Chapter 5
Functionality of Alternative Proteins in Gluten Free Product Development: Case Study

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Abstract Coeliac disease (CD), dermatitis herpetiformis (DH), non-coeliac gluten sensitivity (NCGS), and gluten ataxia (GA) are some of the most important problem, auto-immuno, and lifelong intolerance disorders in human. These disorders are found by the ingestion of gluten in our body. Gluten is mostly present in wheat, barley, rye, and other related grains. Replacement of gluten in our diet is the best method to reduce the chances the coeliac disease. Gluten replacement presents a major technological challenge, as it is an important protein that creates the structure required to formulate to bake the food of high quality. The functionality of non-gluten protein is the major limitation in the development of gluten free products. Finding of alternative protein is great demand in gluten free food markets. The selection of appropriate protein for gluten-free product is a great challenge for food industry. The current chapter focuses on the uses of alternative proteins to replace gluten. As well as studies related to the functionality and nutritional qualities of these alternative proteins are also discussed.

Keywords Coeliac disease · Gluten-free · Zein · Casein · Whey protein · Chickpea · Bread

5.1 Introduction

Coeliac disease (CD), dermatitis herpetiformis (DH), non-coeliac gluten sensitivity (NCGS), and gluten ataxia (GA) are some of the important, auto-immuno and lifelong intolerance disorders found by the ingestion of gluten in our body. It is mostly seen in genetically susceptible or peoples suffering from gluten

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intolerance. The Prevelencing of CD continuously increasing and almost it affected 0.5–1% of the worldwide population (Gujral et al., 2012; Deora et al., 2014). This gluten intolerance problem leads to the destruction of the villous structure of the small intestine and instigates the inflammatory problem. Gluten is a proteinous mixture of wheat (commonly consumed food), rye, barley cereals, and some varieties of oats. Gluten is made of different prolamins fraction, which is rich in prolamins and glutamine amino acids. Higher proline content in gluten, make it extremely resistant to proteolytic degradation inside of the gastrointestinal tract. Gliadin is another amino acid in gluten that is insoluble in water. And it is responsible for the most adverse health effects since it is a toxics factor for CD patients (Wieser, 1996). To reduce the effect of gluten on peoples and their adverse health effect, need a requirement of several high-quality non-gluten products. Hence researchers are more focusing on Gluten-Free product development. Presently, the consumption of gluten-free diet is alone one treatment for CD (Deora et al., 2015; Jerome et al., 2019). Functionality of non-gluten protein is the major limitation in development of non-gluten products. It has been great demand for finding alternative protein sources for the replacement of gluten and which should exhibit similar or more functional and nutritional properties to gluten. The current chapter focuses on the uses of alternative proteins to replace the gluten. As well as study related to the functionality and nutritional qualities of these alternative proteins are also discussed.

5.2 Protein Sources Other Than Gluten

5.2.1 Cereals

Recent research support that idea of the functionalizing of non-wheat cereal proteins to imitate the viscoelastic nature of gluten is a promising field in the area of the development of gluten-free products (Deora et al., 2014). Cereal technologists around the world have successfully solved the problem of elimination of gluten from bread, biscuits, and other bakery products (Pradhan et al., 2021). Among all gluten-free products, bread is the most complex and commonly used baked product. The reason behind of complexity of bread is due to the role of gluten protein in the development of the bread matrix (Jerome et al., 2019). Rice flour is the most commonly used cereal flour for gluten-free bread making. It is also most suitable for various gluten-free products i.e. pasta, muffins, etc. due to its white color, easily digestible, and low prolamins content make it suitable for the patient suffering from coeliac disease. Various properties of products like the texture, appearance, and volume are improved and created by the use of different additive i.e. hydrocolloids, enzyme, protein acids, and emulsifiers. Storage protein of cereal is a good alternative for gluten replacement. Amino acid composition of zein and kafirin contains less percentage of prolamins and glutamine and it has unique properties like gluten for gluten-free product formulation. And it has been a great demanded research area for gluten-free product development.

5.2.2 Zein

Zein is the storage and functional properties of maize. Maize protein consists of 8–11% of whole weight, and zein is one of the major proteins found in the maize kernel. Zein protein is highly soluble in alcohol i.e. ethanol, ketones, glycerol solution but insolvable in water. Based on solubility and structural differences, zein protein is fractionated in four subclasses (Esen, 1987): α, and β-zein are major protein, γ and δ-zein are minor protein (Deora et al., 2015). α–zein is the highest (71–85% of total protein) protein composition of all zein subclasses (Lending & Larkins, 1989). Due to the presence of a large number of hydrophobic amino acid such as alanine, leucine, phenylalanine, and proline, α–zein significantly shows the hydrophobic properties (Gianazza et al., 1977).

Zein proteins have the ability for development of wheat (gluten) like dough and which has been successfully explored to produce gluten-free bread for CD sufferer's patient (Andersson et al., 2011; Jeong et al., 2017). At room temperature, zein protein is not able to form viscoelastic nature like wheat gluten, but higher temperature made it functional. Glass transition temperature of zein protein plays a vital role in the formation of viscoelastic nature. α–zein has the capability to the formation of viscoelastic properties, if the temperature of mixing and tempering was 35 °C and which is above the glass transition temperature (28 °C at ≥20 moisture content) of zein. Viscoelastic material formation capability of zein protein can suggest because of non-covalent interaction between low molecular weight protein, and in the case of gluten is due to physical and chemical interaction of very high molecular protein (Smith et al., 2014). Viscoelastic properties of zein can also be modified by certain additive or modification to the protein themselves. This improvement in zein protein is possible either in alone protein or with the addition of co-protein.(Andersson et al., 2011) revealed that zein protein alone could not imitate the similar character of gluten dough but adding of hydrocolloids significantly improves the rheological and structural properties of zein dough. Zein protein dough shows a similar function to a wheat dough. By measurement of hyperbolic flow contraction of zein-starch dough with hydrocolloids shows the high extension viscosity. And this dough is best suitable for gluten-free bread development. During the baking process of dough, hydrocolloids supplemented zein-starch bread evolved the improved bread height, volume, and fine structure.

Development of dough by zein-rice starch mixture with amylose content is a possible alternative for the gluten-free product (Jeong et al., 2017). High amylose content in zein-rice paste exhibited great pasting and elasticity. And this mixture was suitable for the preparation of gluten-free noodles. Prepared noodles showed a firm texture.

5.2.3 Kafirin

Kafirin is the storage protein of sorghum and it shows great potential in the research area of alternative protein sources for gluten-free products. Sorghum endosperm contains 77–82% kafirin of total protein (De Mesa-Stonestreet et al., 2010). Kafirins are classified as prolamins and they also contain a high amount of glutamine and proline. It is insoluble in water and highly solvable in alcohol. The hydrophobic capacity of kafirin is higher among average protein and wheat's prolamins (Deora et al., 2015). Oom et al. (2008) found that kafirin protein has the capability for the formation of viscoelastic dough as wheat gluten. Strain hardening and extensive viscosity of kafirin protein were similar to wheat flour dough. They also revealed that kafirin dough exhibited adequate rheological properties for the development of gluten-free porous bread. Heat treatment of sorghum flour also affects achieving a higher volume of gluten-free cake and bread during the baking process (Marston et al., 2016).

5.3 Dairy and Poultry

In the recent year, applications of milk and egg protein have been increased in gluten-free food markets. Both ingredients can be used for alternative protein source in non- gluten product development. Incorporation of these alternative proteins improved the nutritional quality and structure of the non-gluten product. Casein, whey protein, and egg albumin protein provide the strength to gluten-free products.

5.3.1 Whey Protein

Whey protein concentrate (WPC) are extensively used dairy protein in research field of non-gluten products. The percentage of whey protein is 20% of total protein in milk (De Wit, 1990). Essential amino acid score of whey protein is higher than egg and soy protein. It is also considered as natural food additives for thickening functionality and as an alternative thickeners of starch and hydrocolloids (Resch & Daubert, 2002). Rheological properties of dough can be modify by addition of WPC in to dough paste (Lupano, 2003). van Riemsdijk et al. (2011) used the whey protein particle of meso-structured as a substitute of gluten for bread development. This structure of protein was selected based on Gluten free bread prepared by whey protein showed the strain hardening structure. Crumb structure of developed bread was similar to wheat bread (van Riemsdijk et al., 2011). Author also concludes that formulation of more or another protein with additives can improve the properties of the non-gluten bread. Volume of whey powder formulated bread was higher than the vital gluten based bread. Even small of whey amount (2.5%) was sufficient for large volume bread. Whey protein isolate enhanced the emulsifying properties, gelling behavior, and gelatinization of rice starch (Marcoa & Rosell, 2008). Ungureanu-Iuga et al. (2020) developed the gluten free pasta with whey powder and grape peel. Incorporation of whey powder in pasta paste improved the texture and sensory characteristics of pasta. Whey protein also enhanced the microstructure of pasta.

5.3.2 Casein

Casein is a major milk protein and its percentage is highest approximately 80% of total milk protein. Casein is obtained by precipitation of milk at 4.6 pH, supernatant portion is called whey and another substance of precipitated milk is casein protein (Liang & Luo, 2020). It is commonly used as a binding agent for many food product developments. It comes under the group of phosphoproteins (Deora et al., 2015). Most important function of casein protein is used as an ingredient for structure building agents. They can be used in solid and semisolid food material to provide mechanical strength and improved textural properties. Proteins are also used as a thickening agent to boost the consistency and stability of developed food products (Chan et al., 2007; Deora et al., 2015). Caseinate is another important casein-based ingredient. Based on the emulsifying and foaming functionality of caseinate, it can be used to improve the properties and functionality of dough and paste for gluten free products (Luo et al., 2014). A combination of sodium caseinate and whey protein improved the quality of gluten free pasta of pearl millet (Kumar et al., 2019).

5.4 Egg Protein

Eggs are common food additives for food product development. In recent years, its application is increasing for gluten free product developments. Egg proteins are highly functional protein, and can be incorporate for gluten free dough formation (Ziobro et al., 2013; Crockett et al., 2011; Pico et al., 2019). Egg white protein is good alternative source for gluten. Based on functional properties i.e. foaming characteristics, improvement in crumb structure can replace the gluten from bakery products. For example, egg protein helps in the improvement of dispersion capability and stabilization of gas bubbles in the non-gluten dough system (Deora et al., 2015).

Egg albumin and whey protein with parboiled rice were used for the development of non-gluten pasta (Marti et al., 2014). The addition of protein significantly reduced the roughness of uncooked pasta. These proteins can be used as texturing agents in gluten free pasta production without addition of chemical agents. During pasta development, hydrophobic interaction and disulfide bond between rice starch and protein make the pasta stable. During the cooking of pasta, the formation of a disulfide interprotein bond takes place and result in significantly enhanced textural and structural properties of products (Marti et al., 2014).

Matos et al. (2014) developed the muffins with various protein sources. The emulsifying activity of rice flour was effectively increased by the addition of egg albumin. The best appearance of muffins was observed in the case of egg albumin and casein protein. The incorporation of egg white protein increases the height and volume of muffins. The author also found that animal-source protein produced chewy, springy, and more cohesive muffins than vegetable protein.

5.5 Legumes

Legumes are the plant seed in the family of Leguminosae. It is one of the most prominent sources of food protein. Primarily, legumes are grown for human consumption and but also for livestock feeding. Legumes are a good alternative and supplement grain for cereals based food products. Due to the presence of higher content of essential amino acid i.e. arginine, aspartic acid, lysine, glutamic acid, in legume, it can provide well sufficient diet with consumption of other cereals (Deora et al., 2015; Miñarro et al., 2012). Leguminous protein also has functional properties that play major role in the formation and processing of food (Boye et al., 2010). Legume protein has been used in development of various food product i.e. bakery product, soup, and several ready to eat snacks. Nutritional benefits of legume consumption suggest it's for alternative of gluten flour to the development of non-gluten food products (Miñarro et al., 2012).

Addition of legume flour or protein explored as a substitute for nutritional quality improvement as well as physical attributes and overall qualities of gluten free products (Crockett et al., 2011; Miñarro et al., 2012; Foschia et al., 2017; Pico et al., 2019). The functional properties of soy protein and pea protein isolate were used to make and developed various gluten free products (Deora et al., 2015).

5.6 Soya Protein

Soybean is one the most commonly grown and used oilseed. It is also rich source of protein and its percentage approximately 40% of total weight (Sharma et al., 2014). Also of a higher content of protein, the nutritional value of this protein is high. Its protein digestibility-corrected amino acid score is also in the region of egg white protein (Deora et al., 2015). Due to foam stabilization capacity of soya protein isolates, its incorporation in gluten free products are studied (Marcoa & Rosell, 2008; Crockett et al., 2011; Miñarro et al., 2012).

Rice flour-based muffins were developed by (Matos et al., 2014) with the addition of various gluten free protein sources for modification of properties of non-gluten muffins. The addition of soya protein isolates significantly increases the storage modulus of developed batter. It also altered the textural properties of baked products. In addition, its incorporation also modified viscosity and elastic component of the rice based batter, and results in inducing the hardening effect. In study of (Crockett et al., 2011), addition of soya protein increased the crumb structure and volume of bread. Soya protein and hydroxypropyl methylcellulose as an ingredient for rice flour bread development produced the bread with similar porosity like wheat gluten (Srikanlaya et al., 2018).

5.7 Carob Seed Germ Protein (Caroubin)

Caroubin is separated from the carob germ powder. Carob is evergreen flowing tree in the legume family of Fabaceae. Carob tree commonly cultivated for its edible pot. Germ of carob seed is rich source of protein and it's known as Caroubin. It is a mixture of great amount of protein number which varies in their size and degree of polymerization, ranging from one million to several thousands of molecular weight. Physico-chemical properties of this protein are thoroughly similar to gluten protein of wheat (Feillet & Roulland, 1998). Caroubin is not similar to wheat gluten but its function behaves like in the manner of wheat gluten (Smith et al., 2012). Due to the presence of disulfide bond between the high molecular proteins, caroubin germ flour produced a similar dough like wheat flour (Smith et al., 2012). And also the rheological properties of developed dough were similar to the gluten dough. The concentration of carob powder and water give significant viscoelastic characteristics of dough (Tsatsaragkou et al., 2014). Appropriate ratio of both parameters can produce dough with equilibrium viscosity and elasticity.

5.8 Chickpea Protein

Chickpea is one of the most consumable and important legume crops in Indian states. India is the highest production county of chickpea around the whole world. Chickpea plays a leading role in world food safety by resolving the problem of deficiency of protein in our daily diet (Kaur & Singh, 2007; Merga & Haji, 2019). Chickpea is a common and good source of protein and carbohydrate, and quality of protein is better than other legume crops such as pigeon pea, green gram, and black gram. Chickpea contain almost 40% protein of total mass. Due to such amount of protein, make it unique legume for food consumption. Besides protein content, it also has potential health benefits for reducing the cardiovascular, cancer, and diabetic risks (Kaur & Singh, 2005). Chickpea protein has various functional protein such as emulsifying and foaming characteristics (Boye et al., 2010). Due to functional properties, good nutrional qualities, and excellent baking capabilities of chickpea protein, it is good alternative protein source for gluten free products (Boye et al., 2010; Kaur & Singh, 2007; Aguilar et al., 2015). Addition of chick pea flour significantly increases the specific volume as well as storage modules of bread (Aguilar et al., 2015). The incorporation of chickpea flour with tiger nut flour can replaced the emulsifier and shortening agents for non-gluten-free product development.

5.9 Pseudocereals

The dicotyledonous plants which resemble the true cereals in their functions and composition are called pseudocereals. These pseudocereals can be classified as legumes, oilseeds, cereals and nuts, etc. Now a day's these pseudocereals are grabbing the attention of researchers to use them in gluten free product formulations since they have many health promoting effects as well as they possess lot of proteins, fibers, calcium and iron. If these pseudocereals are used for the formulation of gluten free product, there will no need to fortify the product by external addition of the minerals. Hence the usage of pseudocereals will make the product gluten free and will improve its nutritional quality (Alvarez-Jubete et al., 2010; Aghamirzaei et al., 2013).

Due to the high nutritional value and absolute free from the toxins while it possesses a considerable amount of protein. Majorly the amaranthus, quinoa and buckwheat are pseudocereals used for the formulation of gluten free products (Ballabio et al., 2011). In the studies conducted by many researchers, they have concluded that buck wheat and quinoa possesses high quality of protein which has good digestibility, balanced efficiency ratio. The protein from these sources resembles the qualities similar to milk proteins (Ranhotra et al., 1993; Repo-Carrasco et al., 2003). Some studies also showed that amaranth and quinoa are rich in bioactive compounds such as γ- and β-tocopherol as well as polyunsaturated fatty acids - high linolenic: linoleic acid ratio (Comino et al., 2013).

5.9.1 Amaranth

The amaranthus belongs to Amaranthaceae family; it has more than 60 species which are grown across globe. *Amaranthus cardates* is one of those 60 species which is selected and used majorly for consumption. It is widely grown in Peru and other South American countries (Caballero et al., 2003). *Amaranthus cruentus* is grown in Guatenmala while *Amaranthus hypochondriacus* in Mexico. In India the pseudocereal of amaranth is widely consumed during fasting. It is very rich source of calcium, magnesium, and iron. It improves the hemoglobin in the blood. It is also high in protein content. The amino acids composition in the amaranthus is well balanced than other cereals and grains. The major parts of the protein present in amaranthus are albumin and globulin while prolamines are in lesser proportions. It also comprises with vitamins such as riboflavin, tocopherol (Ballabio et al., 2011; Chand

& Mihas, 2006). In the study conducted by (Gambus et al., 2002), the amaranthus flour was used in the preparation of bread to replace the gluten. At 10% replacement the nutritional properties were improved as compared to the standard composition while the sensory properties remained unaffected.

5.9.2 Quinoa

The quinoa botanically known as *Chenopodium quinoa* belongs to the amaranthus family. Quinoa is a pseudocereal which was a staple food from the ancient civilizations of Andes in South America. But now a ways it grown all across globe in all continents of Europe, Africa, and Asia etc. the quinoa has a similar appearance like oil seeds and possesses high oil content hence it can also be classified as pseudo-oil seed. The quinoa is available in different variety of colors such as white, red, purple, and black (Saturni et al., 2010; Vega-Gálvez et al., 2010; Bhargava et al., 2006). The proteins from the quinoa resemble very similar functional properties to that of milk protein. The quinoa protein completes the nutritional value since its protein possesses a high biological value of 83%. It is possible due the presence of combination of essential amino acids which provides it good functional properties. It also contains minerals in good proportions making it valuable for the human consumptions. The mainly found minerals are magnesium, manganese, iron, zinc and calcium (Repo-Carrasco et al., 2003; Vega-Gálvez et al., 2010). From the various studies conducted by researchers, food technologists (Zevallos et al., 2012; Mäkinen et al., 2013). It can be concluded that quinoa is safe for the replacement of gluten to produce and formulate gluten free product by using alternative proteins. The lower concentration of oat malt less than 1%, improved the bread volume and crumby structure (Mäkinen et al., 2013). The complete in vivo characterization of the developed gluten free product by using the alternative proteins should be done in order to understand its digestibility and the reactivity.

5.9.3 Buckwheat

The buck wheat belongs to polygonaceae family and caryophyllales order while botanically it is classified as fruit. But it is consumed in the form of grain or flour. The buck wheat is toasted before grinding it to flour. Since, the buck heat has reported some allergy cases in korea, Europe and japan (Panda et al., 2010). The toasting of buck wheat will denature the allergen compounds. There are two types/species of buckwheat which are widleychoosen for the human consumption namely *Fagopyrum esculentum* or common buckwheat and *Fagopyrum tartaricum*or tartary buckwheat. The *Fagopyrum tartaricum* is largely cultivated in Asian countries (Ikeda, 2002; Skrabanja et al., 2004). Buckwheat is a rich source for dietary fiber, vitamins, essential minerals, trace elements, rutin. The major factor for choosing the buckwheat for gluten replacement is its favorable amino acid sequence composition which is desirable to improve the protein quality of newly developed gluten free formulation for any product (Panda et al., 2010; Dunmire & Tierney, 1997). Hence buckwheat can be a suitable option to replace the gluten.

5.10 Functionality of Proteins

Apart from the gluten's textural properties and its effect, there are various other properties exhibited by the protein known as functional properties which govern the physical, chemical, organoleptic and nutritional properties of any product. Valuable dimensions have imparted by the functional properties of the protein to the various products in terms of its product's texture, appearance, taste as well as nutrition. The functionalities of protein include solubility, emulsification, foaming, water holding, and oil holding capacity, gelation, surface hydrophobicity, etc. which are described in detail as follows.

5.10.1 Solubility

The index of protein functionality can be measured from the solubility which can be estimated by the aggregation and denaturation of protein. The improvement in the functionality of protein can be achieved by improving its solubility (Chobert et al., 1988a). Since some product require soluble protein and some require insoluble protein. The solubility of protein depends on the various factors such as temperature, pH, isolectric point etc (Chobert et al., 1988b). The solubility of protein plays an important role in the replacement of gluten in the products such as soup powders, instant soups and curries mixes which are having a major component of wheat flour and soy bean flour. In such products the protein solubility is desirable (Mutilangi et al., 1996). Hence such soluble protein can be replaced by the whey protein and whey protein isolates which are soluble in water. While the proteins in the bakery products must be insoluble to impart the textural and rheological properties. The gluten protein is responsible for the structure of bakery products (Garrett & Hunt, 1974). The caseinate protein from milk and some soy proteins which are insoluble can be used as an alternative for gluten in the bakery products . But before replacing the gluten by caseinate and soy protein its rheological properties are to be checked. Both the soluble and non-soluble proteins are important in respect to replace the gluten as per their utility in the final product possessing desirable properties.

5.10.2 Emulsification

Emulsification is a property where the oil water interfaces are hold together to form an emulsion. The emulsion quality of the protein is measured from its water-oil holding and binding (Turgeon et al., 1992). The interaction at the surface of oil and water at their surfaces are studied to know the emulsification property. The emulsifiers are measured for the emulsification activity, emulsion forming capacity and the stability of formed emulsion (Nakai et al., 1980). These properties of emulsion depend on the molecular structure of the protein molecule. The whey protein from milk and soybean protein, their molecular structure is different, hence both exhibits different emulsion properties. As a result they may find various applications as per the product suitability. The soy bean proteins are considered as one of the best proteins possessing best quality of emulsification quality, hence it can be used an alternative protein to replace the gluten.

5.10.3 Foaming

The foaming property is a unique property exhibited by protein generally in the products such as marshmallows and other edible foams. The molecular properties of the protein are related to the foaming characteristics (Kitabatake & Doi, 1982). The capacity to form foam and its stability are the important parameters to check these foam characteristics. The peptide linkages of amino acid sequences numbering from 101–145, 107–153, and 107–145 are important for the formation of superior quality of foam (German & Phillips, 1994). Foams are generally comprised of two phases a continuous aqueous phase and another dispersed gaseous phase. For obtaining food quality foam, segmental flexibility which is unfolding of bonds at the interfaces of the molecular secondary interactions of charged and polar groups is very important (Althouse et al., 1995). The soybean protein and whey protein are mainly used for their foaming property. The protein molecules present in these proteins possess a strong tendency to form the dimers, trimmers, and high order peptide- peptide linkages that are necessary for the generation of foam (Cheftel et al., 1985). Where gluten protein molecules have fewer tendencies to form such linkages and ultimately the low-quality foam is obtained. Hence, these alternative proteins can be used very well in place of the gluten for their better foaming characteristics.

5.10.4 Surface Hydophobicity

The functional properties of the protein are impacted by the structural characteristics which are used to evaluate the protein conformation while can be measured by the surface hydrophobicity of the protein molecules (Nakai et al., 1980). The protein molecules are susceptible to heat treatments, resulting into the surface hydophobicity. The heat treatments given to the protein molecules may result in the denaturation and hydrolysis giving rise to two separate effects (Kato et al., 1983) have studied these effects in the research conducted by him. In the research he concluded that, when the good co-relations are established between the surface tension, emulsifying activity, interfacial tension of the proteins present in the sunflower, rapeseed and soy bean. Due to the presence of the shorter amino acid chains and secondary structures, this surface hydophobicity is affected. The properties of the protein molecules are important for the formation of structures in the bakery products, foams, gels, confectionary products etc. the surface hydophobicity is an important parameter to study for the gluten and non-gluten proteins.

5.10.5 Gelation

The formation of gel by the entrapment of water molecule with the minimum syneresis is the gelation property which is generally referred to the structural strength of the product. When the protein-protein interaction increases with the water, it results in the formation of gel (Matsumoto & Hayashi, 1990). The products' mechanical strength and its viscoelastic properties can be determined from the gel strength. The gel formation and the gel strength may depend on the various extrinsic factors such as pH, ionic strength, temperature etc. The environmental conditions govern the gel characteristics; the characteristics includes such as firmness, rate of gelation, transparency of gel and its microstructure (Aguilera, 1995). The gel forming characteristics of the protein can be used as a replacement for the gluten. Many of the researchers have replaced the gluten by using various gums such as moringa seed gum, guar gum, gum arabica, gum gatti etc. Gum binds all the ingredients well (Kitabatake & Doi, 1993). But there are certain disadvantages of using the gum as replacement of gluten. The gum binds all ingredients but imparts stickiness to the product and increases the calorie content. Gum can't provide the desired springiness to the product. Hence protein can be suitable option to replace both gluten and gum. The protein possesses both the properties i.e. to hold and bind all ingredients together as well as it can impart the desired springiness to the product. The gel forming property of protein will improve the bonding and interactions; it will help to improve the texture of product. The protein will impart good structure and strength to the product. It also adds benefits by improving the protein content of the product.

5.10.6 Water Holding Capacity

The protein water interactions are one of the important characteristics which govern the water bounding and extent of water holding (Fevzioglu et al., 2012). The rheological properties of the bakery products are governed by the water holding capacity of protein molecules. The caseinate and β-lactoglobulin are water-insoluble proteins and possess some similar characteristics to that of gluten (Kinsella et al., 1989). The water holding capacity of protein influences the textural and structural properties of any product. When the water holding capacity of non-gluten protein increases and solubility decreases, such protein molecule can provide similar rheological properties as of gluten (Kneifel & Seiler, 1993). As discussed caseinate and β-lactoglobulin can be alternative to replace the gluten in the bakery products since desired functionality can be obtained.

5.10.7 Oil Holding Capacity

The oil holding property of protein is the most important property for the production of bakery products. Cakes, biscuits, crackers, cupcakes, donuts, muffins, etc. product requires fats as a compulsory ingredient to impart the softness. The fluffiness, springiness of these bakery products depends on the protein and the oil holding capacity of the protein. The protein molecules hold the oil in therewith to provide the desired softness. Oil holding plays an important role in the formation of emulsions, foams, and their stability (Gauthier et al., 1993). Hence when the gluten-free product has to be developed, it should be kept in mind the proteins from soybean can be alternative to gluten, since they possess more oil holding capacity. The oil holding capacity of proteins will govern the softness of the product as well it is also responsible for the flavor holding.

5.11 Strategies for Replacement of Gluten

When the gluten protein has to be replaced, several combinations of the protein from various sources can be used. The functional properties of the protein can be altered and modified to obtain the best results and superior quality products. The functionalities of these proteins can be altered by using different processes such as as enzymatic modification, high-pressure modification, cross linking of proteins, ultrasound treatment to proteins. These processes can be termed as strategies for the replacement of gluten and are discussed as follows.

5.11.1 Enzymatic Modification

The functionality of the proteins can be enhanced by the usage of enzymatic modification in order to achieve the desired properties, which can be used as alternative protein in place of gluten. The enzymatic modification is done by altering the amino acid chain sequence which will yield into the desired functionality of the specific protein. The enzymatic modification can be achieved by proteolysis, it is also termed as peptide linkage hydrolysis (Panyam & Kilara, 1996). The mechanism of the enzymatic modification can be seen by three different effects occurring in the protein molecule. There is decrease in the molecular weight of protein molecule, an increase in the ionizable groups while hydrophobic groups are concealed as a result of enzymatic modification (Kim et al., 1990). All of these results into the improved solubility reduced surface hydophobicity, increment in the gelation, foaming and emulsification capacity of the proteins. While carrying out enzymatic modification of the protein, the enzyme specificity is one of the key factors affecting the modification as well resulting in the faults in achieving the functionality of the protein (Whitaker, 1977). The other factors affecting the modification process are pH, isolectric point, ionic strength, protein denaturation, enzyme concentration, temperature. The molecular size of protein, specific amino acid sequence, structure of protein molecule will govern the functionality of protein (Casey et al., 1991). To achieve the desired functional properties, such as emulsification, foaming. Surface hydophobicity, water holding, oil holding and flavor binding capacity of the protein molecules can be modified enzymatically in order to utilize them as a replacement for gluten. The enzymatic modification of protein finds potential option to improve protein functionality which can be alternative to be used in place of gluten.

5.11.2 High Pressure Modification

The functionality of the protein can be modified by the application of high pressure. (Messens et al., 1997) have successfully carried research on the modification of the functional properties of proteins from the milk, egg, soy and meat proteins by the use of high pressure. The high pressure modification process carried at a pressure of 1000 mPa can confront the protein to modify resulting in desirable changes in its functional properties. The mechanism for modification can be explained as, since the high pressure is applied; the volume of the protein molecule decreases due to the compression of the internal cavities. It also results in the rupture of covalent bond interactions and establishment of new intra and inter molecular bonds within the protein molecules. As a result of these secondary, tertiary and quaternary protein molecules are stabilizes. In the research conducted by (Puppo et al., 2004) it was found that the hydophobicity of the protein can be reduced by using high pressure. The pressure of 150 mPa can be applied to stabilize the hydrophobic interactions between the protein molecules. This will contribute to enhancing the water holding capacity of the protein. 200 mPa pressure can be used for the hydrophobic interaction stabilization of secondary, tertiary protein molecules. From the study of (Singh & Ramaswamy, 2015) it was included that the β-lactoglobulin present in the casein-a milk protein can be modified for its gel-forming capacity. The interactions between the β-lactoglobulin and α-lactoglobulin can be improved; resulting in an improved gel-forming capacity. The high pressure can also be applied to whey proteins as well. The emulsification efficiency of soy protein has been improved due to the application of high pressure.

5.11.3 Cross-Linking of Proteins

The food biopolymers play an important role in the formation of effective structure in the bakery products. The cross-linking of protein is an important tool to alter the structure of the protein molecule by which the functional; properties will be enhanced. The cross-linkage of protein molecules may be achieved by the enzymatic treatment and non-enzymatic Maillard reaction. The enzymes used for the cross-linking are transglutaminase, tyrosinase, lactases, peroxidase, and sulfhydryl oxidase (Buchert et al., 2010). The specificity of cross-linking the proteins is affected by the enzymatic catalyst and the mild reaction conditions. The reaction condition includes temperature, pH, isolectric points etc. the disulphide linkages govern the thermo-rheological properties of dough (Gerrard, 2006). The cross linking can be achieved by the heat treatment, enzyme treatment, ultrasound treatment, which will create the intentional inter and intra molecular bonding of sulfhydryl groups of cysteine in the protein molecules. The creation of cross linking may lead to enhanced stability of the protein and will resist to proteolysis (Matheis & Whitaker, 1987). The cross linking of the disulphide's in the protein will strengthen the stability, firmness of protein and also enhances the viscoelastic properties. The cross linking of the proteins may also enhance the digestibility and reduces the allergenicity of the protein (Thalmann & Lötzbeyer, 2002). The non–globular proteins may be more susceptible for the cross linking while the cross linking in globular proteins may enhance its properties and functionality may be altered. The modified protein by cross linking may be used in place of gluten for development of gluten free product. The modified protein by cross linking will exhibit the similar characteristics to that of gluten due the cross linking and network formation. The cross-linking is a cost-effective and rapid tool which can be used for the modification of protein with good efficacy.

5.11.4 Ultrasound Treatment to Proteins

Ultrasound treatment is a new, safe, and one of the effective methods utilized for the modification of the functionality of the protein. The mechanism of the ultrasound modification is based on the shear stresses and turbulence created by the high-intensity ultrasound (Jambrak et al., 2009). The ultrasound treatment given to soybean protein has to yield good results for the viscosity and elastic characteristics which were recorded on the stress rheometer by (Arzeni et al., 2012). The impact of high-intensity ultrasound treatment was studied by (Riener et al., 2009). In the study conducted by (Mishra et al., 2001) egg protein, whey protein, and whey protein isolate were treated at 750 W, 20 kHz frequency and 20% amplitude. The satisfactory changes in the gelation, solubility and viscosity are closely related to the modification done on the molecular level of the protein molecule. Thus the functional properties of the protein are widely affected by the ultrasound treatment which makes ultrasound as a potential option to alter the functionality of protein and making it suitable for the further development of the desired gluten free product.

5.12 Gluten Free Products

The gluten free product available in market are enlisted in the Table 5.1, comprising with the alternative proteins.

5.13 Challenges for Gluten Replacement

When gluten is replaced from any product, it is necessary to check the functional, nutritional and organoleptic properties of the product and protein. The gluten protein influences all of these properties of the product. Hence the replacement of gluten by an alternative protein can face these challenges, which are discussed as follows.

5.13.1 Nutritional Challenges

Now a day's consumers are aware of the product they are consuming. Whether the product is nutritionally rich, does it contains essential nutrients is checked by the consumer before purchasing it. Since the gluten is replaced from the products, it has created nutritional imbalance. Limited numbers of evidences are available regarding its nutritional inadequacies (Kinsella, 1982). These nutritional deficiencies can be fulfilled by the combination of two or more proteins from varying sources. These

Table 5.1 Gluten free products prepared from alternative proteins

S.N.	Product name	Alternative protein	References
1.	Bread	Whey protein	Storck et al. (2013)
		Soybean protein isolates	Crockett et al. (2011)
		Maize protein	Fevzioglu et al. (2012)
		Albumin	Schoenlechner et al. (2010)
		Soy protein isolates, Pea protein, lupine	Ziobro et al. (2013)
		Carob flour (Caroubin)	Tsatsaragkou et al. (2014)
2.	Pasta	Casein + Egg whites	Sozer (2009)
		Egg albumin	Marti et al. (2014)
		Whey protein	Kumar et al. (2019)
		Whey protein+ Grape peel+ corn starch	Ungureanu-Iuga et al. (2020)
3.	Cupcake	Amaranth + egg protein	Egorova and Reznichenko (2018)
4.	White sauce	Soy bean protein	Gularte et al. (2012)
5.	Muffins	Pea protein, Soy protein isolates	Matos et al. (2014)
		Casein	Hubbell et al. (2007)
		Chick pea protein	Herranz et al. (2016)
6.	Donuts	Egg protein + Whey + casein	Melito and Farkas (2013)
7.	Bakery products	Peas protein isolates	Mariotti et al. (2009)
8.	Fermented products	Pea protein + soy bean protein isolates	Marco and Rosell (2008)
9.	Crackers	Buckwheat + protein isolates	Sedej et al. (2011)
10.	Noodles	Zein + Rice flour	Jeong et al. (2017)

combinations of the alternative proteins which have replaced the gluten are having some of the important essential amino acids such as lysine, and tryptophan. (Thompson, 1999), which are missing in the gluten fractions. Thus the new alternative protein combinations can fulfill the nutritional demands of the protein. Along with it, gluten-free products can be fortified with iron, niacin, folate, riboflavin, and thiamine (Thompson, 2000). The replacement of gluten by the combination of proteins brings a challenge to keep the glycemic index or the calorie count in the limit. If the glycemic index or the calorie goes beyond the limits, the replacement is of no use.

5.13.2 Functional Challenges

The gluten protein provides the essential structure building property to the product. This structural property of gluten is one of its important functional properties. It also governs the visco-elasticity of the dough. The gliadin and gluten in ratio present in the gluten are responsible for these functional properties. In gluten-free products, it is a major challenge to replace gluten by the alternative protein to provide similar resembling functional properties (Smith et al., 2014). The loaf volume of bread, softness of cakes, muffins and cupcakes, the springiness of noodles, spaghetti, and pasta; all of these are the functional properties imparted by gluten. The consumer is habitual to consume these products due to its functional properties. If the gluten is replaced by the alternative protein and it does not meet the similar characteristics of functional properties imparted by gluten, the product will fail and will not meet consumer satisfaction.

5.13.3 Organoleptic Challenges

When the gluten-free product is developed, organoleptic factors should be always considered to obtain a product which is having similar properties to that of gluten-containing products. The most important property imparted by the gluten is its texture; the people are habitual to the texture of gluten imparted in various products such as cakes, biscuits, bread, cookies, etc. (De Wit, 1998). The organoleptic characteristics of any product are judged by color, flavor, texture, appearance, taste, etc. Making the gluten free product of the similar sensory characteristics is one of the most important organoleptic challenges faced by the researcher (De Wit, 1990). Where the consumer has an impact of the taste, texture, color and flavor of the gluten based product, denies the acceptance of the product. The perfect combination of the alternative source of protein which will provide perfect texture, structure, product appearance, taste, color is most important and difficult challenge faced by the researchers. In case of breads, the gluten free bread should possess same loaf volume as that of gluten containing; the gluten free cakes should impart same softness as that of gluten containing cakes; the gluten free sauces, and premixes should provide same consistency, visco-elastic properties and appearance to that of gluten containing.

5.14 Conclusion

The development of gluten-free products by using alternative protein is not a straight and easy process. It involves many steps and complex processes. Many of the food technologist, researchers, and scientists are working on the molecular chemistry of the non-gluten proteins to produce a perfect combination which will impart the functional as well as nutritional properties to the newly developed gluten-free product. In products such as breads, biscuits, cookies, and cakes many of the researchers have found out a perfect combination of alternative proteins which are providing similar characteristics to gluten in terms of texture, structure of product, taste, overall appearance and enhanced with more nutritional properties.

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