Chapter 4
Role of Microbial Fermentation in Gluten-Free Products

R. Anand Kumar and Winny Routray

Abstract Gluten intolerance is one of the significant symptoms associated with different health disorders, which has become an increasing concern worldwide. A gluten-free diet is considered a curative product for the problem, which has been steadily increasing in the market. Gluten plays a key role in developing gluten-containing products with desired attributes. Elimination of gluten in staple food is not an easy task and it is difficult to provide the gluten-free product with similar characteristics as gluten-based products. Gluten-free products are produced from gluten-free cereals with different kinds of additives that modify the product according to the desired properties. Gluten-free cereals such as rice, sorghum, maize, and corn are some of the raw materials that are the major replacement cereals for producing gluten-free products. Fermentation is also an avital step in the preparation of the gluten-based product for attaining optimum texture and sensory properties. Sourdough fermentation is an important process employed in the fermentation of gluten-free products for creating resemblance with the gluten-based product. Lactic acid bacteria species have been mostly used in the fermentation process to produce gluten-free products of equivalent quality. Enzymes are also utilized in the production of non-gluten products such as gluten-free beer. The major drawbacks of gluten-free products include the cost of production, nutritional deficiency, and lack of simpler methods to produce the final products. Lack of nutrients in gluten-free products is due to the replacement of raw materials that can be recovered by the incorporation of nutrients from different sources such as vegetables and grains containing vitamins, minerals, and high-level dietary fiber. The modification of physical, chemical, and aromatic properties by the incorporation of additives also influences the final product quality. The future market scenario mainly depends on the adaptation of new lifestyle diets by the respective consumers for the respective abnormality and disease.

Keywords Gluten intolerance · Gluten-free · Sourdough fermentation · Lactic acid bacteria · Textural properties · Sensory and aromatic properties

R. Anand Kumar · W. Routray (✉)
Department of Food Process Engineering, NIT Rourkela, Rourkela, Odisha, India
e-mail: routrayw@nitrkl.ac.in

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4.1 Introduction

Gluten-free products, low-carb diets, and low-fat diets have become some of the most common and important terms used in the new dietary diaspora, where the trend of gluten-free diet consumption is increasing worldwide, attributed to the increased consciousness about health and dietary lifestyles. The market value of gluten-free products has been anticipated to reach 6.47 billion USD by 2023, which was 4.48 billion USD in 2018 (Markets, 2018). In the Indian market, it is expected to reach 189 million USD by 2024. It was 8.62 million USD in 2018 (Markets, 2019). Baked products are some of the most commonly consumed gluten-free products in the diet, wherein gluten-free coconut cookies and choco-chip cookies are some of the popular products in the Indian market and possibly throughout the world (Markets, 2019). Furthermore, e-commerce is one of the major players in the market of gluten-free products, which has given a common platform for the local and global manufacturers and augmented the selection of products for the consumers, combined impacting the market and the consumer wellbeing.

Gluten intolerance is one of the major concerns in recent days. Also, celiac disease is one of the commonly observed autoimmune diseases, mainly caused due to genetic disorders and environmental factors. It can be identified through gastrointestinal symptoms and extraintestinal manifestations (Torres et al., 2007). The disease is triggered mainly due to consuming food that contains gluten such as food made of wheat, barley, oat, and rye. Symptoms of celiac disease include bloating, vomiting, and diarrhea (Skerritt et al., 1990). There is also another condition called non-celiac gluten sensitivity, which incurs similar symptoms such as bloating, gas, abdominal pain, weight loss due to malabsorption, anemia, and fatigue (Murray et al., 2004; Ghadami, 2016). Celiac disease occurs due to particular genes, including HLA – DQ2 and DQ8 Haplotype. Allergens present in wheat protein have been characterized by a food IgE-mediated allergy. The important allergen and main toxic component present in wheat gluten are ω5-gliadin that induces anaphylaxis (Balakireva & Zamyatnin, 2016). The untreated celiac disease leads to osteoporosis, epilepsy and genetic problems such as Turner syndrome, Down syndrome and IgA deficiency (Ghadami, 2016).

Fermentation of food enhances the flavor and nutritional quality of food, where microorganisms play a vital role. Since, Louis Pasteur observed lactic acid fermentation in 1857 and 1837, and proposed that yeast is responsible for the conversion of sugars into ethanol and carbon dioxide, which are some of the main components contributing to the physicochemical properties of fermented products, the fermentation process has been diversified and has become an innate unit-operation for the processing of various food products. Egyptians, 4000 years ago, fermented the dough for the preparation of bread, which is currently available in different versions as a major staple food all over the world. Wheat bread, beer, pasta, cakes, cookies, and similar pastries are some of the fermented products containing gluten as one of the main raw materials. Furthermore, currently, different other fermented products are also available in the market. Sauerkraut is produced through the fermentation of cabbage, wherein the present method of sauerkraut fermentation was developed by Vaughn (1981).

The market value of the baking industry is expected to be 17 billion USD by2022, which was about 13 billion USD in 2017 worldwide. In India, the market value was 7.22 billion USD in 2018 (India 2020). India is the second-largest producer of biscuits and is expected to reach a market value of 12 billion USD in 2024. The market value of beer was 593 billion USD in 2017. It is expected to reach 685 billion USD in 2025. In India 13 billion USD is the current market value of beer (Statista, 2020). Hence, based on the popular consensus regarding baked and fermented products, it can be deduced that the demand for gluten-containing fermented and baked products will also further grow. However, attributed to the negative effects and health complications observed in cases of consumption of gluten-containing products, the development of gluten-free products is being continually encouraged.

This chapter has summarized different aspects and considerations for the development of gluten-free products, the microorganisms involved in the fermentation of gluten-free products, properties of these products, and the corresponding process and composition modifications required for obtaining consumer acceptable products, which have also been simultaneously briefly compared with the gluten-containing products. Prospective future perspective of the gluten-free products and challenges encountered for successful development of the acceptable products has also been discussed.

4.2 Different Currently Available Gluten-Free Products with Fermentation

4.2.1 Traditional and Non-traditional Products Derived from Gluten-Containing Grains

Traditionally a wide array of gluten-containing products have been available, which are still some of the major sample subjects of studies. For the past many centuries, several baked products have been developed with different flavors. Gluten is the most important constituent in baked products, which can deform, stretch and trap air molecules inside the dough, useful for the production of bread and other similar fermented products. Bread is a major traditional product that is made of raw material containing gluten. Controlling bulk fermentation is the most important factor for the quality of bread (Cauvain, 2015). Cakes are batter-based and chemically leavened products and currently, there are several varieties of cakes, which have been developed through variation of formulation of the ingredients. The pasta varieties include macaroni, vermicelli, lasagna, spaghetti, and noodles. Semolina wheat is a major raw material for manufacturing pasta. Pizza, which is originally from Italy and is a flat leavened bread with different kinds of toppings, is produced from hard wheat flour, where dough develops a gluten network for entrapping carbon dioxide. Empanada is another food item, which can be combined with paneer and prepared using refined wheat flour, milk powder, shortening agent, yeast, and salt; this is also available inspired and sweet forms (Mallikarjuna, 2013). The tortilla is produced from wheat flour, shortening agent, salt, baking powder, and other essential additives with water, wherein the dough structure is maintained by gluten matrix. Gluten is a major factor for the development of a good quality product with desired texture and shelf stability (Alviola et al., 2008). Biscuits, cookies, and crackers are some of the other major products produced from refined wheat flour.

Though wheat-based gluten-containing products are the most commonly available products in the market other gluten-containing grains, from which several products have been developed include rye, barley, and triticale (a hybrid and rye). Barley has higher dietary fiber (2–11% β-glucan) than wheat (0.5–1%) (Feng et al., 2005) and has been recognized as a healthier alternative. Barley bread is made from 80 % of the whole meal barley flour and 20% of white wheat flour. These bread are consumed as part of breakfast by the healthy participants and have been assessed for glucose tolerance effect. It was found that insulin level was maintained at lower quantity for the participants (Östman et al., 2002). Rye bread is prepared in four varieties such as endosperm rye bread, whole-grain rye bread, whole-grain rye bread with lactic acid, and rye bran bread. These products have a significant effect on maintaining low blood glucose levels and stimulated low insulin (Rosén et al., 2009).

Apart from the regular popularly available gluten-based products, there are also several other products developed through fermentation. Fermentation of cracker dough has been observed to modify the protein network, wherein fermentation of dough is followed by cutting and sheeting (Zydenbos et al., 2004). In a different study, barley was used for enriching Tarhana with high β-glucan content, which is traditionally a fermented product prepared from the mixture of wheat flour and yogurt, and has originated from Turkey (Erkan et al., 2006). Awad and Salama (2010) added fermented barley in cheese produced from buffalo milk and buttermilk powder, to develop a product similar to the labneh-fermented (Greek Yoghurt) product.

There are also several traditional and non-traditional beverages derived from gluten-based grains. American rye whiskey is an important fermented alcoholic beverage product of rye grain. It has unique characteristics compared to other whiskeys, and are dry and spicy (Lahne, 2010). The raw spirit produced from the grains such as rye and wheat has been used for the manufacturing of the Vodka after rectification of the spirit (Lachenmeier et al., 2003). Benzoxazinoids is introduced in the barley-fermented beer by the addition of rye or wheat malt. It is a nitrogen-containing secondary metabolite compound, which has a positive effect on health including weight reduction, central nervous system stimulatory, antimicrobial, and immune-regulatory effects (Adhikari et al., 2015; Pihlava & Kurtelius, 2016).

4.2.2 Gluten-Free Products Developed from Gluten-Free Grains

Cereals not containing gluten include rice, maize, sorghum, and millets, which are also used for the production and formulation of gluten-free products. Correspondingly, celiac patients can consume gluten-free pasta made from buckwheat flour (Alamprese et al., 2007). Channa flour, soy flour, sorghum flour, and whey protein concentrate are the raw materials for producing gluten-free pasta (Susanna & Prabhasankar, 2013; Gao et al., 2018). These cereals have been considered as the major replacements of gluten-containing raw materials for producing gluten-free diets. In gluten-free biscuits, refined wheat flour is replaced by other raw materials, which should consist of not only starch but also the equal amount of protein fractions as present in the original recipe with wheat. Gluten-free biscuits produced from starch contain raw materials such as corn, rice, millet, potato, and buckwheat, mixed with fat like palm oil, low and high-fat dairy powder. The quality of biscuits is comparable to wheat flour biscuits when it is prepared with rice, soya, corn, and potato with high fat powder (Gallagher, 2008).

Different non-traditional food commodities have also been developed as healthy alternatives by several research groups, with enhanced sensory properties. Gluten-free empanada and pies were developed from cassava starch, dry egg, whey protein concentrate, gums, and water by Lorenzo et al. (2008). The addition of gum produced dough that had high elasticity properties and lesser hydration. These properties were reported as almost similar to the gluten-containing dough used in industries. Sorghum flour was used by Winger et al. (2014)for preparing gluten-free tortilla by hot press procedure, where sorghum flour completely replaced the wheat flour, and xanthan gum, baking powder, citric acid, sugar, monoglycerides, shortening agent, salt, and water were used for the preparation of tortillas. Rice-based muffins have also been prepared by adding different protein sources such as soy protein isolate, pea protein isolate, casein, egg white protein, which were compared with samples containing wheat gluten, for the assessment of samples win terms of conventional muffin properties. Muffins with pea protein isolate were found to be softer; whereas, casein containing muffin was harder. The texture quality of the muffin was greatly dependent on the protein sources (Matos et al., 2014).

Malting and brewing of gluten-free cereals such as sorghum, rice, and maize, is a common process for producing alcoholic beverages (Zweytick & Berghofer, 2009). Sake is an alcoholic beverage manufactured from rice and water. Sake is traditionally produced in Japan with steamed rice. The broken rice from the milling industry is also used for producing beer, wherein the sweet taste of rice beer changes to sour when sorghum is added to it; the taste is acceptable after successful organoleptic assessment (Phiarais & Arendt, 2008). There are several other low alcoholic beverages such as Braga, darassum, cochate (Phiarais & Arendt, 2008), and uphutsu, which is a traditional beer brewed using Pearl millet in Mozambique (Pelembe et al., 2002; Phiarais & Arendt, 2008).

4.2.3 Gluten-Free Products Derived from Gluten-Containing Grains

The gluten content in bread is reduced by sourdough fermentation. Sourdough fermentation has been proven to be ideal for improving the shelf life and nutritional value of bread, where after mixing water with flour, the mixture is fermented with yeasts and lactic acid bacteria. The metabolic activities of the corresponding microorganisms (Lactobacilli) in the sourdough cause positive effects, including fermentation of lactic acid, proteolysis, production of exo-polysaccharides, and antimicrobial compounds synthesis (Corsetti & Settanni, 2007). Sourdough fermentation helps in reducing the glycemic index of the bread (Scazzina et al., 2009). Physio-chemical properties of the dough correspondingly modify, as sourdough fermentation also causes higher resistance to deformation after gelatinization of the batter (Schober et al., 2007).

Refined triticale flour has been used for bread making and other baked products. Triticale flour does not produce good quality dough due to low gluten content, high levels of alpha-amylase activity, and inferior gluten strength. These limitations have been overcome by mixing at low speed, shorter fermentation times, and blending with wheat flour to produce bread with acceptable quality by Naeem et al. (2002). Onwulata et al. (2000) also used triticale flour to produce extruded high fiber snacks and a nutritious bar containing 20–40% wheat and oat bran. However, oriental noodles manufactured from triticale flour possessed poorer properties, including the greyish color of noodles caused due to high ash content in flour creating an undesirable property (Shin et al., 1980).

Worldwide, there are several products developed from gluten-containing grains, which possess lower concentrations of gluten attributed to the fortification with other components. Miso is a Japanese product prepared by fermentation of barley and soybeans with *Aspergillus oryzae*, and the corresponding thick paste-like Miso product is a regular diet of the Japanese people. Routine consumption of miso has been reported to decrease the risk of gastric cancer (Murooka & Yamshita, 2008; Hirayama, 1982). However, due to the high level of salt, it possesses the risk of blood pressure (Kawano, 2007). A modified version of Tempeh (Indonesian soy product) is produced employing barley, wherein *Rhizopus oligosporous* and *Lactobacillus plantarum* are used for the fermentation process to produce barley tempeh (Feng et al., 2005).

Based on the above-mentioned sections, it can be observed that fermentation improved the quality of products, both with gluten and without gluten. Hence, it can be deduced that fermentation is an important and essential processing method for the development of gluten-free products, which are derived through reduction of gluten in products derived from gluten containing grains and products derived through formulation with gluten free grains.

4.3 Properties of the Products to Be Considered

The quality of products, including bread, biscuits, pastas, etc. mainly depends upon the raw material and processing methods. There is no individual characteristic that can be used for the sole identification of the quality of the product. There are many properties to be considered that decides the final quality of the product.

4.3.1 Compositions and Chemical Properties

Traditional compositions and preferred gluten-containing products are generally affected by the component flour properties. Gluten network formation is the major process in bread making. The protein content of the flour determines the bread quality, where higher protein content enables to trap the carbon dioxide and retains the greater volume of bread. Dough properties such as specific volume, spread ratio, color, smoothness, texture depend on the protein quality (Zhu et al., 2001). Yeasts are available in different forms and the major type of yeast used in the baking is the baker's yeast (*Saccharomyces cerevisiae*) (Bell et al., 2001). Yeast feeds on sugar to release carbon dioxide (Ali et al., 2012) and produces carbon dioxide during the fermentation process for leavening of bread dough. Adding fats like margarine increases the carbon dioxide retention in the dough. Also, attributed to the formation of hydrogen peroxide by yeast, the dough becomes more elastic. However, insufficient hydration of dough leads to incomplete formation of gluten network (Faridi & Faubion, 2012); hence, textural properties of dough are modified through the amount of water added (Cauvain & Young, 2007). Salt controls the fermentation process by strengthening the network formed by gluten and increase of the effect of lipid peroxidation reaction (Toyosaki & Sakane, 2013).To improve the product quality, additives like oxidizing agents, reducing agents, emulsifiers and enzyme active materials are also added (Cauvain, 2015). Emulsifiers such as sodium stearoyl lactylate, lecithin and distilled monoglycerides are also used in bread making. Similarly, other products including biscuits and cookies are also produced with flour, sugar, fats and oil.

In gluten-free breads, soya flour is used to replace protein content and starch is added through rice and maize, which replace the essential amylopectin content (Taghdir et al., 2017). Eggs are often added to the gluten-free compositions as an emulsifier, which also contains proteins that enable strong cohesive viscoelastic films that are necessary substrates to trans glutaminase for the formation of stable foaming (Moore et al., 2006). Gluten-free cookies have also been made by using germinated amaranth flour, which has high antioxidant activity and total dietary fiber content (Chauhan et al., 2015).

Apart from regular compositions with other grains for starch and protein replacement, supplementary additives are also added with bioactive and textural properties. In a separate study, gluten-free rice muffins were developed using black carrot pomace dietary fiber concentrate and xanthan gum, which demonstrated higher water and oil absorption capacity and consequently increased flour paste viscosities (Singh et al., 2016).

Fermentation of gluten-free materials with microorganisms and sourdough fermentation in baked goods also enhances functional properties such as an increase in free amino-acid concentration, increase in antioxidant activity (Gobbetti et al., 2014; Curiel et al., 2015), and increase in mineral (free Ca^{2+}, $Zn^{2+}$and Mg^{2+}) availability (Di Cagno et al., 2008).

4.3.2 Physical Properties

Based on the available reports and scientific papers, it can be concluded that some of the different desirable physical properties required for the bakeries and confectioneries and other related food products include textural properties such as hardness and fracturability values (Kadan et al., 2001), loaf volume and color (Sciarini et al., 2010) of the product.

During the past decade, different gluten-free grains have been used to prepare bread. In gluten-free bread, during fermentation, CO_2 is released due to the absence of the gluten network, which influences the specific volume, oven spring, and other characteristics of bread. This contributes to the gas retention properties and water absorption properties. Hence, the absence of gluten leads to bread with low volume and dense structure (Ayo, 2001). In bread made with rice flour (wherein amylopectin is a major component, which imitates the gluten properties), milled defatted bran, yeast, sugar, and salt, the hardness, and fracturability value are tenfold higher than the whole wheat bread. The values of properties, such as springiness, cohesiveness, and chewiness have been observed to reduce in whole rice bread during the storage period. Rice bread was not found to be acceptable for sandwich preparation by Kadan et al. (2001), as the rice bread is more brittle in nature. In a different study, Sciarini et al. (2010) prepared gluten-free bread with different formulations consisting of a combination of rice, corn, and soy, wherein the loaf volumes of the gluten-free bread were found to be lower than the conventional wheat bread. Furthermore, the addition of soy flour (10%) to the rice bread reduced the crumb hardness of the gluten-free bread, and the addition of cornflour darkened the color of the crust of the rice flour bread; however, the color was less dark than the normal wheat bread. Gluten-free donut have also been made with a combination of regular rice flour, pre-gelatinized rice flour, and reduced vanilla content in the ingredient mix. Xanthan gum and methylcellulose were added for replacing gluten. Methylcellulose had increased the L* and b* values in the crust color of the donut and a* value remains the same as the wheat donut (Melito & Farkas, 2013). In the case of cookies prepared with amaranth- oat composites significant water holding capacity was demonstrated (Inglett et al., 2015).

In pasta, strong gluten is necessary for maintaining products with less sticky properties and enhanced textural properties (Padalino et al., 2016). However, to achieve better sheeting properties, there is a need for weaker gluten and better extensible dough, as gluten matrix plays a vital role in providing desirable properties of the product. In gluten-free pasta, the desirable physicochemical properties are obtained by the application of additives, including hydrocolloids, proteins, and enzymes (Padalino et al., 2016). Similarly, in pizza, appearance, texture, and taste are important physical properties identified by a consumer, wherein the quality of pizza mainly depends on the characteristics of the dough, controlled by the leavening process. Gluten-free pizza is prepared with potato starch, wheat starch, maize starch, cornflour, rice flour, gums, and emulsifiers. Elastic properties of gluten-free dough were observed to increase with the amount of water and hydrocolloid in the flour mix (Onderi, 2013).

Other functional properties often considered during product development from gluten-containing and gluten products include emulsification, foaming, fat absorption, and thickening, which vary extensively with different compositions. Emulsification has been reported to decrease with an increase in soy protein content in the flour blends, which has been further reduced with pea protein content in the ingredient (Tömösközi et al., 2001).

Apart from the blends of different grains, the addition of different specific chemical additives and modifiers also affect the physical properties of the corresponding developed products, which include hydrocolloids, gums, and enzymes. Hydroxypropyl methylcellulose (HPMC) has been reported as one of the best replacements for gluten and it has been observed to enhance the properties of bread, including volume, moisture content, and decrease of the hardness of bread (Hager & Arendt, 2013). However, enzymes such as transglutaminase have been observed to decrease the foaming stability and foaming activity attributed to the increase in molecular activity as observed in the case of rice flour-based gluten-free product development (Marcoa & Rosell, 2008; Marco et al., 2007). Other additives and supplements have been discussed in detail in Sect. 4.6.

4.3.3 Aromatic Properties

The different aromatic components which contribute towards the traditional aromatic properties of the gluten-based products include ethyl acetate, ethyl butanoate, diacetyl, dihydrocoumarin, butyric acid, decanoic acid, benzaldehyde, vanillin, and propylene glycol, which are further modified with different additional additive components (Pozo-Bayón et al., 2006). The pyrazines and 2-acetyl-1-pyrroline are the most significant compound for producing the desirable aromatic properties of bread (Pacyński et al., 2015). 2-acetyl-1-pyrroline and methyl propanal are key odorants in the baguette crust (Cho & Peterson, 2010). In wheat bread crumb (E) – 2-Nominal is responsible for green tallow odor. γ – Nonalactone is responsible for the coconut-like aroma and (E, Z)-2,6- non-adienal leads to cucumber-like aroma in bread. 2-Pentylfuran is formed during baking due to the process of fermentation, Maillard reactions, and lipid oxidation. 2-Pentylfuran is aromatic factor responsible for the floral fruity notes in the wheat bread crumb (Pico et al., 2017). In the rye bread crust, 3-methyl butanal is aroma producing factor.

Further addition of other gluten-free grains, such as oats, as an extra adjunct, provides modified flavor properties. In a study on assessment and improvement of the gluten-free bread aroma, about 33 volatile compounds detected in GC-MS from the gluten-free bread prepared using a bread mix made available by Glutenex (Pacyński et al., 2015). The volatile compounds identified included alcohols, aldehydes, ketones, pyrazines, and furans. However, pyrazines have been reported to be absent in gluten-free bread, which is traditionally found in wheat flour bread. Incorporation of precursors like proline in combination with glucose or fructose in gluten-free bread has been reported to enhance the aromatic properties by producing acetyl pyrazine. Furthermore, the presence of other components such as methional has also been identified in gluten-free bread. In a different study by Annan et al. (2003), during the production of kenkey (staple dish of West Africa and Ghana) from maize dough, about 76 aromatic compounds were identified within the period of the fermentation process. Esters like Ethyl acetate and Ethyl lactate were also produced in higher concentrations during the fermentation period of 2 days.

Hence, it can be observed that significant changes occur in the physiochemical and aromatic properties, during the production of gluten-free products; however, products with desirable properties can be obtained through the controlled formulation of food ingredients and additives.

4.4 Microbial Strains Useful for Gluten-Free Products

4.4.1 Traditional Microorganisms (Benefits and Characteristics)

Gluten-containing fermented food items are some of the oldest developed and consumed grain-based food commodities; hence, a wide array of microorganisms is used for the synthesis of these. *Saccharomyces cerevisiae* is the most commonly used microorganism for the fermentation of bread dough and has also been employed in the production of wine. Lactic acid bacteria (LAB) has also been used for fermenting wheat and rye flour dough and traditional LAB strains reduce toxic and anti-nutritive factors in cereals (Holzapfel et al., 2006). The corresponding strains have been observed to remove stachyose, raffinose, and verbascose from soy-based products, which are the main cause of flatulence and intestinal cramps in case of higher oligosaccharides content (Dworkin et al., 2006; Holzapfel et al., 2006). *S.boulderi* and *pombe* are also some of the yeast used in the fermentation of traditional beverages, including white grape wine, cashew wine, red grape wine, banana beer, date wine, tepache, jackfruit wine, palm wine, and colonche (Battcock, 1998). The other fermenting bacterial species for producing several locally available exotic products from gluten-based cereals are *Leuconostoc*, *Pediococcus* and *Lactobacillus*

(Blandino et al., 2003), which have been often employed for the production of boza, dhokla, hamanatto, kecap, shoyu, and kenkey.

Fermentation also increases the bioavailability of mineral constituents and contributes towards the development of better organoleptic and aromatic properties. Application of the above-mentioned organisms has been mainly associated with the aromatic properties; however, other physiochemical properties are also affected by the fermentation methods and the chosen microorganism. Gamel et al. (2015) fermented with three different dough-making processes leading to the straight, sponge, and sourdough. The bread prepared from straight and sponge dough had higher molecular weight and decreased β glucan content compared to sourdough, which was attributed to the fermentation of dough with microorganisms such as yeast and lactic acid bacteria in sourdough bread leading to lower pH (4.2–4.6), wherein high acidity in dough maintained the molecular weight of β-glucan and decreased the activity of degrading enzymes of β-glucan (Bhatty, 1992). However, an increase in fermentation time decreased the molecular weight of β-glucan and viscosity in the bread, as increased fermentation time leads to greater contact time with degrading enzymes. Due to enzyme degradation of amylose and amylopectin during fermentation of wheat starch, a reduction in molecular weight of wheat and amylose has also been observed (Nowak et al., 2014).

Fermentation has also been associated with an increase in the content of bioactive compounds such as phenolics, as often observed in the cases of alcoholic beverages. Fermentation with *Cornus Officinalis* has been observed to increase phenolic compounds from 441.06 to 496.00 mg/L in wines. An increase in the amount of gallic acid observed during the fermentation process has been correlated with metabolites produced by yeast (Zhang et al., 2013).

4.4.2 Microorganisms Utilized in Gluten-Free Products

Lactobacillus plantarum has been used for producing gluten-free bread, consisting of buckwheat flour, cornstarch, soy flour, and xanthan gum through the method of sourdough fermentation. *L. Plantarum*also produced antifungal compounds that enabled shelf life extension of the bread (Moore et al., 2008). It produces acetic acid from pentose sugar and lactic acid from hexoses. In sorghum flour pentose sugar content is very low as compared to hexoses; hence, lactic acid production is higher during the sorghum flour fermentation (Schober et al., 2007). Instant dry yeast has also been used for the preparation of gluten-free bread from rice and maize flour. *Aspergillus oryzae* and yeast have been used to produce gluten-free bread from the rice flour by Hamada et al. (2013), where the batter was fermented at 38 °C for 60 min.

In a different study, *Lactobacillus reuteri* and *Weissella cibaria*were used for the production of fermented sorghum and quinoa flours for further food applications. The fermentation process produced fructo-oligosaccharides and gluco-oligosaccharides. *Lactobacillus buchneri* released hetero polysaccharides, which significantly controlled the rheology properties of sorghum sourdough, according to a study by Galle et al. (2011). In a separate study, *Leuconostoc argentinum*, *Pediococcus pentosaceus,* and *Weisella cibaria* were the species identified for the fermentation of oat sourdough for preparing oat bread, wherein *Lactobacillus coryniformis* was also identified and shown to cause a higher reaction rate during the fermentation process at 37 °C (Hüttner et al., 2010). In the case of sorghum fermented for the towga production, which is a traditional Tanzanian lactic acid fermented gruel, lactic acid bacteria are the predominant microorganism, which also leads to the production of various flavor compounds (Zannini et al., 2012). Select lactic acid bacteria can also be used for enriching the gluten-free products with γ-Aminobutyric acid, which is a major non-protein amino-acid acting as an "inhibitory neurotransmitter of the central nervous system" (Coda et al., 2010).In a separate study by Di Cagno et al. (2008), *Lactobacillus plantarum* and *Lactobacillus sanfranciscensis* LS40 and LS41were used for the fermentation process of gluten-free flour, where the gluten content decreased from 400 ppm to 20 ppm.

Hence, though lactic acid bacteria have been extensively exploited for the development of gluten-free products, several other bacterial strains have also been identified and employed for gluten-free product development.

4.5 Processing Methods and Apparatus Employed in Case of Gluten-Free Product Development

Sourdough technology is a traditional method used for the production of wheat and rye breads, wherein overall the process consisted of mixing flour with water followed by fermentation using lactic acid bacteria and yeast, causing the release of lactic and acetic acid and producing sour-tasting end product (Chavan & Chavan, 2011). Lactic acid bacteria are applied in desired proportions for enhancing volume, texture, nutritional value, and flavor along with the enhancement of the shelf life of the bread. Although the standard method of sourdough production is a simple method, researchers have modified and optimized the process parameters for achieving different targets in the case of the production of gluten-free products. During a study by Moore et al. (2008) on the development of sourdough preparation method from gluten-free flour mixture, cultures of *Lacto bacillus* were inoculated at 1% concentration levels in 40 ml of broth, which was incubated for 1 day at 30 °C and was centrifuged (4000 rpm for 5 min) to prepare concentrated harvested culture. This extracted strain was again dispersed in 1 ml of broth and mixed with water followed by mixing with flour, after which, the well-mixed batter was incubated at 30 °C for about 24 h.

Most of the processing methods and apparatus used in gluten-free product development are similar, the part that varies most is replacing the raw material, and inclusion of new additives for the development of the product that is equivalent to the gluten-based product. Extrusion processing has been used for enriching gluten-free products with high-level dietary fiber. Processing conditions of the extruder considered by Stojceska et al. (2010) included feed rate of 15–25 kg/h, barrel temperatures at hopper side as 80 °C and 80–150 °C at die point along with screw speed between 200 and 350 rpm. During this study, the product from the die output was cut using a knife attached at the end of the die, wherein the product was later cooled and left at room temperature, and subsequently packed. The process was similar to the processing of pasta, spaghetti, and other similar products obtained through the extrusion process, with enhanced nutritional value through the addition of different sources of raw materials (Stojceska et al., 2010; Zhang et al., 2011). Apparatus used for this process was a co-rotating twin-screw extruder with a barrel diameter of 37 mm and an L/D ratio of 27:1. The diameter of the die was 4 mm and a volumetric feeder was used for feeding the dry mixture (Stojceska et al., 2010).

Germination and enzymatic treatment of the respective grains and their corresponding extracts have also been employed for the enhancement of nutritional value and decrease of gluten concentrations, which are also useful processes for producing gluten-free products. In a different study by Knorr et al. (2016), barley was germinated using a proofing cabinet during the batch process, after which the steeping process was carried out for producing gluten-free beer through peptidase treatment. After germination, malt was extracted from sprout, which was followed by the milling process. Milled malt was extracted with water using a magnetic stirring process, which was subsequently filtered and the filtered extracts were concentrated using a rotary evaporator set at 50 °C and 65 mbar. The final product was obtained at the concentration of 40° brix, wherein, the final enzyme active malt extract was used for the production of beer. Beer was also produced using barley malt and hop pellets by fermenting for 7 days using yeast at 10 °C, where secondary fermentation processes were carried for 4 weeks at 4 °C after the addition of wort, which was incubated with enzyme active malt extract (10% (v/v)) at 50 °C for 1 day (Knorr et al., 2016). Enzyme treatment in the production of gluten-free beer is essential. Enzyme hydrolyses the peptide linkages (peptide sequences are responsible for producing the celiac disease) that occur during downstream processing of proline residue. Beers have also been produced from gluten-free cereals such as sorghum, corn, millet, and rice (Hager et al., 2014).

4.6 Additives and Modifiers Used for Gluten-Free Products

4.6.1 Different Additives and Modifiers

Different kinds of additives, such as emulsifiers, enzymes, and hydrocolloids have been used for the development of gluten-free products (including breads), which are also useful for enhancing the quality of the final product. Hydrocolloids have been increasingly used in bread making process. These compounds act as additives to slow down the retrogradation of starch and are also used for the replacement off at, for the enhancement of viscoelastic properties and texture. These components bind water molecules and contribute towards the maintenance of the quality of the product during storage. The quality of bread produced from rye flour was enhanced through the addition of guar gum and carboxymethyl cellulose (CMC) by Ghodke Shalini and Laxmi (2007). Other components extensively employed in product development include the emulsifiers such as diacetyl tartaric acid ester of monoglyceride and sodium stearoyl 2-lactylate, enzymes such as glucose oxidase and α – amylase, and other hydrocolloids like xanthan gum, carboxymethyl cellulose, carrageenan and alginate (Sciarini et al., 2012).

Microorganisms are mainly added for fermentation during product development; however, in many cases, they are also added for fortification. LAB has demonstrated antifungal activity and these microorganisms have been reported to prevent the staling of bread; hence, application of these organisms can be proposed as a better alternative for chemical preservatives (Lowe & Arendt, 2004). Specifically targeted extracts can also be prepared, which demonstrate beneficial properties. The Amaranthus seeds extract contain peptides that have been reported to demonstrate antifungal activity, wherein it was observed that the substrate significantly retarded the growth of *Penicillium roqueforti* for 21 days of storage (Giuseppe Rizzello et al., 2009); hence, the addition of these extracts in the composition of gluten-free product formulation can also be further extensively exploited.

4.6.2 Modification of the Chemical and Biological Properties of the Gluten-Free Products Attributed to the Additives

Chemical and biological additives modify the physio-chemical properties of the raw, intermediary and final products, affect the storage life as well consumer acceptability of the final products. Furthermore, the extent of the effect is also affected by the combination of the effects of individual components and effects of other processing and surrounding factors, where the final product properties are the resultant of the amalgamation of all the different factors.

Chemical properties of the products and their overall makeup, significantly affected by the additives, include the chemical and physical structures/ transformations, intermediate conversions, bonding and stability of the components. HPMC and starch hydrolyzing enzymes have been observed to reduce amylopectin retrogradation (Bárcenas & Rosell, 2007) in the corresponding compositions such as rice-based breads (Gujral et al., 2003a, b).Transglutaminase, which is another helpful additive, has the capability of linking proteins originated from different sources. Transglutaminase has been used in gluten free bread by Moore et al. (2006), which consisted of soy, egg and skim milk powder, wherein transglutaminase helps in forming stable protein network without the gluten compound. The protein network helps in developing the good quality gluten free bread with desirable loaf volume, crumb characteristics and texture.

Stability at different stages of processing and storage are also significantly affected by the components and their proportions. Xanthan gum and pectin have been reported to improve cooking stability (Kohajdová et al., 2009),whereas, calcium propionate used for preventing fungal growth at time of bread storage (Tosh et al., 2012). Inulin is a prebiotic compound digested by colon bacteria, which at 5% concentration with oligosaccharide syrup and chicory flour has been observed to decrease the staling rate for 3 days, as reported by Korus et al. (2006). It also retarded rate of crumb hardening and subsequently improved the loaf volume. Enzymes like α – amylase and cyclodextrin glycosyl transferase extracted from *Bacillus* species reduced staling rate of rice bread (Gujral et al., 2003a, b). Similarly, the components produced by the microorganisms during the process of fermentation not only affect the physiochemical and sensory properties relevant for the product quality in terms of consumer acceptance, but also the storage quality of the developed product through the production of disparate biochemicals. Bacteriocin produced by lactic acid bacteria has been observed to delay the growth of *Aspergillus flavus* and *Aspergillus niger* in the gluten free cereal product called *agidi* (Dike & Sanni, 2010).

The different additives also contribute towards further enhancing the health beneficial effects of these gluten-free products. Inulin has been observed to enhance the dietary fiber content in the gluten free foods, which subsequently enhances the digestive health of the consumer (Korus et al., 2006). Also, in other cases, essential health beneficial components are enriched through fortification of the additives. Folate content is very low in gluten free product compared to gluten containing product and it is essential to maintain the concentrations of folate and other vitamins in the diet for maintaining good health. Hence, for the development of gluten free products, pseudo-cereals like quinoa and amaranth species can be included, where these components can provide ten fold higher concentrations of folate as compared to other cereals such as spring wheat (Schoenlechner et al., 2010). There is need of vitamin and mineral source to be added in gluten free product, as it provides less concentrations of these nutrients as compared to conventional diet.

4.6.3 Modification of Physical Properties of the Gluten-Free Products Attributed to the Additives

Physical properties of the developed gluten-free products are significantly affected by the absence of gluten, as discussed in the previous sections. However, consumer acceptability of these products can be significantly improved through the addition of modifiers improving the physical properties of these products.

Stability of dough is an essential qualitative entity for the indication of flour strength. Incorporation of hydrocolloids like carrageenan, xanthan gum and hydroxy-propyl methylcellulose (HPMC) has been observed to improve the stability of wheat dough at the time of proofing (Sahraiyan et al., 2013). Hence, firmness of breadcrumb has been observed to reduce through the addition of κ-carrageenan or HPMC. Addition of HPMC has also led to the reduced hardening of the bread, enhanced specific volume index and width/height ratio. In a different study, addition of 2% xanthan gum reduced porosity of the bread that provided good textural quality of bread (Lazaridou et al., 2007). Crumb elasticity of the gluten free bread improved by adjunct of CMC, pectin and xanthan gum that improved textural properties (Arendt et al., 2008). Furthermore, Schober et al. (2005) observed increased loaf volume and crumb hardness for the bread produced from sorghum with addition of xanthan gum, wherein increase in crumb hardness was as an undesirable quality of the bread, which demonstrated the occurrence of negative effects of factors as well in certain cases. Addition of hydrocolloid in bread with 65% water also led to inferior properties such as dense crumb structure, low specific volume and high firmness, which could be recovered by the addition of higher water content (Sciarini et al., 2012). Hence, though there are negative effects of absence of gluten and certain additives, through optimized combination of different components, the desirable properties of the commodities can be retrieved and/or achieved.

4.6.4 Modification and Enhancement of Aromatic Properties

Modification of aroma and flavor greatly depends on the raw material, type of starter cultures, fermentation and baking conditions. Change in raw material from wheat to other similar ingredients leads to lack of aromatic compounds such as pyrazines and 2-acetyl-1-pyrroline, which were originally present in gluten based products (Zehentbauer & Grosch, 1998). These limitations of aroma can be overcome by manual addition of different kinds of amino acids and sugar pairs to the raw materials before processing. In order to produce aroma, pairs of precursor compounds for targeted aroma were added by Pacyński et al. (2015), including proline/glucose, leucine/glucose, proline/fructose, ornithine/fructose and cysteine/rhamnose. Also, incorporation of high level of sodium chloride (1.5–3%) has been associated with decrease in the amount of 2-phenylethanol, a compound causing smell of yeast (Raffo et al., 2018). Addition of different grains and their corresponding compositional proportions can also lead to different aromatic profiles of the products. In a study by Wolter et al. (2014), where buckwheat, quinoa, sorghum, teff and wheat breads, quinoa and sorghum flour (and teff crumb) were observed to produce "cooked potato and pea" like and "cooked tomato and pea" like odors, respectively. Overall, the different additives and their corresponding concentrations used for producing aroma is based on the consumer likeability and preference.

Microorganisms employed during the different unit-operations of the product development have also been associated with development of various aromatic compounds. Increase in amount of yeast during the preparation of the product has been observed to enhance the production of 2-acetyl-1-pyrroline and methional. These compounds have been identified as the main factors responsible for causing roast like aroma (Zehentbauer & Grosch, 1998). In a different study by Wolter et al. (2014), *Weissella cibaria*has been observed to produce the 'popcorn –like roasty' aroma in gluten-free breads . Quinoa flour was observed to produce 'cooked potato and pea' like and 'cooked tomato and pea like odour produced for sorghum and teff crumb (Wolter et al., 2014).

4.7 Future Perspectives

4.7.1 Combined Benefits of Fermentation and Gluten-Free Compositions

Gluten free diet has less fiber compared to the normal diet. Hence, food technologists and dieticians have been increasingly recommending the consumption of gluten free baked products with the addition of dietary fibers. The ancient grains like amaranth seeds have higher beneficial effects that satisfy the requirement for wheat replacement (Fornal, 2000); however, there is a need for the optimization of the fermentation process for newer as well as non-traditional raw materials. Established methods such as fermentation and extrusion are being further optimized to obtain high quality gluten-free products. Fermentation increases digestibility and absorption of nutrients, which is also one of the low-cost preservation methods (Beyene and Seifu). The process also detoxifies the products and provides better textural properties. Gluten free composition in fermented products provides the combined effect of fermentation benefit and gluten free raw materials used in the product (Chojnacka, 2010).

Subsequently, these benefits are further increased through the addition of other novel and non-traditional components; studies on these topics have also increased recently. The incorporation of bee pollen in gluten free bread has been observed to increase total carotenoids, proteins, minerals, soluble and bio-accessible polyphenols and antiradical activity at all levels (2–5%) (Conte et al., 2020). Most of these bioactive components have added benefits, which include both primary and secondary metabolic functions. Carotenoids are the precursor of vitamin A and provide protection against most of eye-related chronic diseases (Rodriguez-Concepcion et al., 2018; Johnson, 2002). Dietary polyphenols are essential for the maintenance of gut health and balance of gut microbiota. It enhances the growth of beneficial bacteria and prohibits pathogenic bacteria (Cardona et al., 2013). They also provide other beneficial effects including antioxidant activity and effectiveness against several other chronic disorders such as diabetes, different cancers, neurodegenerative and ocular disorders (Orsat & Routray, 2017).

4.7.2 *Possible Future Market Scenario*

Gluten free diets can be used for the treatment of medical conditions such as bowel syndrome, arthritis, dermatitis, diabetes mellitus and other neurological disorders (Wahnschaffe et al., 2007; Badsha, 2018; Sanchez-Albisua et al., 2005). Many consumers choose gluten free diet for their healthier life style. Gluten free products have been recognized as high quality products. An increase in number of consumers for gluten-free diets has led to novel products and corresponding process development to achieve the various qualities of the products based on consumer demand and acceptability. Hence, it can be deduced that gluten-free diets have to be designed and prepared, according to the different medical conditions observed in people along with consumer demand. For example, for diabetic consumer, gluten-free diet with a low glycemic index should be developed. In accordance with the medical conditions, the ingredients and the processing techniques employed in the manufacturing process will be different for obtaining the final desired product. It is expected that in the future, gluten free cereal-based probiotic products will achieve huge growth in market and these products will target consumers with either or both the gluten and lactose intolerance.

4.8 Conclusions

Attributed to the higher growth rate in industrialization and urbanization, there is a large demand for processed foods. Food consciousness in consumers has increased which has also led to higher consumption of gluten free food products. Gluten concentration influences a wide range of properties subsequently affecting the overall final product quality. There is a need for the supplementation of multiple ingredients to replace the single wheat flour and to avoid the gluten introduction in the product manufacturing process. Hydrocolloids are important additives required for the production of good quality gluten-free products. Both, starch-based and protein-based ingredient are necessary for the formulation of gluten-free products; hence, the addition of the old grains and a wide array of other raw materials for the replacement of wheat flour is essential, wherein the old grains and other dietary additives will have higher nutritional value and satisfy the dietary needs. Most of the lactic acid bacteria generate bacteriocins that can be used for the replacement of chemical preservatives. Also, new microorganism strains have to be produced with help of genetic engineering for achieving further innovation in this sector and further optimization of the parameters of the fermentation process is also necessary to achieve high-quality gluten-free food products. Further research is also required to analyze the consequences of consuming a gluten-free diet containing additives such as xantham gum, HPMC, and other similar components that help in providing good quality gluten-free diet.

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