated rice (Liu et al., 2022) and Wang et al. (2020).

19.4.2 Swelling Power and Solubility

Starch changes its composition and structure after the germination process, which confers it different properties. Starches can be used as binding agents and thickeners in the development of foods. Still, these parameters depend on the interaction between amylose and amylopectin chains, as well as crystalline and amorphous areas of starch granules, polymerization degree, branching, and molecular weight (Zhang et al., 2005; Ratnayake et al., 2002). The swelling power in native starch is closely related to amylopectin concentration because amylose is a diluent and swelling inhibitor (Singh et al., 2003).

Germination modifies the swelling power of starch granules due to the degradation by enzymatic action, which affects granule hydration. In barley germinated for 24, 48 and 72 h, the swelling power has decreased. This is because of amylose release during germination, due to starch degradation by enzymatic action (AL-Ansi et al., 2021). The amylose released creates a gel structure on the surface of the starch granule, preventing hydration (Yang et al., 2020). A similar behavior was found for germinated mung bean (25 °C for 12–72 h); however, this capacity increases along with the temperature (50–90 °C) (Liu et al., 2020). This occurs since starch granules are partially disintegrated during germination, which exposes the starch chain, increasing hydrophilicity and thus the swelling power (Su et al., 2020). The starch of germinated quinoa (0, 24, and 48 h) has the same behavior as temperature rose, proving that germination deeply affects this property (Xing et al., 2021). However, swelling power is also controlled by intrinsic factors of starch, as the presence of minor compounds as phosphate monoesters and phospholipids (Srichuwong et al., 2005).

Furthermore, solubility is a parameter determined by factors such as particle size, distribution, structure, and granule morphology. These factors are modified by germination, affecting solubility (Xia et al., 2020). In quinoa, the solubility of starch granules increases along with the germination time due to a progressive starch hydrolysis by enzymatic action (AL-Ansi et al., 2021; Xing et al., 2021).

19.4.3 Thermal Properties

Gelatinization, an irreversible transition process, occurs when starch is subjected to high temperatures in excess of water. Starch gelatinization parameters in food processing are of great importance for specific applications. These properties have been evaluated through different techniques as differential scanning calorimetry (DSC), nuclear magnetic resonance (NMR) spectroscopy, and Fourier transform infrared (FTIR) spectrometry. Nevertheless, DSC is most commonly used to analyze the thermal characteristics of starch in foods (Annor et al., 2014).

During germination, branched starch chains are reduced while sugar concentration increases because of the degradation of the double helix bonds of starch (Wu

Table 19.3 Thermal and pasting properties of native and sprouted starches from different seeds

	Pasting properties				Thermal properties									
	Native starch		Sprouted starch		Native starch				Sprouted starch					
Starch source	Peak viscosity	Paste temp. (°C)	Peak viscosity	Paste temp. (°C)	T_0 (°C)	T_C (°C)	ΔH (J/g)	Tp (°C)	T_0 (°C)	T_C (°C)	ΔH (J/g)	Tp (°C)	Germination time and temp.	Reference
Rice	1306.1 (mPa*s)	61.3	1063.7 (mPa*s)	61.9	55.22	77.61	14.44	–	57.16	78.39	12.97	–	2 days, 35 °C	Wang et al. (2020)
			1156.0 (mPa*s)	61.7					57.02	78.07	13.21	–	2 days, 30 °C	
			1194 (mPa*s)	61.4					56.7	78.15	13.81	–	2 days, 25 °C	
Barley	Esmeralda variety												6 days, 25 °C	Gutiérrez-Osnaya et al. (2020)
	2730.50 (cP)	95.33	510.50 (cP)	95.28	–	–	6.46	61.36	–	–	1.56	63.40		
	Perla variety												8 days, 25 °C	
	3101 (cP)	95.43	828.50 (cP)	95.19	–	–	7.93	61.83	–	–	4.96	62.08		
Rice	ZX variety												5 days, 25 °C	Wu et al. (2013)
	1276 (cP)	–	1324 (cP)	–	63.24	74.37	9.68	69.35	62.33	73.81	9.71	68.92		
	NJ variety													
	1451 (cP)	–	1503 (cP)	–	61.66	72.69	10.04	66.78	60.95	72.38	9.97	66.16		
	YN variety													
	1914 (cP)	–	1926 (cP)	–	60.68	71.71	10.15	65.87	60.67	71.57	10.12	65.55		

	Pasting properties				Thermal properties									
	Native starch		Sprouted starch		Native starch				Sprouted starch					
Starch source	Peak viscosity	Paste temp. (°C)	Peak viscosity	Paste temp. (°C)	T_0 (°C)	T_C (°C)	ΔH (J/g)	Tp (°C)	T_0 (°C)	T_C (°C)	ΔH (J/g)	Tp (°C)	Germination time and temp.	Reference
Sorghum (*Sodamchal*)	2466 (cP)	72.7	196 (cP)	72.3	65.3	79.4	17.4	70.3	66.7	79.9	18.4	70.9	2 days, 30 °C	Li et al. (2017)
Millet (*Samdachal*)	2485 (cP)	75.5	972 (cP)	73.5	61.9	82.1	17.5	72.6	62.4	83.4	19.9	71.7	2 days, 30 °C	
Brown rice (*Ilpum*)	990 (cP)	89.9	1076 (cP)	93.2	58.0	72.2	15.8	63.8	58.8	71.7	14.5	64.2	2 days, 30 °C	
Oat (*Choyang*)	914 (cP)	91.5	941 (cP)	94.7	57.4	67.3	13.8	62.2	57.8	68.0	13.9	63.1	2.5 days, 25 °C	
Rice (*Oryza sativa* L.)	3293 (cP)	65.30	2726 (cP)	63.55	59.07	77.98	8.34	65.85	58.74	71.97	6.72	63.97	1 day, 30 °C	Xu et al. (2012)
Mung bean	656 (cP)	78.25	629 (cP)	72.25	61.97	92.71	18.74	69.67	62.01	93.62	22.20	70.18	12 h, 25 °C	Liu et al. (2020)
			588 (cP)	79.05					62.94	96.12	17.53	70.39	1 day, 25 °C	
			653 (cP)	78.65					62.74	95.06	21.64	68.86	1.5 days, 25 °C	
			619 (cP)	79.88					62.75	96.30	15.31	69.55	2 days, 25 °C	
			655 (cP)	79.13					62.87	96.39	15.30	70.72	2.5 days, 25 °C	
			549 (cP)	79.10					63.44	92.55	13.54	70.78	3 days, 25 °C	
Bambara Peanut	1473 (cP)	86.2	1529 (cP)	86.63	67.84	83.05	4.62	72.39	66.9	85.16	2.8	73.48	3 days, 28 °C	Chinma et al. (2021)

(continued)

Table 19.3 (continued)

	Pasting properties				Thermal properties									
	Native starch		Sprouted starch		Native starch				Sprouted starch					
Starch source	Peak viscosity	Paste temp. (°C)	Peak viscosity	Paste temp. (°C)	T_0 (°C)	T_C (°C)	ΔH (J/g)	Tp (°C)	T_0 (°C)	T_C (°C)	ΔH (J/g)	Tp (°C)	Germination time and temp.	Reference
Pea (*Pisum sativum* L.)	Xiwan 1 variety													Gao et al. (2022)
	3205 (cP)	76.75	2453 (cP)	76.58	53.91	74.41	6.40	62.96	59.02	73.95	6.32	64.96	1 day, 25 °C	
			4325 (cP)	74.45					55.44	71.55	7.10	62.49	2 days, 25 °C	
			4735 (cP)	74.25					55.10	71.15	7.00	62.65	3 days, 25 °C	
			2611 (cP)	75.05					61.08	75.52	8.05	67.66	4 days, 25 °C	
	Xiwan 2 variety													
	4608 (cP)	75.88	5261 (cP)	75.00	58.17	72.22	5.94	64.18	58.89	73.55	6.89	63.81	1 day, 25 °C	
			4357 (cP)	73.83					58.29	72.59	8.16	63.56	2 days, 25 °C	
			4235 (cP)	73.48					59.18	74.16	8.23	64.95	3 days, 25 °C	
			3135 (cP)	75.20					61.55	77.19	8.04	67.49	4 days, 25 °C	
Barley	Hot air dried (60 °C, 4 h)													Su et al. (2020)
	2995 (cP)	88.40	3079 (cP)	89.25	55.02	65.23	7.56	58.51	55.67	67.81	9.30	59.65	12 h, 25 °C	
			3243 (cP)	88.38					55.57	65.97	8.66	59.25	1 day, 25 °C	
			4478 (cP)	86.35					55.92	64.94	7.52	59.23	1.5 days, 25 °C	
	Infrared dried (600 W/m2 radiation intensity, 20 °C inlet air, velocity of 0.5 m/s)													
	2995 (cP)	88.40	3140	89.23	55.02	65.23	7.56	58.51	56.40	67.21	8.68	60.19	12 h, 25 °C	
			2490	92.88					56.35	68.26	7.19	61.05	1 day, 25 °C	
			1241	90.83					58.63	70.47	6.24	63.21	1.5 days, 25 °C	

et al., 2013). The alteration of the structure and molecular order of the starch granule led to an increase in gelatinization temperatures and modifications in ΔH values given the differences in the distribution of the size particle, amylose content, and relative crystallinity (Zhang et al., 2020).

When the germination time increases, a degradation of starch double-helical structure is generated, causing a greater demand for energy for the gelatinization the starch, which causes an increase in gelatinization temperature. This modification is due to the changes in granule size and shape, amylose content, and length of amylopectin chain (AL-Ansi et al., 2021). It must also be considered that the presence of some amino acids and peptide fractions can increase the gelatinization temperature (Xu et al., 2017).

Lipids play an important role during gelatinization, since they form a coating on the starch granules, restricting swelling, this causes the gelatinization temperature to increase with germination (Biliaderis & Tonogai, 1991), because the lipids are degraded during the first days of germination. Additionally, the amylose/amylopectin ratio also affects the behavior of gelatinization (Varavinit et al., 2003). In Esmeralda variety barley, the gelatinization temperature has increased after 4 days of germination and Perla variety barley decreases after 2 days of germination (Table 19.2) (Gutiérrez-Osnaya et al., 2020).

The constant increase in gelatinization temperature, as related to germination time, is also linked to sugar accumulation. This behavior occurs in rice, sorghum, and millet at 24 and 48 h of germination (Li et al., 2017). An increase in gelatinization temperature found in adlay (*Coix lacryma-jobi*) seeds germinated for 12, 24, 36, 48, and 60 h (Xu et al., 2017). However, as in several studies shown in Table 19.3, gelatinization enthalpy was significantly reduced due to the germination time. There was a modification in the molecular structure of the amylopectin chains of the starch, representing a loss of double helix order in the crystalline and amorphous regions (Cooke & Gidley, 1992). The type of sugars accumulated because of starch hydrolysis during germination can also affect the behavior of gelatinization enthalpy. Certain sugars, as monosaccharides, reduce gelatinization enthalpy (Baek et al., 2004).

19.4.4 Pasting Characteristics

Pasting properties are closely related to starch functionality. To assess them, starch is heated in an excess of water to obtain a pasting curve and visualize peak viscosity, breakdown, setback, and final viscosity. Peak viscosity indicates the water absorption capacity and how easily starch granules can disintegrate, while breakdown refers to viscosity during a heating period at a constant temperature to have granules swell and break. Setback is the cooling stage and final viscosity indicates the capacity of starch in a solution to create a viscous paste after heating and cooling. The minimum temperature at which viscosity increases is known as pasting temperature (Dhaka & Khatkar, 2015). These properties entirely depend on moisture content and type of starch; still, it has been proven that germination can modify these properties. In brown rice germinated for 48 h decrease in peak and final viscosities was found (Wang et al., 2020). This effect is likely due to the disruption of the granular structures, amylose leaching, amylopectin degradation, and a disorder of the hierarchical structures of starch (Zhang et al., 2015; Qiao et al., 2019). In addition, a reduction in retrogradation, possibly to the low rearrangement between the molecular chains of starch.

There is a relationship between the reduced viscosity and the morphological changes in starch (Table 19.3). The morphology of the starch granule is modified along the germination time. The structure is lost, and the granule shows small holes with porous texture due to the enzymatic activity that makes starch granules lose water retention capacity while viscosity is reduced in consequence (Simsek et al., 2014; Uthumporn et al., 2010; Xu et al., 2012; Wu et al., 2013).

Several works have demonstrated a decrease in peak viscosity as a result of germination caused by the fractioning of long amylose chains and amylopectin branching in native starch (Oseguera-Toledo et al., 2020; Liu et al., 2022; Wang et al., 2020). The effect of germination on the pasting properties of legumes like lentil, chickpea, and yellow bean has also been studied. Similarly, a progressive reduction in viscosity has been found because of changes in starch granule by the hydrolytic enzymes (Xu et al., 2019; Gutiérrez-Osnaya et al., 2020).

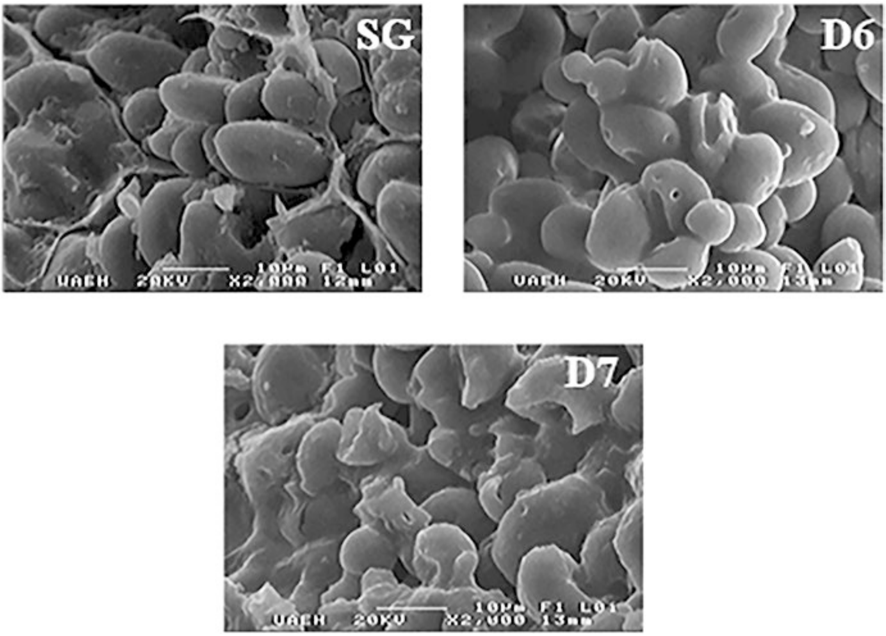

Fig. 19.2 Micrographs of barley variety Perla starch granules: (SG) without germination, (D6) 6 days of germination, (D7) 7 days of germination. (Guitérrez-Osnaya et al., 2020)

19.4.5 Structural and Morphological Changes

The morphology of starch granules is analyzed using different microscopy techniques. The process of starch hydrolysis by enzymatic action produces sugars and modifies and alters granule morphology. Enzymes can penetrate granules and hydrolyze them, creating surface pores and erosion as well as degraded granules (Li et al., 2017). This means starch has been modified by enzymatic action through germination. Some studies have proven that starch granules from cereals are eroded during germination; they show pores and rough surfaces as a result of enzymatic activity (Fig. 19.2) (Gutiérrez-Osnaya et al., 2020; You et al., 2016). In corn and triticale, the enzymatic activity produces large holes on the surface of starch granules (Li et al., 2011, 2012). Correia et al. (2008) evaluated the effect of germination in *Sorghum bicolor* (L.); electron microscopy confirmed the enzymatic hydrolysis of starch granules, which appeared eroded on day 7 of germination at 26 °C. Additionally, the starch granule is released from the existing protein matrix in the seed. The morphology of brown rice starch granules has an irregular polyhedral shape with a relatively smooth surface (Wang et al., 2020). Still, the germination process (35 °C, 48 h) alters granule homogeneity and integrity, and surfaces

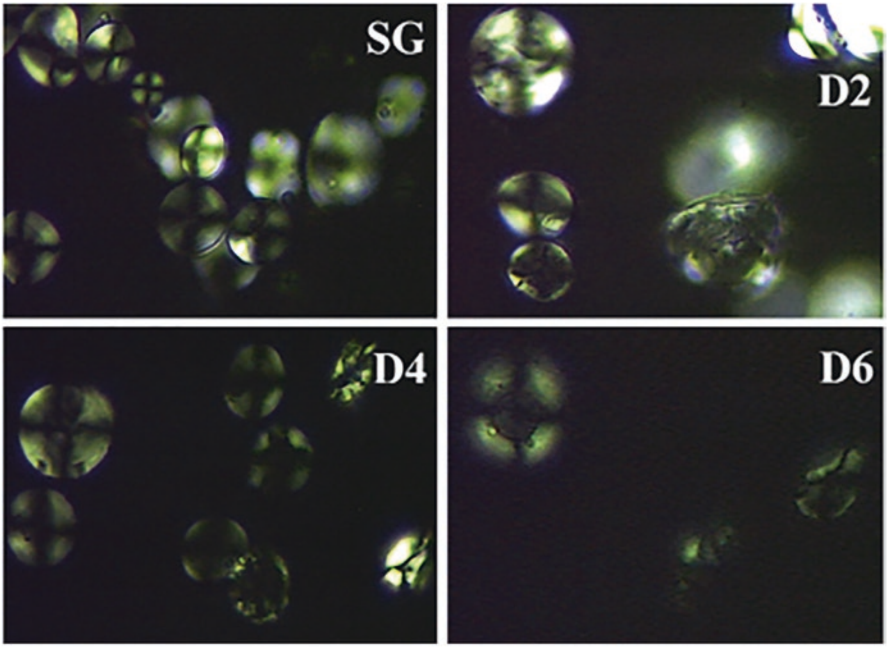

Fig. 19.3 Starch granules of barley variety Esmeralda through polarized light (SG) without germination, (D2) 2 days of germination, (D4) 4 days of germination, (D6) 6 days of germination. (Guitérrez-Osnaya et al., 2020)

show pores and damages as well as a smaller particle size. The compact and homogeneous structure of native starch it is destroyed as the germination time increased, generating a rough and eroded morphology, irregular shapes are generated in starch granules, including dents and holes on the surface. This effect has also been observed in adlay (*Coix lacryma-jobi*), in rice, millet, oat, and sorghum after germination (48 h) (Xu et al., 2017; Li et al., 2017).

On the other hand, the maltese cross or birefringence in starch granules is also modified by the germination process. This morphological characteristic can be visualized under polarized light microscopy and indicates a high order in the structure of amylose and amylopectin layers. The action of α-amylase during germination causes modification in the maltese cross. This morphological change increases as the germination time does (Fig. 19.3), which leads to significant changes in viscosity and gelatinization parameters. From day 4 of germination, birefringence of starch granules is lost, indicating a loss of molecular order in the crystalline region (Jane et al., 2003).

The modification of the starch granule morphology creates a porous surface and reduces viscosity and swelling capacity. This has been observed in barley, mung bean, and quinoa (Gutiérrez-Osnaya et al., 2020; AL-Ansi et al., 2021; Liu et al., 2020; Xing et al., 2021) due to a higher enzymatic activity during the germination process (Wu et al., 2013).

The structural changes in starch by germination can be identified through X-ray diffraction, and starch crystallinity can be calculated from the peaks (Cheetham & Tao, 1998). Several works report a decrease in starch crystallinity in different seeds because of germination conditions (Oseguera-Toledo et al., 2020; Liu et al., 2022; AL-Ansi et al., 2021). The long and organized amylopectin chains in starch represent a relative crystallinity, which can be modified by germination. Relative crystallinity it has been reduced from 24.7% to 23.6% in barley at day 4 of germination when compared against native starch (Gutiérrez-Osnaya et al., 2020). In quinoa, the relative crystallinity also decreases with germination. The MQS variety reduce from 38.62% to 35.24%, while ZQS variety was reduced from 37.41% to 35.46% and YQS, from 38.59% to 37.34% (Xing et al., 2021). Reduced crystallinity has also been reported in other cereals: 35.18% to 32.495% in brown rice, 27.44% to 25.05% in oat, 30.24% to 26.35% in millet, and 35.70% to 32.09% in sorghum (Xing et al., 2021). In legumes like mung bean, relative crystallinity is also decreased from 34.52% to 12.72% after 72 h of germination (Liu et al., 2020). The reduction is a consequence of enzymatic hydrolysis; the presence of shorter amylopectin chains is attributed to the loss of hydrogen bonds in starch helices in the crystalline region (Tarr et al., 2012). Therefore, when the crystalline regions are progressively hydrolyzed, the microcrystalline structure is modified and the interactions between the molecular chains are reduced, this causes a reduction in crystallinity (Liu et al., 2020).

19.4.6 In-Vitro *and* In-Vivo *Digestibility*

Starch digestibility is affected by several factors, such as the concentration and diffusion of the enzyme to the substrate and absorption to the starchy material. Digestion can generate several glucose molecules that are absorbed in the body, it is related with the glycemic index. According to the kinetics of starch digestion, it is classified into rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). RDS is a starch fraction that rapidly hydrolyzes into glucose molecules, releasing them after 20 min, according to the method proposed by Englyst et al. (1992), based on the *in vitro* digestion of starch by simulating the stomach and intestine. SDS is the fraction of starch that is slowly hydrolyzed to sugar molecules in the small intestine, in an average time of 20 and 120 min. RD is the portion of starch that remains unhydrolyzed by digestive enzymes and reaches the colon becoming short-chain acids. Native starch digestion can vary depending on the botanical source, granule morphology, presence of pores and channels, amylose/amylopectin content, crystallinity, lamellar density, growth rings, and the presence of other compounds such as proteins, lipids, phenolic compounds. Native starch with a B-type X-ray diffraction pattern shows a high resistance to enzymatic hydrolysis because the long chains form longer and more stable helices, which means that this type of starch has a high content of starch RS. Unlike the native starch of cereals that have a semi-crystalline type A structure, with a high level of short chains and branches, that give them slow starch digestion properties (Zhang et al., 2006).

A thermally, chemically, or enzymatically modified starch undergoes structural changes that will affect its digestibility, depending on the degree of molecular disorder. During germination, the amylopectin chains are reduced (Table 19.2), producing a less crystalline structure due to the loss of hydrogen bonds between the chains, which can favor hydrolysis and increase in digestibility. The enzymatic activation that is generated during the germination process modifies the surface of the starch granules (Fig. 19.2). Through the germination time larger pores appear. The appearance of these perforations in the starch granules can improve the penetration efficiency of the enzymes and favor their digestibility. As germination time progresses, the molecular weight and relative crystallinity of the starch decrease, indicating a partial degradation of the starch, these phenomena may indicate better digestibility (Ma et al., 2020). In maize sprouts at 25 °C, 24 h it has been reported that the RDS contents increase, while the SDS and RS contents decrease with prolonged germination time. In brown rice the SDS increases 10.09%, however the RDS and RS decrease 11.95 and 11.71% respectively at 24 h of germination at 30 °C (Xu et al., 2012).

In other grains the RSD content increased 11.3% in wheat, 12.1% in rice, 20.1% in oats and 24% in corn after 72 h of germination as a result of starch hydrolysis by enzymatic action of amylases and phosphorylases (Kaur & Gill, 2020). The increase in the digestibility of starch during germination is also due to the decrease in interactions of starch with other molecules such as fiber, protein, and lipids.

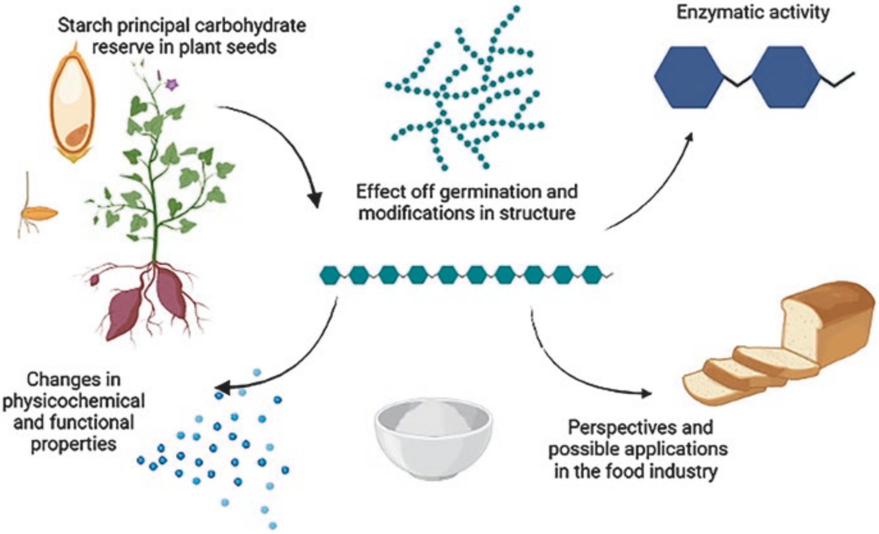

Fig. 19.4 Changes in starch digestibility due to germination

In legumes, in addition to the characteristics of starch, the presence of antinutritional compounds also influence starch digestion (Jeong et al., 2019). In germinated Bambara groundnut flour, a decrease in phytic acid, tannins and trypsin inhibitor activity has been reported with germination time and an increase in starch digestibility from 8.83% to 10.18% at 48 h of germination (Chinma et al., 2021). In mung beans, an increase of 80.1% to 90.6% digestibility at 20 h of germination has also been reported. In chickpea from 79.7% to 88.1% at 60 h and in Cowpea from 80.9% to 91.9% at 24 h (Uppal & Bains, 2012).

The increased digestion of starch with sprouting produces more glucose molecules that can be absorbed into the body. *In vitro* studies reveal a behavioral approach to an *in vivo* system. In the human body, starch digestion through the gastrointestinal tract is mediated by salivary and pancreatic α-amylases hydrolyzing starch to disaccharides and subsequently glucose, which are then hydrolyzed to glucose by the dual action of two border enzymes in brush, maltase-glucoamylase and sucrase-isomaltase (Fig. 19.4) (Quezada-Calvillo et al., 2007). In this sense, starch digestibility has been related to the glycemic index by the amount of glucose released. However, there are different aspects to consider during starch digestibility, the plant cell wall, for example, constitutes a diffusion barrier for amylases as in the case of legumes (Noah et al., 1998). Analyzing the interactions between starch and other macromolecules is of interest, to establish its application in the food industry and its possible benefits to human health.

19.5 Interaction Behavior with Other Polysaccharides and Hydrocolloids

Starch has been widely used as an ingredient in the formulation of different foods to achieve a pleasant texture for the consumer. However, the interaction that it may have with other components modifies the characteristics of the final product. Hydrogen bonding and water competition between starch and other polysaccharides and/or proteins affect the swelling and retrogradation of starch molecules that directly influence the texture of foods. The interactions between soluble polysaccharides and starch in a system help to form a strong gel and increase the viscosity of the gel. The abundant hydroxyl groups of soluble polysaccharides can absorb more water molecules, giving starch granules greater ability to swell (Tu et al., 2021). The use of germinated flours can favor the formation of a strong gel, due to the greater availability of soluble polysaccharides. A higher concentration and accumulation of soluble sugars such as fructose, galactose, melibiose, sucrose, stachyose and verbascose with germination, which allows a greater number of molecules that can interact with other compounds such as hydrocolloids (Goyoaga et al., 2011).

There is a positive correlation between a resistant starch content of native starch and high amylose content (Lin et al., 2021); therefore, starches obtained by germination where the amylose content increases (Table 19.2) can be used to produce resistant starch. Generally, one way to achieve this is through the interactions of starch with polysaccharides or hydrocolloids such as xanthan gums, guar gum, chitosan, arabic, carrageenan, etc. however, by combining starch with these polysaccharides during food processing, texture parameters such as viscosity, gelatinization and digestibility are also modified. The combination of starch with another hydrocolloid such as xanthan gum decreases digestibility. However, another parameter to consider is the viscosity of the mixture, since it increases due to the thickening effect of the hydrocolloid, the leaching of amylose and the swelling power of the granules are reduced (Sasaki, 2020).

By modifying the viscosity, the digestibility is altered due to the limited accessibility that the enzymes have to the starch granules (Brennan, 2005). The digestibility of mung bean starch in combination with hydrocolloids, finding a decrease in the digestibility of resistant starch dependent on the percentage of substitution of the hydrocolloid, reaching a reduction from 44.97% to 10.76% when mixed with xanthan gum at 0.30% and 3.48% when mixed with 0.30% konjac gum (Lin et al., 2021). These results are beneficial and have an impact on human health since these starch/hydrocolloid mixtures can be used in the control of postprandial glucose and insulin response (Qiu et al., 2017; Lim et al., 2003).

On the other hand, carrageenan has a greater effect compared to xanthan gum, pectin, gum arabic and guar gum in restricting the hydrolysis of corn starch during digestibility; In addition, by inhibiting the rapid digestion of starch, the fraction of SDS and the content of RS increased (Tester & Sommerville, 2003). The combination of starch with pectin causes a decrease in the digestibility of starch in the presence of pectin due mainly to two factors: the binding of the enzyme with pectin, inhibiting its access to the starch granules and the increase in viscosity since the speed is decreased enzyme diffusion (Bai et al., 2017). However, the viscosity parameters depend on the properties of the hydrocolloid used, such as molecular weight and flexibility, concentration, degree of branching, distribution, and charge (Sasaki & Kohyama, 2012). During mung bean germination, starch viscosity parameters tend to decrease depending on process conditions (Liu et al., 2020). The mixture of native mung bean starch with xanthan and konjac gum has been shown to achieve higher values of maximum viscosity and final viscosity compared to native starch, thus modifying its possible application and functionality. In addition, the mixture with hydrocolloids allows to strengthen the formation of gels (Lin et al., 2021). A similar behavior was found in corn starch where xanthan gum interacts with starch granules (Zhang et al., 2018).

Different types and concentrations of hydrocolloids have different effects on starch paste properties. For example, the addition of guar gum decreases the maximum viscosity of wheat starch (Funami et al., 2008). Fenugreek gum increases the peak viscosity time, setback viscosity and final viscosity in corn starch (Ravindran & Matia-Merino, 2009). ß-glucans increased the setback and final viscosity in rice starch (Banchathanakij & Suphantharika, 2009). However, the viscosity and gelatinization properties will depend on the structure and type of hydrocolloid used, due to the possible molecular interactions that they establish with the starch used, as well as the starch, the proportion of the mixture and the method of preparation (BeMiller, 2011).

The functionality of the starch when interacting with other polysaccharides and hydrocolloids can be modified; in addition to modifying the physicochemical properties, its application is diversified. Germinated wheat starch has been used to produce aerogels in combination with polyethylene oxide, due to the increase in water absorption capacity and high degradation temperature, caused by the starch/hydrocolloid interaction, becoming a material of interest for the food industry in the development of absorbent materials and packaging (da Silva et al., 2020).

19.6 Possible Applications in Food Industry

Starch as a food represents the most abundant and relevant digestible polysaccharide. In the food industry, it is mostly used as an ingredient to modify texture, viscosity, adherence, humidity retention, and creation of gels and films. In general, the chemical composition and physicochemical and functional properties, including starch granule morphology, are the major factors to determine a possible application. Germination has proven to be an efficient and cost-effective technique to modify and improve the physicochemical and functional properties and diversify the use and application in foods (Waterschoot et al., 2015; Muñoz-Llandes et al., 2022).

During germination a significant starch degradation is triggered, reducing the viscosity and gelatinization properties. These modified starches are useful in the development of emulsions as sauces, creams, mayonnaises, and tart fillings (Xing et al., 2021).

Starch hydrolysis during germination involves the accumulation of simple sugars, mostly monosaccharides and oligosaccharides. This concentration is proportional to amylose content; therefore, these starches are desirable in the beer industry. There, these carbohydrates are more available to yeasts during the fermentation process. Additionally, they can be used in foods with a high caloric value, such as candy, infant formulas, and energy drinks for athletes.

The enzymatic activation involved in the process results in the production of depolymerized starches with a greater number of short linear chains that can be used in the creation of stronger thermoreversible gels. These starches can replace the use of fats in formulations that require these gels, significantly reducing the caloric intake. In addition, they produce a texture similar to that of baked goods, noodles, gelatins, emulsion stabilizers as ice-creams, and water-oil emulsions (Liu et al., 2017). The use of these modified starches as fat substitutes is highly relevant to the ice-cream industry due to the presence of discrete domains that imitate the behavior of microstructures in fat particles (Alting et al., 2009). They can also act as an efficient enhancer of creaminess in foods like yoghurt.

On the other hand, the generation of pores on the surface of starch granules, resulting from damage by enzymatic activation during germination, creates a greater capacity for oil absorption. This is a desirable parameter in the development of foods that will undergo a frying process, like nuggets and potatoes, to obtain a crunchy texture that is desirable for consumers (Purcell et al., 2014). Extending the germination time involves a greater capacity to modify starch granules, producing dents on the smooth surface of native starch. This allows for a higher degree of crystallinity, solubility, and swelling power as well as a reduction in viscosity and pasting temperature. This leads for a potential application of modified starches in the innovation of food formulas that require low viscosity, as baked products like cookies, tarts, and pastry (Liu et al., 2020).

19.7 Conclusion

Starch is the most used natural polymer at industrial level; however, the current need and demand for techniques that allow its modification to diversify its use and efficient application is greater. Germination is an environmentally friendly alternative that is simple, efficient, and cost-effective that promotes multiple changes in starch characteristics. It has been proven that the conditions of the germination process are an alternative to modify the structure, morphology, and physicochemical properties of native starch. Then, there is a higher degree of molecular disorder in starch granules when the germination time is increased.

This technique can be used to improve the characteristics of native starches from botanical sources already used and unconventional. The aim is to optimally define their use and application, increasing the quality of the final product in terms of texture, viscosity, adhesion, springiness, and water retention, among others. Still, the major parameters to consider in germination so that starch granules are modified by enzymatic activity are time and temperature of the process as well as the previous soaking of the seeds used, the botanical source, and starch composition.

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