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Gluten-free Bread Technology

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Gluten Intolerance

Anesa Majeed

1 Gluten

Gluten is defined for legislative purposes as "a protein fraction from wheat, rye, barley, oats or their crossbred varieties and derivatives thereof, to which some persons are intolerant and that is insoluble in water and 0.5M NaCl" CODEX STAN 118-1979 (Codex Alimentarius, 2008). The proteins that form gluten are major storage proteins of wheat and represent between 70% and 80% of the total protein content of the grain (Rzychon et al., 2017).

Gluten can be readily prepared by gently washing the dough under a stream of running water. This removes the bulk of the soluble starch and particulate matter to leave a proteinaceous mass that retains its visco-elasticity upon stretching. These proteins are reffered to as prolamins based on their higher proportion of proline and glutamine aminoacids, comprising of 15% and 35% of the total amino acid composition (Shewry & Tatham, 1990). Wheat gluten comprises of two fractions which are distinguished based on their solubility in aqueous alcohol solutions as the alcohol-soluble monomeric gliadin proteins and the alcohol-insoluble polymeric glutenins. As gliadin and glutenin subunits are closely related in their amino acid sequences, glutenin can also be soluble in aqueous alcohols after reduction of interchain disulphide bonds. It is therefore acceptable to classify the gliadins, glutenins and the related proteins in other cereals as prolamins. Gluten comprises the major storage proteins of wheat and related cereals such as rye and barley; these proteins are defined as prolamins based on their high contents of the amino acids proline and glutamine which respectively comprise 15% and 35% of the total amino acid composition. During bread-making, these proteins form a three-dimensional network capable of retaining carbon dioxide. This imparts viscoelasticity to dough mass, improves its structure and the honeycomb texture to breads (Gujral & Rosell, 2004).

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Gluten is found in many staple foods in the Western diet, and due to its unique functional properties, it is widely used in the food for preparation of high-quality baked products.

In spite of such important functionality of gluten, its use has been limited in food industry from recent years. This is because, the ingestion of gluten has been associated with various celiac and immune related disorders, which include:

(i) Celiac disease or gluten intolerance, which is a permanent autoimmune disease affecting about 1% of the world's population (Lamacchia et al., 2014);
(ii) Wheat allergy with a prevalence of about 0.5% of the world population (Pamela & Alessio, 2009);
(iii) Non-celiac gluten sensitivity (NCGS) excluding celiac disease and wheat allergy recently discovered with estimated prevalence of 6% of the US population (Rosell et al., 2014);
(iv) Gluten-induced enteropathy with the symptoms of diarrhoea, malnutrition or a malabsorption syndrome (indicated by weight loss, steatorrhoea and oedema secondary to hypoalbuminemia);
(v) Gluten ataxia, and
(vi) Dermatitis herpetiformis

The spectrum of clinical manifestations includes gastrointestinal symptoms such as abdominal pain, nausea and diarrhoea, inflammatory disease of the small intestine which can result in nutrient malabsorption and/or allergic reactions including anaphylaxis. The thresholds for Wheat allergy (WA) and Gluten sensitivity (GS) have not been established yet. However, in the case of CD patients, damage to the intestinal mucosa can be induced by even the trace concentrations of gluten (Sapone et al., 2012). For patients suffering from gluten-related disorders, the only available treatment is the life-long elimination of gluten from their diet. The prevalence of CD is approximately 1% in the regions populated by individuals of European origin. However, due to the increase in popularity of a western style diet, rich in gluten, the diagnosis of CD is increasing globally (Bai & Ciacci, 2017). WA affects roughly 1% of the world's population, but, the prevalence of GS is unknown, with the rough estimates ranging from 0.6% to 6% of the population (Czaja-Bulsa, 2014).

1.1 Celiac Disease

Gluten sensitive enteropathy commonly called as celiac disease, is an autoimmune inflammatory disease of the small intestine. It is triggered by the ingestion of gluten in genetically susceptible and clinically diagnosed persons. The genetic predisposition to celiac disease is linked to the presence of Human Leucocyte Antigen (HLA) DQ2 and DQ8 molecules. These HLA-DQ genes account for approximately 40% of the genetic risk of celiac diseases. However, merely the presence of HLA risk alleles is, not sufficient for its onset and it needs to be accompanied by other genetic and environmental factors which include ingestion of wheat, rye, and/or barley derived gluten (Nylund et al., 2016).

A defective enzyme system responsible for splitting gluten and the atrophy of jejunal mucosa may also be among the specific causes of celiac disease. It usually develops within the first 3 years of life. In 2011, a panel of 15 experts announced a new classification of gluten-related disorders (Fig. 1) and expressed the opinion that the term "gluten-related disorders" is the umbrella term to be used for describing all the conditions related to ingestion of gluten-containing food. The classification covers a wide range of disorders including allergies (food allergy, anaphylaxis, wheat-dependent exercise induced anaphylaxis, baker's asthma and contact dermatitis), autoimmune diseases (celiac disease, dermatitis herpetiformis, gluten ataxia) and the diseases that are likely to be immune mediated (gluten sensitivity). At the Second Expert Meeting on GS that was held in Munich in 2012, it was discussed that immunoglobin E (IgE) and non-IgE mediated WA have similar symptoms as that of NCGS making these difficult to be distinguished from the latter (Catassi et al., 2013). Gluten-related disorders are manifested not only by disturbances in the gastrointestinal tract, but also by the dermatological, haematological, endocrinological, rheumatological, gynaecological, dental and neurological symptoms. It has been found that after the administration of gluten free diet (GFD) in patients the symptoms gradually disappear. On contrary, when the diet containing gluten is reintroduced, all the symptoms recur (Sapone et al., 2012). For making right diagnostic decisions, it is important to carefully define the symptoms and choose such

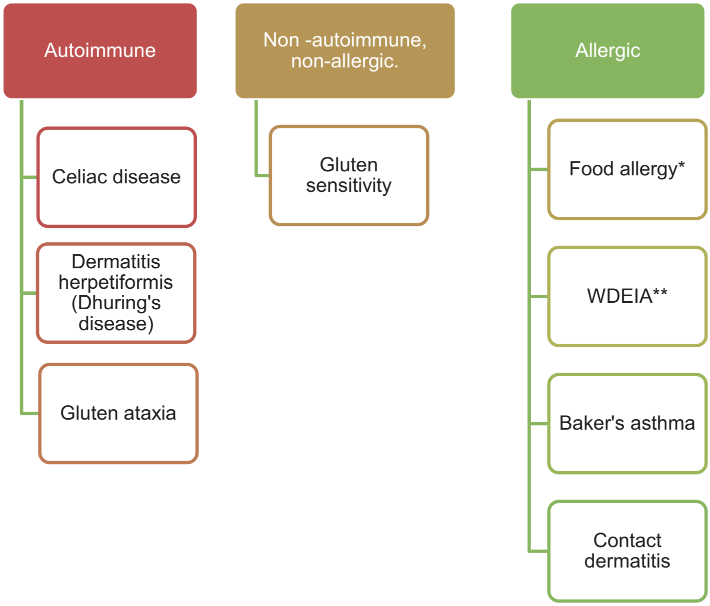

Fig. 1 Classification of Gluten related disorders (Czaja-Bulsa, 2014). * IgE-mediated and non IgE-mediated, ** wheat dependent exercise induced anaphylaxis

serological tests and histological imaging of duodenal mucosa that makes possible to distinguish between different gluten-dependent disorders with their varying courses, diet protocols, prognoses and complications (Czaja-Bulsa, 2014).

Children with celiac disease fail to thrive, loose appetite and have a pot belly. Stools are large, pale and with offensive odour (stetorrhoea) due to the presence of fat in the form of fatty acids. Child is usually anaemic with the symptoms of paleness, fatigue and tachycardia (fast pulse). The microscopic cross section of small intestine shows flattening of the villi. However, as the gluten free diet is given to children with such clinical symptoms, a substantial recovery is soon observed. Celiac diseases are also associated with numerous neurological disorders, including epilepsy, cerebral calcifications, and peripheral neuropathy.

The other symptoms that indicate the consumption of gluten containing diet by sensitive patients include:

- Digestive disorders such as diarrhoea, abdominal pain, bloating, passing pale and foul-smelling stool and flatulence.
- Behavioural changes such as depression in adults and irritability in children.
- Nutritional deficiency disorder such as weight loss, delayed growth, failure to thrive in infants, missed menstural periods, anaemia and fatigue. Anaemia is the most common laboratory manifestation of celiac diseases. This is because of the malabsorption of iron in the proximal small intestine, where celiac manifestations are most prominent. Such manifestations often lead to deficiency of iron, cynacobalamin (vitamin B12) and/or folate.
- Impaired bone health, joint pain, seizures and muscle cramps.
- Some non-specific disorders such as, tingling sensation, numbness in legs due to nerve damage, painful skin rash, tooth discolouration and enamel loss.

- Patients suffering from severe form of celiac diseases for prolong period are at risk for several complications due to impaired nutrient absorption often leading to malnutrition. Until recently gluten intolerance has been believed to be a typical type of celiac disease (CD) and wheat allergy (WA). In the recent years, however, several studies reported the manifestation of gluten intolerance with the symptoms other than those seen in above mentioned disorders. The syndrome is termed Non-celiac gluten sensitivity (NCGS) or simply gluten sensitivity (GS), and has been recently included in the list of gluten-related disorders (Czaja-Bulsa, 2014).

1.2 Wheat Allergy

Wheat allergy (WA) represents another type of adverse immunologic reaction to proteins contained in wheat and related grains, with different clinical presentations which depends upon the route of exposure. In this setting, IgE antibodies mediate the inflammatory response to several allergenic proteins like alpha-amylase/trypsin inhibitor, non-specific lipid transfer protein (nsLTP), gliadins and/or glutenins (Elli et al., 2015). Depending upon the route of allergen exposure, WA is classified into occupational asthma (baker's asthma) and rhinitis. Food allergy (FA) affects the skin, the gastrointestinal tract, the respiratory tract, wheat dependent exercise-induced anaphylaxis (WDEIA) and contact urticaria. Ingested wheat can cause IgE-mediated wheat allergies in both children and adults. Although the sensitization to wheat assessed by serum IgE is more prevalent in adults, WA shows greater prevalence in children (Vierk et al., 2007; Matricardi et al., 2008). Immediate wheat allergy is mainly seen in children of school-age, in a way similar to egg or milk allergy. The majority of wheat allergic children suffer from moderate-to-severe atopic dermatitis and it may elicit typical IgE mediated reactions, including urticaria, angioedema, bronchial obstruction, nausea and abdominal pain, or in severe cases systemic anaphylaxis (Ramesh, 2008). In adults, food allergy to ingested wheat is infrequent and the most common variant is WDEIA, where symptoms result from the combination of causative food intake, physical exercise as well as the consumption of non-steroidal anti-inflammatory drugs or alcohol. On contrary the gastrointestinal symptoms could be mild and difficult to recognize, among which the most common are diarrhea and bloating.

1.3 Non-celiac Gluten Sensitivity (NCGS)

"Non-celiac gluten sensitivity" is a proposed term for the condition in which gastro-intestinal and extra-intestinal symptoms are triggered by gluten consumption. Typical gastrointestinal symptoms include abdominal pain, bloating and altered bowel habit while the most oftenly reported extra-intestinal symptoms include fatigue, headache, joint or bone pain, mood disorders and skin manifestations (Nylund et al., 2016). Non-celiac gluten sensitivity (NCGS) is a newly classified syndrome of gluten intolerance. The first consensus definition of CD was published in Acta Paediatrica in 1970. This publication defined CD as a permanent condition of gluten intolerance with mucosal flattening that reversed upon the consumption of gluten-free diet (GFD) and then relapsed on re-introduction of gluten. However, the scientific community has come to recognise that there is a spectrum of disorders related to gluten ingestion. In the First Expert Meeting in 2011, of a multidisciplinary task force of 16 physicians from seven countries with particular expertise in diagnosis and treatment of CD, its name was proposed as "Gluten Sensitivity" (GS) (Sapone et al., 2010). Later, a group of 16 experts who announced a new definition (the Oslo Definition) of celiac diseases suggested that instead of GS the disorder should be named NCGS, which made it distinguishable from CD (Ludvigsson et al., 2013). The first reports about this disease dates back to 30 years and since then numerous reports have appeared primarily about the adults. It was observed that among a group of patients whose symptoms disappeared with gluten withdrawal from the diet that they were neither affected by celiac disease (CD) nor by wheat allergy (WA) (Cooper et al., 1981). The first case report of NCGS in children was described in 2012 (Mastrototaro et al., 2012). NCGS can be diagnosed in patients with gluten intolerance who do not develop antibodies that are typical of CD or WA and do not suffer from lesions in the duodenal mucosa. The overall prevalence of NCGS in the general population is still unknown, mainly because many patients are self-diagnosed and start a gluten-free diet (GFD) without medical advice or consultation (Catassi et al., 2013). The disorder is more common in females and in young and middle aged adults (Catassi et al., 2013). Some researchers hypothesize that the incidence of NCGS can be higher than CD and WA, with estimated numbers reaching upto 6% of the world population (Di Sabatino & Corazza, 2012).

1.3.1 Clinical Manifestation

NCGS is characterized by symptoms that usually occur after gluten ingestion, disappear with gluten withdrawal from diet and relapse following gluten intake (Sapone et al., 2010; Biesiekierski et al., 2013). Patients suffering from NCGS are a heterogenous group composed of several sub-groups, each characterized by a different pathogenesis and clinical course. The typical presentation of NCGS is a combination of irritable bowel syndrome (IBS)-like symptoms, and systemic manifestations such as headache, joint and muscle pain, muscle contractions, leg or arm numbness, chronic fatigue, foggy mind, weight loss and anaemia. These may also they can include behavioural disturbances such as reduced attention and depression (Biesiekierski et al., 2013). IBS-like symptoms, on the other hand include, abdominal pain, nausea, bloating, flatulence, diarrhea or constipation. In children, NCGS manifests with intestinal symptoms like abdominal pain and chronic diarrhea, while the extra-intestinal manifestations seem to be less frequent. However occasionally, the most commonly appearing extra-intestinal symptom is tiredness (Mastrototaro et al., 2012). NCGS is more often than not diagnosed in patients with IBS, especially in those with diarrhea. In this case, it is referred to as gluten-sensitive irritable bowel syndrome (Armstrong et al., 2011). NCGS is also frequently observed in the subjects with allergic disorders. Many researchers have also proved an increased frequency (13%) of the CD in the offsprings of the patients already suffering from this disorder (Volta et al., 2012).

1.3.2 Symptoms of Non-celiac Gluten Sensitivity Disorder (NCGS)

- Intestinal Disturbances like abdominal pain, diarrhea, nausea, body mass loss, bloating and flatulence.
- Cutaneous disorders like erythema and eczema.
- General headache.
- Bone and joint pain, Muscle contractions and numbness of hands and feet.
- Chronic tiredness.
- Haematological problem like Anaemia.
- Behavioural disturbance which include inattention, depression, hyperactivity and ataxia, and

- Dental issues like Chronic ulcerative stomatitis.

1.4 Irritable Bowel Syndrome (IBS)

IBS is a group of problems having similar symptoms as that of CD, which include abdominal cramping, diarrhea, steatorrhoea, malnutrition, nausea and anorexia. It is, therefore, recommended to perform serological screening for CD in patients with IBS. This is particularly important for the patients with diarrhea as one of the symptoms as in their case, the incidence of CD is four times higher (Brandt et al., 2009; Spiegel et al., 2004). This also is a well-known fact that persistent minor inflammation of duodenal mucosa can lead to IBS. Such lesions are observed in patients with both WA and NCGS, leading to increased prevalence of IBS. Arranz and Ferguson (1993) confirmed that some IBS patients could develop NCGS and gluten-sensitive diarrhea, without a CD specific enteropathy. The disorder was then called as gluten-sensitive irritable bowel syndrome. In a large study performed by Carroccio et al. (2012) 30% subjects with IBS-like symptoms were found suffering from wheat sensitivity, whereby GFD improved the symptoms of IBS (Vazquez-Roque et al., 2013). The patients suffering from IBS who respond well to a GFD can suffer from one of the three diseases, that is, CD, WA or NCGS (Fig. 2). The presence of IgE for wheat, a positive food challenge and minimal histological lesions confirm WA, while the absence of markers typical of CD and WA and minor histological lesions accompanied by a good response to a GFD indicate NCGS (Verdu et al., 2009). The presence of AGA antibodies is an additional marker for NCGS in such patients.

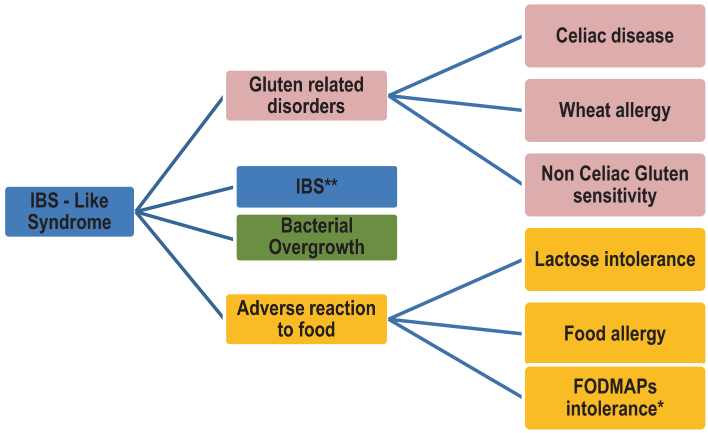

Fig. 2 Clinical presentation of irritable bowel syndrome (IBS) (Czaja-Bulsa, 2014). *FODMAP fermentable oligosaccharides, disaccharides, monosaccharides and polyols, **IBS irritable bowel syndrome

1.5 Gluten Ataxia

Gluten ataxia can be defined as idiopathic sporadic ataxia and positive serum antigliadin antibodies even in the absence of duodenal enteropathy. Gluten ataxia is one of a number of neurological manifestations attributed to CD. Defining criteria for gluten ataxia include idiopathic sporadic ataxia in association with positive AGA (IgG or IgA, or both), with or without enteropathy on duodenal biopsy (Ludvigsson et al., 2013).

1.6 Dermatitis Herpetiformis

Dermatitis herpetiformis (DH) is a cutaneous manifestation of small intestinal immune mediated enteropathy precipitated by exposure to dietary gluten. It is characterised by herpetiform clusters of pruritic urticated papules and vesicles on the skin, especially on the elbows, buttocks and knees, and IgA deposits in the dermal papillae. DH responds to a GFD and strict adherence to it shows patients can stop drug treatment completely (Ludvigsson et al., 2013).

2 Diagnosis of Celiac Disease

It is recommended to assess serology and duodenal histology while the patient is still on a gluten-containing diet. Patients with suspected but unproven CD who are already on a GFD at the time of referral may not show histologic changes or antibodies consistent with CD due to improvement of diagnostic symptoms caused by the GFD itself. In order to diagnose CD accurately, such individuals should be tested for the presence of HLA DQ2/DQ8 genotype. In case of positive test for such genotype, gluten should be reintroduced under medical supervision via the so called "gluten challenge" before planning any serologic testing and duodenal biopsies (Elli et al., 2015) (Fig. 3). Nevertheless, in order to achieve a correct diagnosis, the required duration of these tests and recommended amount of gluten per day for such patients are yet to be researched. For a long time, the guidelines have recommended to prescribe 10 grams of gluten per day for a duration of 6–8 week. However, some recent studies have shown that lower doses of gluten over shorter periods (3 g per day for 2 week) determine diagnostic changes in histology and/or serology in up to 90% subjects. The new proposed low-dose 14 day long gluten challenge has shown higher compliance. NCGS is mainly a "diagnosis of exclusion" made after other wheat-related and non-wheat-related disorders have been ruled out. In fact, NCGS has often been described as an IBS-like disorder, in view of apparent functional nature of both syndromes and the evident overlap of symptoms (Verdu et al., 2009). Moreover, it has been observed that both patients with self-reported NCGS and IBS

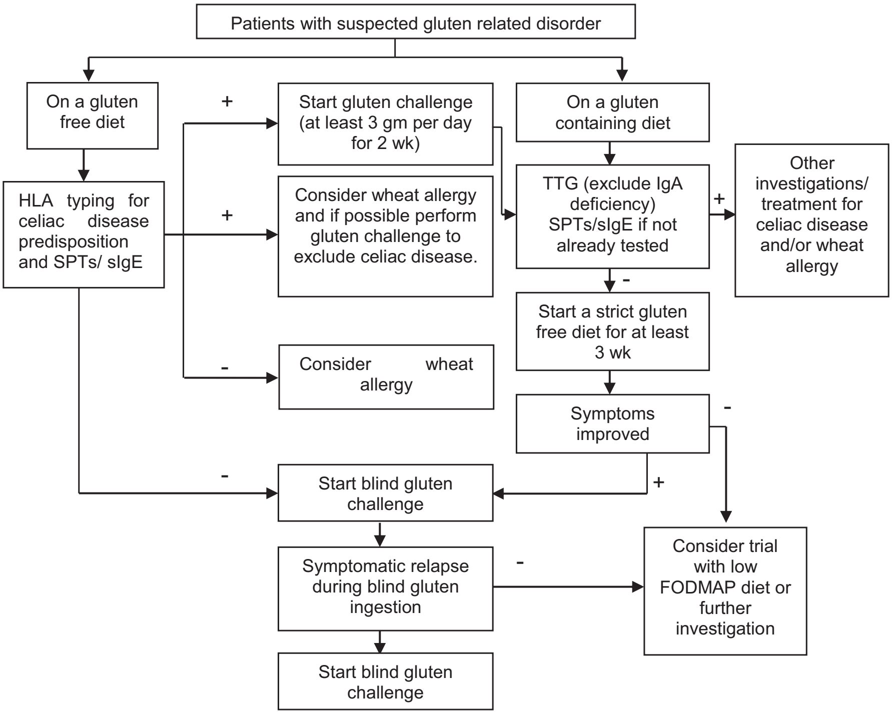

Fig. 3 Diagnostic flowchart in case of Suspected gluten related disorders (Elli et al., 2015). HLA Human Leukocyte antigen, SPTs/sIgE Skin prick test/specific immunoglobulin E, HLA+ and SPTs/SIgE+ = (+), HLA– and SPTs/SIgE– = (–)

improve after the dietary reduction of FODMAPs (fermentable oligo, di, mono-saccharides, and polyols) (Shepherd et al., 2014). Also, the IBS patients, especially those with the IBS-D (diarrhea) subtype have been seen to benefit from a GFD (Vazquez-Roque et al., 2013). The recent evidence about the efficacy of a low FODMAP diet in such patients suggests the hypothesis that some components of wheat other than gluten may be responsible for triggering the symptom (Shepherd et al., 2014). In fact, oligosaccharides like fructans, contained in wheat and related grains, have proven capable of exerting an osmotic effect in the intestinal lumen and increase gas production from bacterial fermentation (Gibson & Shepherd, 2012; Murray et al., 2014). Patients with self-reported NCGS on a GFD showed further improvement when placed on a low FODMAP diet and blinded gluten re-introduction led to no specific or dose-dependent effect. However, the reintroduction of both gluten and whey protein in their diet probably had an effect similar to other groups, who might have concealed the true effect of gluten re-introduction. A more appropriate standard for the confirmation of NCGS would be an elimination diet followed by double-blind placebo- controlled gluten challenge, in which the patient receives increasing doses of the suspected food allergen and a placebo (a harmless substance made to appear like a drug) (Fig. 3). The food allergen and placebo are given separately, either hours apart or on separate days. Because the allergen and placebo look alike, neither the patient nor the doctor is actually aware which one the patient is receiving, hence the term “double-blind” is applied (Sapone et al., 2012). This method can be particularly useful in order to differentiate NCGS from IBS.

3 Diagnosis of Wheat Allergy

The diagnosis of WA is classically based on skin prick tests (SPT), in vitro specific Immunoglobulin E (sIgE) assays and functional assays. SPTs and sIgE in vitro assays are the first-level diagnostics for WA. However, they are affected by a low predictive value. In particular, their low sensitivity can be explained by the fact that the commercial test reagents are mixtures of water/salt-soluble wheat proteins that lack allergens from the insoluble gluten fraction.

Food labeling legislation exists in many countries to assist the patients with gluten-related disorders in making sound dietary choices. These classify the foods according to the gluten content contained in them. The Codex Alimentarius standard defines foods as ‘gluten-free’ if the gluten level does not exceed 20 mg/kg in total and recommends the threshold of 100 mg/kg for the labelling of low gluten foodstuffs that have been specially processed to reduce their gluten content (Codex Alimentarius, 2008). Therefore, 20 mg/kg gluten free threshold has been adopted for foods bearing a ‘gluten-free’ label by the regulatory bodies in European Union, United States of America and Canada (Rzychon et al., 2017). A GFD can demonstrably normalize the intestinal damage and reverse malabsorption and as of now, it represents the only proven and available treatment for CD. But there are certain adverse effects associated with a GFD, which is mainly due to specially designed gluten-free products available on the market. They often contain high amounts of rice flour or other rice products that can lead to the high concentrations of heavy metals like arsenic or mercury. The association of higher heavy metal concentrations and GFD found in the recent studies of Raehsler and Bulka are so far only associations. They are to some extent plausible, at least explainable with higher rice intake and the possibility of arsenic or mercury intake through rice. However, the health impact of increased heavy metal concentrations in blood and urine found in GFD followers has not been investigated yet and deserves further studies. A GFD might contribute to micronutrient deficiencies of Vitamins D, B12 and Folate; also concentration of minerals like Iron, zinc, magnesium and calcium are low in a GFD (Wunschea et al., 2018).

4 Management of Celiac Disease

4.1 Treatment

The only effective treatment for celiac disease is the complete withdrawal of gluten from the diet. Currently, patients with celiac diseases are required to exclude the products containing wheat, rye, or barley. Patients usually need to follow a strict GFD for the rest of their lives. No food or medications containing gluten from wheat, rye, or barley or their derivatives can be taken, as even small quantities of it may be harmful. Removal of gluten (that is a reduction to below 20 mg/day) from the diet of celiac disease patients mostly results in symptomatic, serologic, and histologic remission. Approximately 70% of patients report an improvement in symptoms within 2 weeks of starting a GFD. Growth and development in children also returns to normal upon feeding a GFD. A strict adherence to GFD is, therefore, a fundamental measure that may prevent various complications of celiac disease. Though with the start of the GFD, the specific antibodies for celiac disease begin to normalize, the villous changes improve months after this. Notwithstanding, the histological resolution may take years or may not be achieved at all in some patients. The safe limit of gluten intake varies from patient to patient and is taken as 10 to 100 mg/day, however, a subsequent study has indicated that the upper limit should not be more than 50 mg/day (Bai & Ciacci, 2017).

Consumption of pure oats has been found free of any toxic manifestations in over 95% of patients with celiac disease, and has been used as a part of GFD in some countries such as in Finland. Adults and children of more than 15 years of age can consume it without any increased risk for enteropathy. In some countries, however, there is a reluctance over the liberal use of oats as a part of the GFD due to the toxic effects seen in a small sub-group of population (approximately 5%). In addition, the difficulty in guaranteeing commercially available oats to be free from contamination, an oat-free diet, at least during the first few months of treatment is recommended. In addition, rice and corn can be included in a GFD. Although there is a rapid clinical response to GFD, the overall rate of response varies among patients. Those who show severe allergic reaction may require hospitalization, repletion of fluids and electrolytes and intravenous nutrition along with iron, vitamins, and occasionally steroids. Patients should be encouraged to eat natural high-iron and high-folate foods, especially if the deficiency in these minerals are documented.

Other foods for a basic GFD include fresh dairy, meats and gluten-free preserved commodities like meat, seafood, eggs, legumes, fruits, fruit juices, vegetables, vegetable juices and liquid vegetable oils. In addition, miscellaneous items allowed in a GFD include sweet commodities (honey, corn syrup, sugar—brown and white), snack foods (plain popcorn, nuts, plain pickles, olives, gluten-free potato chips/crisps) and condiments (natural herbs, pure black pepper, vinegars—apple, grape, or wine).

4.2 Cooking and Food Preparation

Patients suffering from gluten intolerance should be instructed to avoid contaminating their gluten-free food with other commodities containing gluten, for example, by using separate utensils, cooking surfaces, and toasters. The majority of industrially processed foods contain non-allowable ingredients such as flavors. Therefore, labelling of the foods is important and available lists should be checked for the allowable foodstuffs. A GFD is low in fiber. Patients should be advised to eat a high-fiber diet supplemented with whole-grain rice, maize, potatoes, and ample vegetables. Any dietary deficiencies such as iron, folic acid, calcium, and very rarely vitamin B12 should be corrected.

4.3 Nutritionist Consultation

Nutritionist should be consulted after the interval of every 3 to 6 months until clinical normalization and then after every 1 to 2 years. This is particularly important in women of child-bearing age and during pregnancy in order to:

- Assess the patient's prevailing nutritional status.
- Identify macronutrient and/or micronutrient intake, and
- Detect deficiencies and/or excess nutrient intake.

It is important that patients with celiac disease consume a well-balanced diet including vitamins, calcium, and fibers, under the supervision of a specialist (Bai & Ciacci, 2017).

5 Conclusion

The only effective treatment for celiac disease is the complete withdrawal of gluten from the diet. Those with celiac diseases are required to exclude the products containing wheat, rye, or barley and need to follow a strict GFD for the rest of their lives. Food labels need to be checked for the presence of wheat or gluten before buying any product. Any dietary deficiencies such as iron, folic acid, calcium or vitamin B12 should be corrected by consuming proper and balanced diet. Nutritionist should be consulted after the interval of every 3 to 6 months until the clinical symptoms disappear and then after every 1 to 2 years. This is particularly important in women of child-bearing age and during pregnancy.

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Challenges in Development of Gluten-Free Breads

Mudasir Bashir Mir, Sajad Ahmad Mir, Reshu Rajput, and Vandana Sablania

1 Introduction

Gliadin and glutenin are key components for network development and the quality of the final baked product. However, the actual structure and interactions of the protein network are still unexplored. Gliadin has a predominantly viscosity-increasing effect whilst the glutenin plays a major role in the elastic properties of the network and dough development. Gluten helps in batter emulsification and visco-elasticity, dough cohesiveness, gas retention, crumb formation and finally impregnates elasticity to the bread texture. Parameters like water absorption capacity, moisture retention, and elasticity of baked products are influenced by the gluten network (Nascimento et al., 2014). Baking of gluten-free flours' is a challenging task in front of bakers and cereal technologists because resulting dough is non-cohesive and least elastic. The absence of gluten negatively affects the water absorption capacity, viscosity, extensibility, resistance to stretching, mixing tolerance, and gas retention of dough (Wieser, 2007). A wide variety of raw materials and/or additives were attempted to replicate the cohesiveness and elasticity of the gluten-comprising wheat dough. Gluten substitution, therefore is a foremost technical challenge for the development of fine quality gluten-free bakery products. The development of baked products from the gluten-free flours results in dense or crumbly texture with inferior sensory attributes (Kaur et al., 2015). Several models have been designed to improve and reduce the defects associated with gluten-free bread

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(GFB). The quality parameters of gluten-free bread are mostly decided by the choice and combination of structural ingredients (mainly polysaccharides) able to provide stability to the system (by increasing viscosity), as well as prevent an excessive weakening of the protein/starch/hydrocolloids coherent matrix (Scanlon & Zghal, 2001). Water addition to gluten-free (GF) dough results in the formation of batter which possesses different consistency, viscoelasticity, and structural networking as compared to a normal dough (Hager et al., 2012). Gluten-free dough is comparatively less cohesive and elastic, more batter-like, and lacks protein network than normal wheat dough (Cauvain, 2007). It is less elastic, sticky, and difficult to handle, take shape of the baking pan, and require little mixing, proofing, and baking times than wheat counterparts (Zannini et al., 2012). GF doughs containing emulsifiers and enzyme recorded higher G′ and G″ in dynamic moduli values than the control. The higher values of moduli reflect the presence of emulsifiers and enzymes facilitate new interaction between lipids, and are responsible for dough reinforcement.

2 Technological Challenges in Gluten-Free Breads

Gluten-free breads (GFBs) are characterized by a varied recipe, being a blend of different raw ingredients like rice, corn, starch, flour, proteins, fibers, fats, hydrocolloids, and specific enzymes. Removal of gluten is reflected from various defects that appear in the product in terms of structural and quality parameters, nutritional characteristics, and finally consumer acceptance. The commercially available gluten-free breads face both social and scientific challenges in comparison to conventional gluten-containing counterparts in terms of quality and acceptability. Due to the need of using gluten-free raw materials, the changes observed in the technological properties of gluten-free doughs may cause altered processing performance and related quality defects of the finished breads (Conte et al., 2018). The quality and sensory attributes of gluten-free baked products are inferior as they show crumbly texture, poor mouth feel & flavor, pale crust color, unattractive appearance, irregular surface, and a shorter shelf-life (Arendt et al., 2002). Deficiency of various nutrients, poor taste, and inferior quality are main issues related to many GF baked products present in the specialty stores and supermarkets (Pszczola, 2012). Apart from visual texture defects, GFBs tend to have high crumb hardness, low cohesiveness, and elasticity and as a consequence, high brittleness with a pronounced tendency to fracture (Gallagher et al., 2003). Gluten-free breads suffer from various technological quality defects like low specific volume (SV), rough, dry, and crumbly texture and a high staling rate. The main reason behind the occurrences of the reduced bread volume and hard crumb in the gluten-free bread is due to the weak protein network, less gas expansion, and its retention during the leavening of the dough (Mariotti et al., 2009). These gluten-free baked products show dissimilar rheological, textural properties, and baking parameters as compared to gluten-based products. Gluten-free bread developed from the millet flour recorded low specific volume and high bread hardness compared to the control (Sayed et al., 2016). However, the addition of protein isolates at a level of above 8 g/100 g enhanced overall textural profile (springiness, cohesiveness, firmness, and chewiness) of muffins (Shevkani et al., 2015). The addition of milk proteins, protein isolates, linseed mucilage, and proteases in different gluten-free formulations depicted good crumb appearance, improved texture, and enhanced nutritional quality of the gluten-free bread (Korus et al., 2015).

3 Strategies to Counter Technological Problems

Various types of technological problems arise due to the absence of gluten in the development of baked products which can be addressed by the incorporation of various additives and nutritive ingredients to mimic gluten properties. Exclusion of gluten results in the products which are deficient in various nutritional components like dietary fibers, protein, vitamins, minerals (calcium, folate, iron), and calories. Addition of starches (Tsatsaragkou et al., 2014a), gluten-free flours (Wronkowska et al., 2013), hydrocolloids (Nicolae et al., 2016), proteins (Ziobro et al., 2016), enzymes (Palabiyik et al., 2016) and emulsifiers (Lopez-Tenorio et al., 2015) to gluten-free flours are encouraged in order to counter the technological problems by enhancing dough rheological characteristics. Starch granules have the ability to gelatinize, entrap gases, and retain these gases during fermentation, thus play a substantial role in the bread baking process (Hug-Iten et al., 2001). The most widely used basic ingredient for GFB preparation is rice (*Oryza sativa*) flour. This is because of its bland flavor, white color, hypoallergenic nature, high amount of easily digested carbohydrates, and its low sodium content (Rosell & Marco, 2008). Hydrocolloids are used as thickening agents which bind with water, and result in increased dough viscosity, better volume, texture, and overall quality of the finished bread (Mir et al., 2016). The incorporation of hydrocolloids to gluten-free bread (GFB) formulae has several effects on both intermediate and end products. In GF dough, they act as water binders, enhance viscosity, increase viscoelastic properties and thus improve the gas-holding capacity of dough. They further alter swelling and starch gelatinization. However, in GF breads, they prevent moisture loss, retard starch retrogradation, and shelf life enhancement of the products, thus preserving their overall quality over time. Add to these, they too affect other bread quality attributes, like specific volume, crumb structure, texture, and sensory properties (Jnawali et al., 2016). Various strategies to combat technological challenges are presented in Table 1. Different types of hydrocolloids of both natural (agar-agar, carrageen, pectin and β-glucan, psyllium fiber, gum arabic, locust bean and guar gum) and synthetic origin (hydroxypropylmethyl cellulose (HPMC), carboxymethylcellulose (CMC), and methylcellulose (MC); xanthan gum) have been used as gluten replacers in GFBs (Demirkesen et al., 2014). Protein addition enhances the functional and nutritional properties of GF products, besides improving sensory attributes by Maillard browning and flavor generation (Deora et al., 2015). Different types of

Table 1 Strategies to counter technological challenges in gluten-free breads

S.No	Ingredients Used	Effect on Gluten-free bread and dough Characteristics	Reference
1.	Proteins	Milk proteins result in gluten like matrix that improves crumb texture and retards staling	Moroni et al. (2009)
2.	Starches	Produces bread with a higher volume and softer and more cohesive crumb structure	Lazaridou et al. (2007)
		Slowed down starch retrogradation and extend shelf life of bread	Jnawali et al. (2016)
3.	Hydrocolloids (xanthan gum, locust bean gum, CMC, HPMC)	Increased viscosity of the system and improves viscoelastic properties of the dough	Rosell and Marco (2008)
		Decreased the loss of moisture content during storage and eventually slowing down starch retrogradation and crumb hardening	Mohammadi et al. (2014), Mir et al. (2016)
		Produced breads with finer and homogeneous crumb structure with low porosity and higher number of pores	Demirkesen et al. (2014)
4.	Gluten-free flours (corn and rice) and hydrocolloids	Produced bread with higher specific volume and improved cell area fraction. Also during storage, had a positive effect on crumb colour	Naji-Tabasi and Mohebbi (2015)
5.	Use of sourdough	Enhanced elasticity and reduced stiffness of dough. Moisture was better retained in GFBs compared to control bread	Nami et al. (2019)
6.	Enzymes	Use of transglutaminase may improve protein structure which would improve crumb characteristics, loaf volume and overall acceptability of GFB	Gerrard (2002)
		Addition of glucose oxidase to rice flour enhanced elastic and viscous modulus. Produced bread with better specific volume and texture	Gujral and Rosell (2004)
		Addition of glucose oxidase with different fibres improved specific volume of bread	Aprodu and Banu (2015)

proteins of both plant (cereals, pseudo-cereals, and legumes) and animal source (dairy proteins and egg albumins) have been introduced to produce protein-enriched GFBs (Collar et al., 2015). Fortification in gluten-free bakery products by protein-enriched flours and protein isolates is a viable option to improve the nutritional status of these products. However, parameters like texture and density may be negatively influenced by the addition of these ingredients. The use of various enzymes enhances the GF dough-handling properties, which in turn results in fresh quality bread with longer shelf life. The use of enzymes to the gluten-free systems in the current scenario is aimed to cause modification in the protein functionality (Renzetti et al., 2008). Due to the gelling properties of pectins, they are being widely added in the gluten-free baked products as a thickening and gelling agent. Use of aggregated casein-based ingredients fortified with calcium results in the formation of a gel network similar to that of gluten network (Stathopoulos & O'Kennedy, 2008). To further upgrade the quality of GF dough and bread, various other ingredients like emulsifiers (Lopez-Tenorio et al., 2015), acidic food additives (Villanueva et al., 2015) and sweeteners (Alencar et al., 2015) are being used presently. Acidic food additives (acetic acid, citric acid, lactic acid, and monosodium phosphate) used in several non-gluten based formulations have caused a positive effect on dough volume, appearance, odor, taste, and texture. Incorporation of soluble and insoluble fibers in various non-wheat based recipes has resulted in improvement in the specific volume, brighter crust, and crumb of the baked product (Gularte et al., 2012). Addition of cereal fibers (maize and oat) into non-gluten formulations (corn starch, rice flour, and HPMC) resulted in the development of GFBs breads with higher loaf volume and crumb softness. Dietary fiber introduction not only compensates nutritional loss occurring due to wheat flour exclusion, but results in the addition of ingredient with tremendous water-binding, viscosity-increasing, and gel-forming capacities. A wide variety of dietary fibers incorporated in gluten-free products are apple pomace, β-glucan, bamboo & carob fiber, inulin, linseed mucilage, oligofructose, polydextrose and resistant starch (Pastuszka et al., 2012). Amaranth and buckwheat flour are rich in fiber, lipids and a range of vitamins, minerals, amino acids and phytochemicals are being used in a certain proportion in the gluten-free breads (Badiu et al., 2014). The addition of flours from amaranth, buckwheat, flaxseed, or pearl millet to gluten-free recipes can increase the mineral content (Badiu et al., 2014). Inclusion of different vitamins, folates, microelements into a GFB formulation is one of the promising methods for the improvement of the nutrition value of GFB without compromising its sensory quality. Above all, use of highly nutritious naturally gluten-free ingredients such as pseudo-cereals, minor cereals, legumes, and protein from various sources have been suggested as an important and dietary method for the improvement of the nutritional value of GFB. It is possible to reduce technological inadequacies in gluten-free bread by adopting novel technologies like hydrothermal treatments, high-pressure processing, improving aeration, sourdough fermentation, extrusion technology and microwave baking to improve the texture and quality of GF dough and bread (Therdthai et al., 2016). The application of protein hydrolysis and sourdough strategies have resulted in gluten-free bread products. Sourdough fermentation has the potential to enhance GF bread making, with improved bread texture, flavor, retarded staling, protecting bread from spoilage, and extended shelf life of GF bread (Ganzle & Gobbetti, 2013). It can also foster bread nutritional value, in terms of mineral bioavailability, starch digestibility, low GI, and concentration of bioactive compounds.

4 Nutritional Inadequacies/Challenges in Gluten-Free Breads

Gluten-free breads (GFB) and other bakery products are prepared by using non-gluten flours like maize, buckwheat, legumes and rice which is most commonly used. These non-gluten flours does not contain the two important gluten proteins present in wheat which are glutenin and gliadin. These two proteins provide elasticity, viscosity and water retention in breads and other bakery products (Nascimento et al., 2014). Gluten-free bread from nutritional point of view, lacks protein, vitamins and minerals and therefore it is required to find effective ways to enhance the fibre, protein, vitamin and mineral content of GFB while maintaining low glycaemic index. Nutritional deficiencies of gluten-free breads are summarized in Table 2. Nutritional value of GFB is a reason of major concern as it lacks these vital nutrients. Gluten-free breads are mainly prepared from starches obtained from different sources and they are deficient in many macro and micronutrients providing lesser amounts of vital nutrients required for the healthy and balanced diet (Gallagher et al., 2004). Commercial gluten-free bread and bakery products contain a lower amount of protein and insufficient amount of B-group vitamins (Yazynina et al., 2008) as compared to their gluten containing counterparts and minerals such as calcium, iron and zinc (Saturni et al., 2010). Nutritional inadequacies can be studied under different headings which are as follows:

Table 2 Nutritional challenges/inadequacies in gluten-free breads

S.No	Nutritional ingredient	Effect on Gluten-free breads	Reference
1.	Protein	Low content in bread i.e. 4.4 g/100 g as compared to control 10 g/100 g	Nascimento et al. (2014)
		Low average protein content i.e. 3.91%, much lower than the wheat flour control bread	Roman et al. (2019)
		Low protein content i.e. 9% as compared to control wheat bread which was 17%	Allen and Orfila (2018)
2.	Fibre	Low content in rice flour and corn starch based bread i.e. 0.7 g/100 g as compared to wheat bread 4.3 g/100 g	Nascimento et al. (2014), Rosell and Matos (2015)
3.	Minerals and vitamins	Low content of Fe, Cu, Ca, vitamin A, B_{12}, B_6 and D	Suliburska et al. (2013), Badiu et al. (2014)
		Low mineral bioavailability	Suliburska et al. (2013), Rosell and Matos (2015)

4.1 Protein Content

Protein is the building block of the body and is required for providing structure and strength to the body, therefore a proper quantity of protein intake is required daily for maintaining healthy well-being. Protein deficiencies are prevalent among coeliac patients especially in developing countries as approximately 25% of the protein comes from cereals and other cereal based products. Bread is an important source of protein and is consumed in various forms but with the presence of gluten-free flours the value of protein is very inferior. Nascimento et al. (2014) tested a number of products and observed that the protein levels of gluten-free bread, cookies, cake mix and pasta were found to be low when compared with conventional products. The protein content of GFB found to be 4.4 g/100 g that was much lower than the amount present in conventional bread containing gluten which was 10 g/100 g. To resolve this problem, protein-enriched flours and protein isolates are potential option to enhance the protein level in gluten-free bread and other bakery products. However, the incorporation of such ingredients has negative impact on sensory attributes such as density and texture. The breads prepared with flours containing gluten like wheat, rye and barley flours, the gluten protein along with starch forms a continuous network that traps the carbon dioxide gas produced during fermentation and helps in the rising of dough (Badiu et al., 2014). A glutinous network also affects moisture retention, water absorption capacity and elasticity of breads and other bakery products. Thus, removing gluten results in bread having crumbly or dense texture with poor sensory attributes such as taste, colour and flavour. During baking, these non-gluten flours act in a different way due to different structural starches and protein. The viscoelastic character of dough is due to the amylose content and the starch internal bonding within the flour (Rosell & Matos, 2015). For preparing gluten-free bread, rice flour is commonly used as a substitute even though it has low protein content. The reason is its digestibility, desirable white colour and bland taste that do not have any effect on characteristics of finished product (Badiu et al., 2014). The gluten-free bread prepared from rice flour have poor specific volume and density which is due to the poor viscoelastic properties and it also contributes very little to the daily requirement of protein with a protein amount of 6.14–7.30 g/100 g (Rosell & Matos, 2015).

4.2 Dietary Fibre

Fibre deficiency is most commonly noted in coeliac disease patients and is a common health problem. Dietary fibre is composed of polysaccharides, oligosaccharides and lignin. The major function of dietary fibre is to assist in digestion which is very important for coeliac patients. Gluten-free breads usually are deficient in dietary fibre and it directly affects its nutritional value which in turn has negative impact on sensory properties. An extensive range of fibres are available that can be used as additive to improve the functionality, sensory properties and nutrition of gluten-free bread (Tsatsaragkou et al., 2016) but these are affected by the particle size and solubility of the fibre. Wholegrain cereals are abundant in fibre content that can be used as a raw ingredient and have the efficiency to enhance fibre content of gluten-free bread but sensory attributes are affected. The particle size of the flour is the critical feature as both the water holding capacity and quality of finished product depends upon it (Tsatsaragkou et al., 2016).

4.3 Minerals and Vitamins

Vitamins and minerals are essential for healthy functioning of human body. The elimination of gluten results in a poor supply of minerals, vitamins, fibers and calories in the diet and also affects sensory properties. Gluten-free breads are deficient in essential minerals such as iron, calcium, zinc and manganese and the level of some vitamins such as B-complex group are reduced in gluten-free products (Yazynina et al., 2008). Therefore, coeliac patients have more possibility to develop mineral deficiencies of iron, zinc, calcium, magnesium and folic acid due to the poor formulations of gluten-free breads (Rosell & Matos, 2015). In a recent study conducted by Rosell and Matos (2015), they have established that most of the gluten-free products available in the market are manufactured from very refined ingredients so they are deficient in nutrients and thus supplies very less nutrients. A study done by Thompson (2000) studied the nutritional composition of a variety of gluten-free products. In this study, the content of iron, folate and dietary fiber of gluten-free cereal products were evaluated and compared with that of gluten containing products. The results suggested that the amounts of folate and iron found to be low in gluten-free breads, cold cereals and pastas. Suliburska et al. (2013) also observed that a variety of gluten-free products are relatively low in mineral content and thus delivers it in an inadequate amount. According to the study, the GFB contained low amount of iron (1.14 mg/100 g) and copper (0.07 mg/100 g) whereas the recommended daily intake of copper for women is 1.2 mg/day and for men is 1.7 mg/day. From these results, it is clear that the formulations of gluten-free breads are inferior in mineral content when compared to wheat or rye based bread. In this study, the products prepared from barley and oat flour are reported to have more nutrients than the gluten-free products. In all the gluten-free products, the bioavailability of minerals was found to be low from 8% to 68% which was due to the ingredients used. The most commonly used substitute flours for gluten-free formulations are corn and rice flours. During milling and refining of these grains, a great fraction of macro and micro nutrients are eliminated which results in poor quality product containing low minerals and vitamins. This is attributed to the uneven distribution of elements in cereal grain component (O'Dell et al., 1972).

5 Strategies to Combat Nutritional Inadequacies/Challenges

From the last few decades, several attempts have been made to overcome these challenges and development of healthier gluten-free breads to improve nutritional value and overall bread quality (Capriles & Areas, 2016). For this, the role of different proteins have been extensively studied and the use of other alternative nutrient rich ingredients has been explored to meet these challenges and for delivering a nutritionally rich product. The following ingredients have been utilized to alleviate the deficiencies in gluten-free breads.

5.1 Proteins

Developing a similar protein network as formed by gluten protein in production of wheat bread, the addition of other polymeric substances which are non-gluten proteins is a crucial thing. Proteins that are primarily known to play a functional role in the development of texture and structure of bread, their addition may result in improving nutritional quality of gluten-free bread (Ziobro et al., 2013). Different types of proteins of both animal and plant origin have been explored for developing protein enriched gluten-free breads. Dairy proteins such as whey proteins, caseinates and skim milk powder were used widely in gluten-free bread formulation because of their functional characteristics which resembles to that of gluten protein and because of their great nutritional value that helps in providing good amount of calcium and protein content with the supply of essential amino acids (Nunes et al., 2009). Krupa-Kozak et al. (2013) studied the effects of the inclusion of 12% and 24% of four low lactose dairy proteins i.e. calcium caseinate, sodium caseinate, hydrolysed whey proteins and dried whey protein isolate on the dough behaviour and quality characteristics of GFBs. They reported that, at all supplementation levels GFBs exhibited higher protein content and the breads prepared with the incorporation of 12% milk powders observed to have 5 times higher protein content when compared to the control bread and the specific volume, softness of crumb and lightness of crumb found to be increased significantly. Another means to incorporate proteins in a gluten-free bread formulation is to use different types of leguminous flours. The legumes such as chickpea, soybean, pea and lentil are being used in the preparation of gluten-free bread. Rosell and Matos (2015) reported that legume flour contains good amount of protein, provide beneficial health effects and have better nutritional profile than maize or rice flours. They are important source of proteins and the content varies from 18% to 25%. Among these legume, soybeans are unique and contains the highest protein content i.e. 40%. Therefore, these leguminous flours have better potential to enhance nutritional profile of gluten-free bread than rice flour. When legume proteins are incorporated into food systems they tend to show a variety of functional properties such as emulsifying, foaming and gelling properties as well as enhance water/oil binding capacity and viscosity

(Maninder et al., 2007). Soybean proteins which are added in gluten-free bread formulation either as soy protein isolates or soy flour has been used to improve textural properties and nutritional value of GFBs.

5.2 Pseudocereals

Under-utilized pseudocereals such as buckwheat, quinoa and amaranth also have excellent nutritional profile than maize and rice and the protein level is similar as that of glutinous flours. Apart from its superior protein profile, they also known to have high resistant starch content, dietary fibre and micronutrients like phenols, minerals and vitamins. Among these pseudocereals, buckwheat is widely used in gluten-free bread formulation than amaranth and quinoa. The nutritional value of buckwheat flour is attributed mainly to its protein composition and its protein has a high biological value. Breads prepared from buckwheat or amaranth flour had significantly higher protein content (Rosell & Matos, 2015). Mariotti et al. (2013) studied the effect of addition of dehulled and puffed buckwheat flour at 40% level of substitution on the nutritional value of two commercial mixtures of GFB. They have reported that the leavening properties of commercial mixtures of GFB improved with the addition of 40% dehulled buckwheat flour. Krupa-Kozak et al. (2011) indicated that the addition of buckwheat flours at different levels varying from 10% to 40% improved nutritional quality and technological properties of GFBs prepared from potato and corn starch. They have also particularly observed that the increasing levels of buckwheat flour resulted in enrichment of finished products with both micronutrients such as copper and magnesium and protein.

5.3 Fibres

Different types of fibres are available commercially that can be insoluble or soluble. Psyllium is water soluble fibre and has shown to have positive results when included in gluten-free bread formulation. It not only enhances value of dietary fibre, but also proves to be beneficial in improving sensory characteristics of GFB at only 2% level of addition (Tsatsaragkou et al., 2016). This was attributed to its ability to make a gel structure like a film that helps in retention of gas and thus increasing specific volume of bread. Additionally, the presence of large amounts of fibre enhance the water holding capacity of the dough which reduces rate of staling during storage, thus increases shelf life (Tsatsaragkou et al., 2016). Various cereal fibres such as corn flour, rice flour and hydroxypropyl methyl cellulose (HPMC) have also been added to different gluten-free formulations to study their effects on quality parameters of finished product. The addition of dietary fibre from maize and oat have been used to develop breads with higher specific loaf volume and softer crumb as compared to the non-fibre containing control bread. With the addition of 3 g/100 g dietary fibre, the overall acceptability scores obtained were the highest (Sabanis et al., 2009). A recent research had been conducted to evaluate the effect of addition of carob flour on the nutritional properties of gluten-free breads (Tsatsaragkou et al., 2014b). Carob germ is obtained after removal of locust bean gum and it is considered as nutritionally rich component that can be used for preparation of GFBs. Carob germ is rich in protein and dietary fibres and addition of varying amounts of carob flour to gluten-free formulations have been observed to enhance nutritional value of GFB.

6 Conclusion

The elimination of gluten protein complex poses both technological challenges and nutritional defects in gluten-free bread making. Several approaches/strategies have been employed to combat these challenges. Incorporation of major ingredients /additives such as starches, proteins, hydrocolloids, enzymes, emulsifiers, dietary fibre, proteins, starch, salts, acids and minerals to gluten-free flours remains to be one of the primary strategies to accomplish the development of gluten-free breads. Use of hydrothermal treatments, high pressure processing, extrusion technology and microwave baking too have been attempted to control various defects in gluten-free breads. These strategies, to a greater extent mitigated the deficiencies in gluten-free breads which is a way forward towards successful gluten-free bread making and its commercialization.

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Technological Aspects of Gluten Free Bread

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1 Introduction

Bread is one of the main staple food products which is consumed all over the world (Wandersleben et al., 2018). The bread is made from wheat flour as a main ingredient however other cereals, pulses and legumes can be milled to flour and used in bread making. Gluten protein of wheat and the prolamins from other cereals such as barley and rye are responsible for the viscoelastic properties for development of strong network structure in bread (Pena-Bautista et al., 2017). Consumers suffering from celiac disease (CD) on ingestion of gluten containing diet can have health related risks (Scherf et al., 2016; Bathrellou et al., 2018). The health related disorders due to consumption of gluten protein can be only avoided by restricting diet to gluten free diets. The gluten-free diet for patients suffering from celiac diseases is the diet restricted therapy for celiac patients and showed improvements in symptoms related to celiac diseases (Haines et al., 2008). The complete restriction of gluten intake is difficult due to gluten availability in variety of diet and hidden sources of gluten. The increased risk of gluten protein in case of celiac patients diverted the attention the food industry for development of gluten free bread (GFB). However, development of bread without gluten shows difficulties in bread making and raised detrimental effect on bread quality (Horstmann et al., 2018). In general, the quality defects in gluten-free baked products including bread include crumbling texture, pale crust colour as well as a faster staling rate (Gallagher et al., 2003). In

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fact, bread quality and sensory parameters of gluten-free bread are lower than gluten-containing bread (Primo-Martín et al., 2006). The quality gap between gluten and gluten free bread can be improved by using nutrient-dense ingredients, additives with functional properties similar to gluten protein and modern technological methods (Capriles & Areas, 2014; Drabińska et al., 2016). This book chapter will include available choices of gluten free flours, characteristics of gluten free bread dough, process technology for development of gluten free bread and approaches for improvement of GFB dough quality, nutritional and sensory properties of GFB.

2 Choice of Alternative Ingredients in Gluten-Free Bread Making

Various products which include cereals flours like maize, rice or sorghum, pseudocereals, legumes, seeds, nuts and fruit are used to replace gluten protein. Sorghum flour has been used in gluten free bakery products with good baking quality (Onyango et al., 2011). Gluten free bread development from millet and teff showed promising properties with acceptable quality. However, the use of millet flour in bread formulation showed low specific volume and high bread hardness (Sayed et al., 2016). Teff possessing the nutrition dense ingredients has been used in gluten free bread to enhance its nutrition profile, organoleptic properties, bread structure and overall bread quality (Hager et al., 2012). Pseudocereals such as quinoa and amaranth flour which are good source of proteins and dietary fiber than cereals, showed good functional properties and can be used as suitable ingredients for replacement of gluten in bread making (Lamothe et al., 2015). Gluten free bread made with whole amaranth flour (Lemos et al., 2012) and buckwheat flour (Wronkowska et al., 2013) showed good nutritional profile and desirable quality. Carob germ flour and chickpea flour also showed their potential use in development of gluten free bread with good nutritional quality. Incorporation of seeds, nuts and corn flour also raised interest in development of gluten free bread. Chestnut flour a non-cereal ingredient devoid of gluten was used in gluten free bread with potential benefits. Sour dough development technique used in chestnut flour based gluten free bread showed good bread quality parameters (Aguilar et al., 2016). Gluten free bread made from debittered acorn flour, pectin and guar gum showed good results related to bread volume, staling, crumb structure and sensory acceptance (Korus et al., 2015). The fruit and vegetables are also be incorporated in gluten free bread to enhance nutritional value in terms of fiber content, vitamins and minerals and overall bread quality. The fruits ingredients such as unripe bananas (Sarawong et al., 2014), orange pomace (O'Shea et al., 2015) and apple pomace (Rocha Parra et al., 2015a, 2015b) in formulation of gluten free bread showed promising with respect to nutritional and overall quality of GFB.

3 Characteristics of Gluten-Free Bread Dough

Gluten-free doughs, which cannot develop a protein network structure due to lack of gluten proteins are less viscoelastic, stickier and have poor handling properties as compared to gluten-containing dough (Ronda et al., 2017). The final quality characteristics of GFB is greatly influenced by selection and combination of structural ingredients that are able to provide stability to the system as well as prevent an excessive weakening of the protein/starch/hydrocolloids coherent matrix (Scanlon & Zghal, 2001). In order to obtain doughs with acceptable consistency and better behaviour during the mixing phase, gluten-free flours and starches require higher level of water content than wheat flour. However, the GFB made with higher amount of water showed quality defects such as lower specific volume, lighter crumb and crust colour, rough, dry and crumbly texture and a shorter shelf life (Gallagher et al., 2004; Jnawali et al., 2016). From a rheological point of view, gluten-free doughs resemble a semiliquid system which greatly varies in terms of consistency, viscoelasticity, and structural networking (Gallagher et al., 2003; Hager et al., 2012). Enrichment of different hydrocolloids in gluten free bread formulation improves both batter consistency and some technological parameters of the final products (crumb hardness, cohesiveness, and resilience). The improvement in rheological properties of gluten free dough with the addition of different levels of hydrocolloids was explored by many authors with their affect on overall bread quality (Ronda et al., 2015). As reported by Pruska-Kędzior et al. (2008), the rheological behaviour of gluten-free doughs may be attributed due to endogenous proteins and basic ingredients used in GFB formulation. Dough rheology of gluten free bread considered has considerable effect on quality of gluten free bread and results reported from rheology data were correlated with the final quality gluten free bread (Matos & Rosell, 2015). Matos and Rosell (2013), in an attempt to determine such quality indicators at dough levels, tested seven different gluten-free complex formulations from corn starch and rice flour and evaluated the rheological properties of the dough, and the technological and sensory characteristics of the resulting breads. They reported that dough Mixolab parameters revealed high correlation coefficients with the physical quality of fresh breads, but relatively low correlations with their sensory characteristics. In gluten-free bread making process, the development of processable doughs able to stretch during release of gases at the time of fermentation, formation of dough films able to stretch without rupturing and with sufficient strength to prevent the collapse of the bread structure, are crucial prerequisites to obtain high quality bread (Mir et al., 2016).

4 Process Technology for Gluten-Free Bread Making

Conventional and gluten-free bread (GFB) making processes differ considerably in terms of the complexity of formulations used (including main ingredients and amount of water), rheological behaviour of dough, and overall quality of the final product (Conte et al., 2016; Morreale et al., 2018). The traditional “bread” usually refers to a yeast-leavened product or sourdough bread which is particularly made with wheat flour. Due unique properties of the wheat protein gluten, formed when flour is hydrated and resulting in the formation of cohesive visco-elastic mass on account of mechanical work input. The gluten has the ability to form visco-elastic dough capable of entrapping gas during proofing and the early stage of baking and is responsible for structure formation in bread (Scanlon & Zghal, 2001; Gallagher et al., 2004). The visco-elastic dough led to formation of bread with a good loaf volume, a typical crumb structure and sponge-like texture, which is highly desirable. The ingredients included in gluten-free bread include gluten-free flour, water, yeast and salt. The sugar addition is not necessary because flour amylases can convert starch to sugars. Because of the absence of gluten in gluten-free bread the continuous three-dimensional protein-starch matrix is not formed which ultimately affects the dough rheology, the production process, and the overall quality of the resulting bread (Ronda et al., 2017). Thus, compared to conventional bread, the fabrication of GFB requires different technological solutions. The development of GFB involves the use of complex formulations which consists of different ingredients and additives able to mimic the viscoelastic properties of gluten. So, in this regard the most common flours and/or starches such as rice, corn, potato, and cassava usually included in GFB formulations, are often combined various other ingredients, such as hydrocolloids and proteins etc. (Capriles & Areas, 2014). Rice (*Oryza sativa*) flour is considered as the most suitable basic ingredient for the preparation GFB. The suitability of rice flour is due to its neutral flavour, white colour, hypoallergenic properties, high amount of easily digested carbohydrates, and its low sodium content. On the other hand, the use of rice flour in the development of GFB is also linked with some technological disadvantages. Due to the its poor functional properties of its proteins and the low level of prolamins, rice flour is not able to form viscoelastic doughs which required to retain the carbon dioxide produced during proofing, and thus the bread formed has low specific volume and a compact crumb (Rosell & Marco, 2008). After rice flour, corn meal (*Zea mays*) is the second basic ingredient which is often used in formation of gluten-free products. Flour/starch obtained from the white maize varieties is most often used in development of gluten-free bread (Hager et al., 2012). During the bread baking process, starch binds with water, forming a gas-permeable structure which in turn influences the water retention and rheology of the dough (Houben et al., 2012; Witczak et al., 2016). Actually it is starch gelatinization which results in the formation paste, able to trap air bubbles (Zannini et al., 2012). Addition of starch in GFB formulae leads to the formation of bread with an elevated loaf volume, and a softer, more cohesive and compact crumb structure (Gomez & Sciarini, 2015). However, it is to be noted that due the difference in their functional properties starches does not behave in the same way (Witczak et al., 2016; Zhang et al., 2017). However, during processing native starch has limited resistance to physical conditions, such as higher tendency to retrogradation and syneresis, loss of viscosity, low thermal stability, and inadequate rheological characteristics of pastes and gels. To overcome these shortcomings modification of starches either by chemical reactions or physical methods have been proposed (Witczak et al., 2012; Yousif et al., 2012).

Compared to conventional wheat bread, the development of GFB is slightly different to that of standard in terms of the regulation of physical parameters and the absence of gluten. Like wheat dough, gluten-free dough is traditionally mixed, bulk fermented, divided/moulded, proofed and finally baked. According to various studies, higher amount of water is required for gluten free dough development and tend to have a fluid-like structure (Bernadin & Kasarda, 1973). So in comparison to wheat counterparts, they require shorter mixing, proofing and baking times. On the basis of formulation used all the ingredients are weighed, blended and hydrated with water for dough development and incorporation of air bubbles. According to Stauffer (1998) the various stages of dough formation as per typical mixogram are hydration, blending and breakdown. During hydration, modification of protein occurs due to the absorption of water from the water-soluble flour components. On the basis of microscopic study when water is brought into contact with the flour, the flour particles seem to explode with the release of protein strands into the aqueous phase (Bernadin & Kasarda, 1973). However, hydration alone is not sufficient for dough making. Requirement of mechanical energy is necessary for dough formation. On blending, the starch granules become less firmly attached to the protein but remain associated with the protein fibres. Blending of all the ingredients results in the formation of homogeneous dough mass. Dough development reaches to its peak when it becomes softer and less resistant to mixing action. On reaching the breakdown stage, flour protein is converted to medium-length protein polymers that help in achieving desirable rheological properties of dough. After this, Punching of dough is done to expel gas and subdivide the existing gas cells, thereby incorporating air into the dough mass. The dough mass is then divided according to the standard process and rounded so as to allow proofing to occur. During the first proof, stresses in the dough relax, resulting in improved handling properties of dough. Prior to a final proofing process, the dough piece is shaped into a cylindrical form and placed into bread pans. During final proofing, CO_2 produced by the yeast makes the dough to rise while in the bread pans. On exposure to heat during baking, loaf structure and, development of baked flavour and colour sets in the bread. The bread is then removed from the pans during the depanning step and allowed to cool for slicing and to prevent any moisture migration on to wrapping or packaging (Ngemakwe et al., 2014).

5 Quality Characteristics of Gluten-Free Bread

5.1 Sensory Quality, Texture, and Shelf-life

In spite of the considerable efforts made in improving the quality of gluten-free bread, there are some major problems related to their technological and sensory quality. Due to the use of non-gluten flours, several changes are observed in the technological properties of gluten-free doughs which may result in various quality defects of the resulting breads like unattractive appearance, poor mouthfeel and flavour, and a shorter shelf-life (Houben et al., 2012; Jnawali et al., 2016; Conte et al., 2018). Crust color is the important visual feature of bread which has a strong effect on consumer acceptance. Compared to conventional wheat bread, GFBs have unattractive and often too white coloration. This could be related to inherent color of the ingredients used in GFB formulations (Conte et al., 2018; Gallagher et al., 2003; Rozylo et al., 2015). In addition to this, low protein percentage and high water contents in GFB hamper the browning reaction, which give bread a desirable brown color (Mohammadi et al., 2014). Besides visual texture defects, various other quality defects occur in GFBs like high crumb hardness, low cohesiveness and elasticity and, therefore, elevated brittleness with a pronounced tendency to fracture or crumble (Gallagher et al., 2003). It is worth to mention that these textural attributes of GFBs are strongly affected by product density and porosity. This depicts that, in dough processing, all the factors that are responsible for change in bread volume and cellular structure could be considered as major determinants of the bread texture (Conte et al., 2019). Low shelf life is another major concern associated with GFBs and these breads can't be kept fresh for a longer time. Actually, during storage there occurs number of physicochemical changes in baked products including hardening of crumb, loss in crust crispiness and organoleptic freshness which gradually decreases the consumer acceptance. These changes are commonly referred to as staling, are thought to be associated with redistribution of moisture, starch retrogradation, polymers reorganization, and interaction between starch and protein (Fadda et al., 2014). Unlike gluten bread, GFB which is more often based on pure starch and require an extra amount of water, is more prone to staling. Due to absence of gluten, in GFB, the transfer of moisture to surface may increase, and thus resulting in a product with softer crust and firmer crumb (Gallagher et al., 2003; Sciarini et al., 2012).

5.2 Nutritional Quality of Gluten Free Bread (GFB)

Nutritional value is another matter of concern regarding gluten free bread. GFB As we know GFB is mainly based on starches from different sources or refined flours, they don't contain various essential macro- and micronutrients which are needed in a healthy and balanced diet (Gallagher et al., 2004; Martin et al., 2013). Unlike gluten containing counterparts, commercial gluten-free products possess low protein content, inadequate amount of B-vitamins (Thompson, 1999; Yazynina et al., 2008), and minerals (including iron, zinc, calcium) (Wronkowska et al., 2008; Saturni et al., 2010). Thus GFB which is recommended for celiac disease (CD) patients may not be nutritionally adequate and well-balanced and can create deficiencies diseases that persist in CD patients. Anaemia caused by deficiency of iron (Theethira et al., 2014) and low bone mineral density (Meyer et al., 2001; Krupa-Kozak, 2014) were commonly found in CD patients at time of diagnosis. Addition of microelements (vitamins and/or minerals) to GFB formulation is one of the promising methods for the improvement of the nutrition value of GFB and could also enhance the nutritional status of CD patients (Saturni et al., 2010; Penagini et al., 2013).

6 Approaches for Improvement of Gluten Free Dough and Bread

From the last few decades, the formulation of novel and healthier GFBs has increased to a considerable extent due to their ability to fulfil all the quality requirements of bakery products (Capriles et al., 2016). However, the most skilled persons in the technology of baking agree with the fact that making gluten-free bread of high quality is a very difficult task. The baker sometimes adds extra ingredients in small amounts to enhance the performance of dough during baking or to have an improved quality product. The main benefit is related to the properties of the finished baked product and the modification of the dough during processing. Each ingredient plays an important role in the development of gluten-free bread with desired quality.

6.1 Additional of Functional and Nutritional Ingredients

In order to enhance the rheological properties of dough and quality of the finished product, different ingredients have been used GF bread making which include hydrocolloids, prebiotics (Inulin, oligofructose), Fibre, resistant starch, proteins and calcium.

Hydrocolloids: Hydrocolloids are high molecular weight hydrophilic polymers having polar or charged functional groups which make them soluble in water (Hoefler, 2004). Hydrocolloids are often used as a thickening and gelling agents, for increasing the viscosity dough, for better loaf volume, texture, and final quality of bread (Mir et al., 2016). They exhibit a range of functions including the primary gelling and thickening, and also emulsifying and encapsulating ones when added in a water-based system (Hoefler, 2004). The addition of hydrocolloids to GFB may affect the quality of both the intermediate and the end products: (a) these increase the viscosity of the dough due to their ability to bind more water and thus enhance the viscoelastic properties of the dough which in turn increase its gas-holding capacity. Moreover, these affect the swelling and gelatinization of the starch granules present in the dough. (b) During bread making, due to their ability to reduce the loss of moisture, slow down the starch retrogradation and extend the shelf life of the products by preserving their overall quality during storage. In addition to this, the other bread quality attributes, such as specific volume, crumb structure, texture, and sensory characteristics are also affected by hydrocolloids (Lazaridou et al., 2007; Jnawali et al., 2016). Several studies have documented the use of different hydrocolloids of both natural origin such as agar-agar, carrageen, pectin and β-glucan, gum arabic, locust bean gum, guar gum and psyllium fibre and as well as of synthetic origin such as synthesized cellulose derivatives (hydroxyl propyl methyl cellulose, carboxy methyl cellulose, and methyl cellulose)and microbial biosynthetic (xanthan gum) as gluten replacers in GFBs (Lazaridou et al., 2007; Mohammadi et al., 2014). However, hydrocolloids do not interact in the same way and their interaction with other food polymers (starch and protein), the specific hydrocolloid and its concentration used (usually up to 2%), as well as the processing conditions, can influence the properties of both the dough and the finished product (Capriles & Areas, 2014; Jnawali et al., 2016). The most commonly used hydrocolloids in GF formulation are HPMC and xanthan gum (XG) due to their ability to enhance the quality of the food products (Hager & Arendt, 2013). Other hydrocolloids including guar gum, CMC, agarose and locus bean gum are also used in GF formulations. Recently, some studies have documented the use of cress seed gum and sodium carboxy methyl cellulose (NaCMC) as novel gluten substitutes which improved final bread quality (Nicolae et al., 2016). The effects of the addition of different gums (xanthan, guar, locust bean, and agar, MC, CMC, and HPMC) and gum blends (xanthan-guar and xanthan-locust bean) on the crumb structure and textural characteristics of rice-based GFBs by has been studied by Demirkesen et al. (2014). The authors revealed in their study that the use of hydrocolloids such as xanthan, CMC, and HPMC and gum blends in breads resulted in homogenous and finer crumb structure in terms of lower porosity and average area of pores, and higher number of pores. It has been reported by Naji-Tabasi and Mohebbi (2015) that the addition of cress seed gum and xanthan gum as gluten substitutes in formulations containing rice flour, corn flour, and corn starch, as the major ingredient led to the formation of breads with higher specific volumes and improved cell area fraction. In addition, a thick layer was formed by the hydrocolloids which enhanced the stability of gas cells, producing more regular solid pores, particularly in breads containing cress seed gum.

Fibre: Research investigations have been carried to study the effects of insoluble fiber on texture and sensory properties of GFB (Martínez et al., 2014). Aprodu and Banu (2015) reported that dough cohesion and starch pasting properties were affected by the addition of pea fiber and oat bran. The results were attributed to high water-binding capacity of fiber which increases dough viscosity and reduces starch gelatinization by competing for the available amount of water (Capriles & Areas, 2014). Addition of soluble fiber to GFB may decrease the glycemic response of the product which is highly desirable for individuals with concomitant CD and insulin-dependent diabetes. Gunness and Gidley (2010) found that addition of functional soluble fibers such as β-glucan and psyllium may assist in gut regulation and decrease serum LDL cholesterol levels.

Prebiotics: Prebiotics such as inulin, oligofructose, and resistant starch are the most extensively studied functional ingredients used in the preparation of GFB's. Capriles and Arêas (2013), reported in their study that addition of increasing concentrations of inulin-type fructans (4%, 8%, 10%, and 12%) to GFB showed higher specific volume below 10%, while observing a decline above 10%. The authors found that inulin-type fructans form a gel network and retain CO_2 in the same way as some other hydrocolloids. The degree of polymerization (DP) of inulin may also affect the quality of the final bread. Generally, lower DP of inulin has stronger effects than those with high DP (Ziobro et al., 2013). Resistant starch plays a vital role in improving the bread quality and also reduces the energy of food and enhances its digestive functions (Witczak et al., 2016). Moreover, RS improves the elasticity and porosity of the bread without increasing the crumb firmness (Tsatsaragkou et al., 2014). Korus et al. (2009) documented that the partial replacement of corn starch with tapioca and corn resistant starch preparations at increasing levels resulted in gluten-free doughs with increased elastic behaviour (increase of both storage and loss moduli, and $G' > G''$) and rheological properties typical of a week gel ($\tan \delta > 0.1$). The GFBs showed decreased crumb hardness values with the increasing concentrations of resistant starch preparations applied. Further, the addition of resistant starch increased the total dietary fibre up to 89%, when compared with the control samples.

Protein based ingredients: The protein-based ingredients from different sources including legumes, egg, and dairy and their addition in GF doughs have been studied extensively. Addition of proteins enhance the functional and nutritional properties of GF products and also improve their sensory quality by increasing the Maillard browning reaction (Deora et al., 2015). To build up a network similar to that formed by gluten during wheat bread production, the inclusion of non-gluten proteins is a critical factor. Addition of proteins in gluten-free formulae may not only help in developing bread structure and texture, but may also confer nutritional benefits to the final products (Ziobro et al., 2016). Several studies have used proteins of both plant(such as cereals, pseudo cereals, and legumes) and animal origin (such as dairy proteins and egg albumins) for the development of protein-enriched GFBs. Dairy ingredients such as caseinates, whey proteins, and skim milk powder have also been used for making gluten-free breads due to their functional properties, similar to those of gluten, and also because of their high nutritional value, which entails an increase in calcium and protein content and supply of essential amino acids (Stathopoulos, 2008; Nunes et al., 2009). Therefore, the addition of dairy proteins in GF breads may influence the overall quality of the intermediate and end products: (a) during dough preparation, these increase water binding capacity and improve the handling properties of dough/batter; (b) at the bread level, these increase the loaf volume, enhance crust colour, improve texture, and aroma, and decrease the staling rate (Houben et al., 2012). However, the patients with celiac disease commonly report a secondary lactose deficiency caused by an inadequate secretion of lactase, therefore the use of high-lactose dairy ingredients for making gluten-free products must be carefully considered. The proteins from egg have shown a great potential in the gluten-free bread making due to their excellent foaming ability, stabilizing effect, and emulsifying properties. However, due to their allergenic character, their use in foods as ingredient should be limited or carefully considered (Phongthai et al., 2016). Addition of different types of legume grains is another way to include proteins in a GFB formulation. Legumes have a high nutritional value, suitable functional properties, and health-promoting effects. These are considered important sources of proteins with contents ranging from 18% to 25% (Tharanathan & Mahadevamma, 2003). Moreover, legume proteins, are rich inessential amino acid lysine which is often deficient in cereal grain food, as well as a concomitant deficiency of methionine and cysteine, are nutritionally complementary to cereal proteins (Duranti, 2006). Legumes are also a good source of minerals (such as calcium and iron), vitamins (especially B-group vitamins), and dietary fibre (both soluble and insoluble fractions). Moreover, these are regarded as low glycemic index foods (Collar et al., 2014). The proteins from legumes when added to food matrices, exhibit a wide range of functional properties, including foaming, emulsifying, water/oil holding capacity, viscosity and gelation capabilities (Maninder et al., 2007). Recently some research studies have reported the use of proteins from legumes such as soybean, carob, pea, and lupine in the development of gluten-free products (Marco & Rosell, 2008a; Crockett et al., 2011; Ziobro et al., 2016; Horstmann et al., 2017). Soy proteins, which are usually added into the bread formulae either as soy protein isolates or high-protein soy flour, have for long been used to improve mechanical behaviour of doughs as well as textural properties, specific volume, and nutritional value of GFBs (Marco & Rosell, 2008b). However, addition of soy flour results in "beany" flavour. Shin et al. (2013) found that the pretreatment of soy flour can reduce the beany flavour and also improve the technological properties of GFBs.

Calcium enrichment: A GFB fortified with calcium was prepared by Krupa-Kozak et al. (2011) who investigated the effect of the addition of individual and combined calcium caseinate and calcium citrate to GFB formulations. The breads containing calcium citrate alone (2%) or a mixture of calcium citrate and calcium caseinate (1.3% and 0.7%, respectively) showed a significant increase in calcium levels when compared with unfortified control GFB. Furthermore, the technological and sensory properties of calcium-enriched GFBs were favourably modified. In a subsequent study conducted by Krupa-Kozak et al. (2012), the suitability of adding different organic or inorganic calcium sources (calcium lactate, calcium citrate, calcium chloride, calcium carbonate) in GFB formulations with inulin was assessed. The GFB containing calcium carbonate, showed the best results which additionally increased the overall consumer acceptability of the product.

6.2 Use of Enzymes

Enzymes have been often used to improve the GF dough-handling properties and to enhance the quality and shelf life of finished products. Starch-modifying enzymes (amylase and cyclodextrin glycosyl transferase (CGT)) and protein modifying enzymes (crosslinking enzymes and proteases) have been added in GFB formulations (Renzetti & Rosell, 2016). Cross linking enzymes including trans glutaminase (TG, EC 2.3.2.13), tyrosinase (EC 1.14.18.1) glucose oxidase (GO, EC1.1.3.4) and laccase (EC 1.10.3.1) have been dominantly used for the preparation of GFBs. Pongjaruvat et al. (2014), reported that the volume of rice bread containing 0.1% TG was increased as compared to that obtained from the wheat control. Conversely, further increasing the TG concentrations produced a deteriorating effect on the bread volume and increased the crumb hardness and chewiness. According to Mohammadi et al. (2015) bread prepared with microbial TG (1 U/g) and guar gum (30 g/kg) possessed desirable quality. Aprodu and Banu (2015) added glucose oxidase at 0.1% to a fiber-enriched GF dough and found an increase in starch gelatinization followed by a decrease in starch retrogradation values. In another study carried by Flander et al. (2011) a high specific volume and increased crumb softness of oat bread combining *Trichoderma reesei* tyrosinase and xylanase was reported. While the combination of *Trametes hirsuta* laccase and xylanase also improved the specific volume of the bread while crumb softness remained unaffected. Proteases including proteinases and peptidases are enzymes that have the ability to hydrolyze the peptide bonds of proteins. Recently, Hatta et al. (2015) documented that the degradation of α- and β-glutelin subunits of rice protein is essential for the improvement of bread texture and quality. They found improvements in the gas holding and textural properties in protease-treated (metallo, serine, cysteine proteases) rice breads, which almost fully degraded the α- and β-glutelin subunits. Glutathione has also been found to break the disulphide linkages between α- and β- subunits of rice glutelins (Yano, 2010, 2012). The authors reported that the bread incorporated with reduced glutathione (GSH) and oxidized glutathione (GSSG) in rice batter had similar structure to wheat bread but with a smoother texture. Moreover, sensory analysis revealed that GSSG bread had significantly lower sulphur odours compared to GSH bread (Yano, 2012).

Other additives including emulsifiers (López-Tenorio et al., 2015), acidic food additives (Villanueva et al., 2015) and sweeteners (Alencar et al., 2015) have also been used to improve the quality of GF dough and bread.

7 Novel Technological Approaches to Improve Gluten-Free Bread

Gluten proteolysis, sourdough fermentation, genetically modified wheat breeding, frozen storage, and partial baking are novel processing techniques used to improve the quality and acceptability of GFB.

7.1 Gluten Proteolysis and Sourdough Fermentation

Gluten proteolysis technique used in gluten free bread making involves the use of proteolytic enzymes to detoxify the gluten. The enzymes like prolyl-endopeptidases are used to break down the peptide bonds next to proline and glutamine-residues and thus are able to degrade the gluten protein to amino acids or nontoxic peptides (Heredia-Sandoval et al., 2016). Germination of cereal grains is also able to degrade the immuno-stimulatory gluten peptides and thus reduce the toxic effects of gluten. Germination of wheat grain at specific conditions for a definite time period reduced the gluten content to a considerable level (Michalcová et al., 2012).

Fermentation of gluten flour by using lactobacilli stains having protease system hydrolyse the proline residue and thus reduce the gluten toxicity. Stefańska et al. (2016) used lactobacillus strains and fungal protease in sourdough development to hydrolyze the albumin/globulin and gliadin fractions in bakery sourdoughs. Bread prepared with flours using selected sour dough lactobacilli and fungal proteases showed similar techno-functional properties similar to gluten containing bread (Rizzello et al., 2014). Because of the metabolites produced by lactobacillus bacteria, the application of sourdough may enhance the quality characteristics of breads including texture, flavor, nutritional value and shelf-life (De Vuyst & Vancanneyt, 2007). Sourdough comes in two forms viz. dried and fresh. Sourdough in powder form offers shorter fermentation time and longer shelf life compared to dried form. Freeze-dried sourdough has been used in GFB formulation to improve the quality of final bread The freeze-dried sourdough from amaranth, buckwheat and rice flour showed better suitability for gluten free bread making compared to fresh sourdough (Rozylo et al., 2015, 2016).

7.2 Genetically Modified Wheat Breeding

Genetically modification in wheat is one the advanced approach in down regulation of toxic gliadins and/or glutenins using RNA interference (RNAi) technology. RNAi is a reverse genetics tool which has the ability to produce double-stranded RNA that causes gene silencing before formation of gluten (Watanabe, 2011). Becker et al. (2007) reported down-regulation of α-gliadins by utilization of RNAi and produced a variety of transgenic lines with the α-gliadins silenced. As per results showed by reversed-phase high-performance liquid chromatography, α-gliadins in transgenic flour were reduced by 60% compared to wild native flour (Becker et al., 2012). RNAi has also been applied to produce transgenic lines with reduced levels of ω-5 gliadins which were good for wheat-dependent exercise-induced anaphylaxis (Altenbach et al., 2014). Results of RNAi technology have showed the down-regulation of only γ-gliadins (Pistón et al., 2011) and α-gliadins, ω-gliadins and γ-gliadins (Gil-Humanes et al., 2010). Bread prepared with genetically modified wheat flour has high lysine content and good overall quality as compared to normal conventional bread (Gil-Humanes et al., 2014). This approach of lowering gluten level could probably be useful for cultivating celiac-safe wheat. However, the resistance to transgenic foods and the lack of clinical trials in celiac patients as such limit the commercialization of the genetically modified low-gliadin wheat.

7.3 Frozen and Partial Baking Technologies

Use of freezing process at different steps of GFB bread making process is another approach in reducing the toxicity of gluten protein. Frozen dough is a value added product that offers improved bread quality. In addition, patients with celiac disease can easily prepare and consume it at home. However, frozen dough breads displayed lower specific volumes due to the reduction in yeast sensitiveness and the change in structure of the gluten free dough (Mezaize et al., 2010).

Partial baking of GFB is also gaining interest with the aim to improve the quality of GFB. Partially baked GFB is a semi-finished product with suitable crumb texture and minimum crust coloration. The partial baking process involves two baking steps. In the first stage bread structure is fixed followed by stored while second baking stage involves development of flavor and crust color in bread (Najafabadi et al., 2014). The other technologies used in improving the quality of gluten free bread include pregelatinized treatment (Pongjaruvat et al., 2014), extrusion technology (Clerici et al., 2009), high-pressure processing (Vallons et al., 2011) and microwave baking (Therdthai et al., 2016).

8 Conclusion

Gluten is considered as a basic ingredient for the successful development of good quality bread. However, the exclusion of gluten from bread formulation has detrimental effect on the bread making process and raises technological challenges in the development of good quality bread comparable with conventional bread. On the basis of current findings mentioned in this review, it may be concluded that the use of combination of different ingredients and additives is necessary for the preparation of GFBs with satisfying quality attributes. However, in spite of the considerable advances made in development of gluten free bread, several quality issues of GFB still remains as subject of concern. Therefore researchers should do further investigations with the key focus on the finding of more innovative gluten substitutes and their application in preparation of quality GFB.

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Dough Handling Properties of Gluten-Free Breads

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1 Introduction

Over the decades, various dough characterization methods, measuring devices and systems have been developed to determine the baking performance of breads (Tietze et al., 2016). The rheological properties of dough governs the quality of the final products (Stathopoulos et al., 2006). Baking without gluten is a challenging process. To surpass this challenge, the gluten-free bread preparation comprises of the use of different gluten-free flours, ingredients and additives as a substitute of gluten to improve the dough handling properties (Mir et al., 2016). The use of wide range of additives such as hydrocolloids, dietary fibers, proteins, enzymes and emulsifiers aim to imitate the visco-elastic characteristics of gluten and produce a better quality product. Other than modifying the flour formulations, the different processing techniques such as sourdough fermentation, dry heat treatment, heat moisture treatment and high hydrostatic pressure treatment employed on gluten-free formulations have resulted in satisfying quality of final baked products (Naqash et al., 2017). A thorough study about the dough properties is crucial for understanding dough processing, dough behaviour and quality of gluten-free breads. Notable changes in the rheology of dough are observed during dough development (Amjid et al., 2013) and the fundamental rheological behaviour shows a strong correlation with the storage stability and the sensory qualities like mouth feel.

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2 Bread Making Technology

Bread making is a complex process that includes various steps like mixing, proofing, baking, and cooling. It comprises of the mechanisms like yeast and enzyme activity, gelatinization of starch, coagulation of proteins, expansion of dough volume, evaporation of water vapour, and finally development of crumb and crust structure to obtain a fully baked product (Sivam et al., 2010). Substantial changes take place in the microstructure of bread during bread making process when a visco-elastic mass of dough is transformed into a soft and porous bread (Autio & Laurikainen, 1997).

Dough is an intermediate stage between flour and bread. Therefore, the quality of dough plays a key role in bread making process. The major ingredients of bread dough comprise of wheat flour and water along with yeast, sugar and salt. The average composition of wheat flour on dry weight basis includes 65% starch, 12% protein, 2% lipid and the remaining is occupied by non-starch polysaccharides and inorganic components (Cauvain & Young, 2007). A too firm or too soft dough is difficult to process and will not yield a consumer acceptable product (Amjid et al., 2013). Ingredients and processing methods together determine the microstructure of the dough which imparts characteristic appearance, texture, flavour and stability to the final products (Autio & Laurikainen, 1997).

A good dough should have good extensibility and sufficient elasticity to retain gases. It addition, it should expand sufficiently during proofing and baking while retaining its original and desired form. To preserve the delicate cell structure during the moulding process, the dough should exhibit little resistance to deformation. This balance between elasticity and extensibility varies with the type of product to be prepared. The suitability of dough for the production of bread relies on the ability of dough to stretch in response to the force of expansion, generated by the effect of leavening gas (Mir et al., 2016).

3 Dough Processing and Rheology

During processing, remarkable changes takes place in the structural as well as the functional properties of dough which determines the quality of breads (Amjid et al., 2013). In general, dough processing for the preparation of breads includes mixing, kneading, moulding, proofing, baking and cooling.

Mixing is an integral part of bread making that involves the processes like combining the ingredients, incorporation of air and kneading. Dough is eventually developed with the formation of gluten network following hydration of gliadin and glutenin proteins. It imparts desirable rheological properties for subsequent processing of dough. During dividing and moulding, dough is subjected to stress and strain to help in uniform distribution of gas cells throughout the dough mass (Autio & Laurikainen, 1997). Proofing comprises of a series of one or more fermentation steps of different time intervals to generate carbon dioxide and provide an aerated structure to baked breads. Heat and mass transfer occurs during baking, thereby transforming dough into bread (Capriles & Arêas, 2014). The remarkable structural changes which take place during baking are the expansion of gas cells, gelatinization of starch, crosslinking of proteins, melting of fats and incorporation of air (Autio & Laurikainen, 1997). When the dough has been fully developed by multiplication and expansion of the entrapped gas cells, the heat of the oven during baking raises the dough temperature to a level that makes gelatinization of the starch and coagulation of the protein. This gives final bread crumb structure on cooling.

Rheology is a significant parameter in bread making as it affects the machinability and gas retention capacity of dough as well as the end product quality (Indrani & Rao, 2007). The dough handling properties are defined by the rheological measurements of dough. Rheology is the study of flow or deformation of a material, in response to applied force (Amjid et al., 2013). The primary goals of rheological measurements are:

- To attain a quantitative description on mechanical properties of dough.
- To obtain knowledge on composition and molecular structure of dough.
- To predict the performance of dough in the course of processing (Cauvain, 2012).

Rheological measurements can be considered as an essential tool in analytical laboratory to assist the process control and design, under a given set of conditions and predict the behaviour of dough. They also play a role to describe the performance of dough during mixing, proofing and baking (Scott & Richardson, 1997).

4 Dough Handling Properties

Dough mixing is one of the important step in bread making as it helps in achieving an optimum balance among dough handling properties by blending all the necessary ingredients. The four major dough handling properties are extensibility, elasticity, resistance to deformation (tenacity) and stickiness.

4.1 Extensibility

Understanding the extensional behaviour of dough is essential for dough processing (Tietze et al., 2016). Extensibility is an important characteristic of dough generally measured using rheometer. It determines the crumb porosity and final volume which are the two main desirable attributes for the preparation of breads. In general, extensibility, bread volume and crumb porosity are interrelated and directly proportional with one another. Quantification of extensibility is mostly done with a Texture Profile Analyzer (TPA) or an extensograph. In both, a measured piece of dough is made to stretch till it ruptures and the force employed at the point of rupture is used as an indicator of dough stretchability and firmness. The total amount of work required to stretch a piece of dough is called dough strength. Extensibility with respect to elastic modulus (G′) can be recorded with the help of shear measurements. Strain hardening, an important dough property that describes the ability of the gas cells to expand further. Dough exhibits strain hardening on uniaxial extensibility tests. So, these tests are mostly carried out in linear visco-elastic region (LVR) to nullify the effect of strain hardening. LVR is the region corresponding to the stress varying linearly with strain for the analyzed dough sample under analysis. The maximum viable dough stretchability cannot be determined as it is not a non-destructive shear measurement (Tietze et al., 2016).

4.2 Elasticity

Elasticity is the ability of the dough to come back to its normal state after a deforming force has been removed. Dough that notably springs back after stretching can be regarded as elastic (Rosada, 2004). Elasticity measures the load of stress which is applied before the dough breaks apart (Cauvain & Young, 2007). Elasticity in the bread making process can be considered as a sensory characteristic that is felt when dough is rapidly stretched and released. It is a desirable property when a baker wants the dough to contract back to its normal state. The formation of a thin membrane with no fissures or breakage when dough is stretched is an attribute of a good quality gluten which indirectly indicates good elasticity of dough. Upon kneading, the dough ingredients start to stick together and get attached to the hook of the dough kneader machine. This process finally makes up the dough by a mechanism called, Weissenberg effect which is an action of elasticity (Kieffer, 2007). Thus, elasticity of dough is an indication of its qualitative nature and leads us to expect a good end product. Dough is known to show visco-elastic behaviour because of its liquid-like (viscosity) and solid-like (elasticity) behaviour. So, it is difficult to measure elasticity independent of viscosity, as both the properties occur simultaneously (Kieffer, 2007). Gluten-free doughs are less viscous, less cohesive, and less elastic when compared to wheat dough. Creep–recovery tests and oscillatory tests are mostly used to evaluate dough viscosity and elasticity and consequently, the visco-elastic behaviour of gluten-free doughs (Ronda et al., 2017). Alessandro Angioloni and Collar (2009) in their study on fibre enriched gluten-free breads applied back extrusion tests to evaluate the visco-elastic properties of dough having very less viscosity.

4.3 Tenacity

Tenacity is the resistance of dough to deformation when being stretched. It influences the degree of elongation in dough processing. It can be described as the property that dough exhibits when the baker tries to make it longer. Tenacity makes longer and stretchable dough if the ingredients are thoroughly mixed during bread making. For example, elastic dough has the ability to resist stretching force and dough with more tenacity shows the tendency to restore to its original form immediately. Thus, tenacity and elasticity are closely related attributes. The balance between extensibility, elasticity and tenacity are taken into consideration to determine the gluten-free dough strength in a laboratory scale (Rosada, 2004).

4.4 Stickiness

Stickiness is the ability of the dough to stick to the surface it comes in contact with. Dough is known to show different degrees of stickiness depending upon the inherent composition and temperature of flour. For better dough handling, a non-sticky surface is preferred. Increased stickiness is represented by an increased viscosity of the dough. Stickiness is associated with the rheological properties of dough but till date no studies have derived a proper relation between the two due to lack of standardized analytical techniques. Most of the tests, however, have shown a relation of stickiness with adhesion and cohesion. Adhesion is a measure of stickiness that can be related to surface tension and is proportional to the rheological properties of dough. In a device developed by Chen and Hoseney (1995), dough stickiness was measured using a flat probe (25 mm diameter) pressed over the surface of a measured piece of curve-shaped dough and released after a short interval of time, 0.1 s. During this analysis, the stickiness of dough was correlated with the releasing force required for lifting of the probe. In another method, the probe was pressed over a flat surface of dough sample and was then withdrawn. In a graph plotted simultaneously between the force (Newton) and distance moved (Meters) by the probe, the peak force was related to adhesive strength or stickiness of the dough and the total work required for complete withdrawal was estimated as the work of adhesion (Wa). It is known that dough stickiness depends on the surface energy of the material with which it is in contact. For example, metals possess high surface energy that exhibit higher stickiness to dough ndwhen compared to plastics (Tietze et al., 2016). On the other hand, viscosity acts as an opposing force to alter the shape but not the surface area of dough. The dough stickiness is measured by a texturometer and the three magnitudes employed to characterise stickiness are:

- adhesive force, which indicates the measure of stickiness,
- adhesive energy, which is the work of adhesion, and
- extended sample distance from probe, which indicates dough strength (Rosada, 2004).

Dough can be classified as strong and weak with reference to the dough handling properties. Strong dough is the one which lacks extensibility and has an excessive amount of elasticity. The dough thus formed is difficult to stretch upon moulding whether done by hand or machine. Strong dough is used to prepare shorter breads with rounder cross sections and poor cut openings. These limitations come into play due to the lack of extensibility and inappropriate proofing. Weak dough shows lack of tenacity and excessive extensibility during moulding. Due to lack of strength, gluten becomes too weak to retain gases during proofing and baking. Finally a product having dense crumb structure, scarcely developed cut openings, flat cross sections and very low volume is obtained. Thus, it is important to maintain a good balance between dough extensibility and elasticity to obtain qualitatively and quantitatively better doughs and breads (Rosada, 2004).

5 Gluten-Free Dough

The sponge-like structure of bread is formed due to the gelatinization of starch surrounded by a continuous network of gluten (Cauvain & Young, 2007). Gluten is the structural and functional protein in wheat flour responsible for the visco-elasticity of dough. It plays a prominent role in bread making improving the texture, appearance and quality of breads (Capriles & Arêas, 2014). Gluten-free dough is the one prepared from one or more gluten-free flours such as rice, corn, soybean and maize. Several additives including hydrocolloids, fibers, proteins, enzymes and emulsifiers coupled with various processing techniques are used to develop gluten-like network structures in dough to produce better quality gluten-free breads (Naqash et al., 2017).

Gluten-free dough can also be called as batter because of its less firm texture in comparison to wheat flour dough rich in gluten (Onyango et al., 2009; Hager et al., 2012; Capriles & Arêas, 2014). For the preparation of gluten-free breads, different gluten-free ingredients are blended along with water and yeast and made into dough. This dough expands gradually as the carbon dioxide formed during fermentation is not fully trapped in air cells. The breads produced as a result have undeveloped cell structure, reduced volume and dried and grainy texture. Therefore, to improve the rheology, texture and baking quality of dough and to ameliorate the quality of breads produced thereof, it is advisable to incorporate additives or to adopt some different processing technologies and/or to incorporate some processing additives to gluten-free flours (Capriles & Arêas, 2014).

6 Factors Influencing Dough Handling Properties

There are many factors which influence the dough handling properties of various flours. The kind of wheat cultivars used, its water absorption capacity of flour, percentage of starch, the quality of gliadin and glutenin fractions have a great impact on dough handling properties of gluten-containing flours. The processing conditions such as mixing (work input, rpm and time), temperature employed, degree of gluten development and proofing period also affect the quality of dough. On the other hand, the presence of bran particles and water-competing ingredients that limit hydration of functional polymers influence the functionality of dough. And most importantly, incorporation of additives such as hydrocolloids, proteins, enzymes, etc. significantly improve the dough handling properties of breads (Naqash et al., 2017).

When dealing with gluten-free dough, dispersive mixing is often used to cause aeration as the mass is devoid of gluten network and exhibits a rheology similar to cake batter. Mixing at an increased speed with a planetary mixer has replaced the kneaders that were traditionally used (Mariotti et al., 2013). Chin and Campbell (2005) in their study found that, increasing the mixing speed causes greater aeration. However, no relation was drawn between the mixing speed and specific volume of gluten-free dough. On contrary, proofing and bread volume is affected by geometry of mixers, mixing speed and duration of mixing (Gómez et al., 2013).

Chesterton et al. (2012) observed in their study that increasing the duration of mixing produced lesser but larger bubbles in the batter and accelerated its aeration during fermentation. Also, the activity of yeast was elevated by increasing dough temperature during long mixing period (Elgeti et al., 2015). A supplementary mixing (knock-back stage) following proofing and prior to baking has been done for the incorporation of more gas cells to the gluten-free dough and to redistribute these cells throughout the dough mass (Sciarini et al., 2012).

The cooking aids used in bread making like hooks, blades, floats and whisks which comes in cylindrical, cruciform or pear-shaped comprises of wires in various thickness. All these cooking aids can be used for the preparation of desired products, depending upon their properties and required functionality. From the studies, it has been found that the wire whip agitator is more suitable than flat or kneading one for the preparation of gluten-free dough. It is because gluten-free dough has higher flour to water ratio (Gómez et al., 2013). Ultrasound aeration is another way to generate micro-bubbles and gas cells in the batter, in addition to mechanical agitation at small scale. Acoustic cavitations from high intensity ultrasounds are seen to denature proteins due to which they form stabilizing film surrounding the gas bubbles (Chin et al., 2015).

The effect of baking in different types of ovens has also been investigated with gluten-free breads. Demirkesen et al. (2013) analyzed the effect of baking the dough formulations prepared with different combinations of tigernut flour and rice in conventional and infrared-microwave combination ovens. Though, the degree of gelatinization of starch in all the types of bread samples was same at the end, the processing time was longer in conventional breads. On the other hand, Sciarini et al. (2012) described the application of partial baking process on quality aspects of gluten-free breads. In spite of baking in single step, a part-baking process was performed which reduced the baking duration consuming only 63% of the time employed for conventional baking. In addition, the baking could be completed whenever needed while storing the partially baked product at low temperature.

However, the researchers revealed that the breads formed in two-step baking process had lower specific volume and higher crumb hardness due to the presence of small gas cells. These breads also exhibited higher amylopectin recrystallization compared to breads prepared by one-stage baking. However, these limitations were mitigated to a great extent by addition of food hydrocolloids as a processing aid.

7 Evaluating Rheological Properties of Gluten-Free Doughs

Numerous techniques can be used to measure the rheological properties of gluten-free doughs. Rheology of dough has a significant role in the final attributes of baked products, and thus may act as an indicator to optimise the concentration of ingredients and preparation procedures used thereof (Lazaridou et al., 2007). The techniques of rheological analysis are classified on the basis of force imposed on dough. For example force in the form of extension, compression, shear and torsion are applied during small and large deformation measurements (Cauvain, 2012). The measurements involving small deformation are generally creep-recovery and frequency sweep tests, while as those involving major deformations comprise of texture profile analysis, uniaxial extension tests, extrusion tests and resistance to penetration.

7.1 Empirical Measurements of Dough Rheology

Traditionally, the dough quality measurements are classified into empirical rheological measurements and fundamental rheological measurements. Empirical measurements are mostly used in the baking industry to evaluate the flour quality and functionality and to understand the effect of additives (Ronda et al., 2017). A point of similarity among all these tests is that the dough samples are subjected to different degree of deformation, pressure, time and temperature as required for specific tests (Tietze et al., 2016). The empirical measurements can be easily carried out when compared to fundamental measurements and have been employed to characterize bread dough behaviour during various steps of processing. Instruments like farinograph, mixograph, alveograph, penetrometer, texturometer, consistometer, extensograph, rheofermentometer, amylograph, Rapid Visco Analyzer (RVA), Mixolab and other viscometers and the devices that measure the degree of fermentation are most commonly used to analyze the dough handling properties during processing (Cauvain, 2012).

A variety of equipments employed for the analysis of dough can be used to evaluate the different rheological parameters. The Brabender farinograph measures the water absorption capacity and records the changes in dough consistency during mixing under standard conditions. This in turn is an indicative of the performance of flour during bread making (Cauvain & Young, 2007) and helps to determine the optimum mixing time (Tietze et al., 2016). The farinograph results are also used to examine the effects of ingredients on dough mixing and to evaluate flour blending requirements to achieve dough uniformity. A mixograph is similar to a large commercial dough mixing machine for analyzing the dough strength by measuring the water absorption of flour formulations in gluten-free bread making. The textural properties of dough can be determined by the instrument like Texture Profile Analyzer (TPA) (Ronda et al., 2017). Angioloni et al. (2008) analyzed the visco-elastic performance of dough on frozen storage by determining hardness, springiness and adhesiveness in a TA.HDi 500 Texture Analyzer with a probe (5 cm diameter) with processing time 75 s and 60% compression.

The instruments like alveograph and consistometer are also used to determine the rheological properties in which the latter is used to test the dough while adding altering amount of water depending upon the water absorption capacity of the flour (Cauvain & Young, 2007). Alveograph is a visco-elastic recorder to measure the dough strength in weak gluten wheat dough or gluten-free dough. The alveograph blows air into a measured piece of dough patty, which expands into a bubble that eventually breaks. The dough strength is measured as the force required to blow and break the bubble of dough. The Brabender extensograph measures the resistance of dough to stretching and records the distance up to which the dough stretches prior to breaking (Mir et al., 2016). This measurement helps to quantify the extensibility and shows a significant correlation with the baking volume of breads. The effect of additives and fermentation time on dough performance in a gluten-free formulation can also be evaluated in Brabender extensograph. Rheofermentometer is a device used to measure the stretchability and gas retaining capacity of dough. Unlike extensograph, it also measures the amount of carbon dioxide produced and the height developed during fermentation of dough. Rheofermentometer comprises of a sealed and heated chamber in which the dough is subjected to proofing and gains height. The gain in height in the cylindrical vessel and the carbon dioxide escaping are the two parameters measured at the exhaust of bottom of the vessel (Tietze et al., 2016). The Kieffer dough and gluten extensibility rig is also used to evaluate the quality of dough, especially in terms of extensibility (Mir et al., 2016). It is a small-scale version of the Brabender extensograph, where a dough sample of about 0.4 g is extended with lower strain rates compared to Brabender extensograpgh and the results are displayed in terms of stress and strain (Dunnewind et al., 2003). The resistance to extensibility of gluten-free dough can be studied by uniaxial extension tests which can be measured by a TPA fitted with a Kieffer dough and gluten extensibility rig. The dough showing higher extensibility and greater stress at the moment of rupture is said to have improved bread making quality (Burešová et al., 2014). Forward extrusion assay is usually done for formulated gluten-free dough that records required compression force by a piston disk to extrude the dough from a standardized 10 mm diameter size outlet (Ronda et al., 2017).

The pasting properties of dough suspension have been studied using the Brabender amylo/viscograph, Mixolab and/or Rapid Visco Analyzer (RVA) (Cauvain & Young, 2007). RVA is widely used to determine the pasting properties of gluten-free formulations in excess amount of water under continuous cycles of heating and cooling. Under controlled temperature, the apparent viscosity of the dough is analyzed using RVA under a suitable degree of shear force (Ronda et al., 2017). Like RVA, Mixolab analysis also depicts the mixing and pasting behaviours of gluten-free dough but only for the samples with minimal amount of water. The Mixolab is used to carry out the complete characterization of the gluten-free dough by measuring the mechanical changes during mixing and heating. All ingredients used to make dough except yeast are put into the Mixolab bowl and mixed under controlled temperature at 90 °C followed by cooling. The torque (expressed in Nm) developed by the passage of dough between the two kneading arms is measured. This helps to study the physicochemical behaviour of gluten-free dough (Matos & Rosell, 2013).

The common shortcomings of the empirical measurements are difficulty in knowing the amount of strain to be applied to the dough and requirement of large amount of sample. It is also difficult to interpret the results in relative units which are not included in the SI system of measurement, and to predict fundamental rheological parameters such as stress, strain or viscosity. Thus, the replication of empirical tests needs a detailed and elaborated description of experimental conditions. The variability and complexity of ingredients in the gluten-free dough mix and their properties are responsible for the hurdles in discovering a specific method for analysing dough handling properties of gluten-free breads (Ronda et al., 2017).

7.2 Fundamental Measurements of Dough Rheology

One of the greatest advantages of fundamental rheological methods is that the tests are precise. They can be smoothly handled and there is an ease in computation of parameters. The results from fundamental tests can be suitably inserted to mathematical equations which acknowledge mechanical models or the instruments used for analyzing the dough handling properties (Tietze et al., 2016; Ronda et al., 2017). Steady/flow tests, dynamic oscillatory tests (stress/strain sweeps and frequency sweeps), creep and recovery tests, extensional measurements, rheometer and flow viscometry are commonly employed fundamental rheological tests for the characterization of gluten-free dough formulations.

Since gluten-free formulations are more like a batter than like dough, some tests can be adopted to measure their flow properties. To analyze the rheological behaviour of gluten-free dough, the constant stress/strain rate applied for a particular time is measured. Such tests are known as steady tests (Ronda et al., 2017). Oscillatory shear measurements are extensively applied for analyzing the visco-elastic properties, i.e. elastic (G′) and viscous (G″) moduli of dough, and are generally used under deformation conditions which are found inappropriate for bread making. Stress sweeps are commonly used to determine the LVR, or maximum stress, represented as τ max, and it is applied in oscillatory or creep tests to adjust the magnitude of strain in order to prevent the breaking of the dough structure (Lazaridou et al., 2007). Rice flour-based dough is observed to behave as a viscoelastic mass with elastic modulus (G′) greater than viscous modulus (G″) (Gujral & Rosell, 2004; Marco & Rosell, 2008; Ronda et al., 2014). Outside the LVR, both moduli decrease but the decrease in elastic modulus (G′) is usually followed by the decrease in viscous modulus (G″). This changes the solid-like behaviour of dough to more viscous one by increasing the loss tangent (tan $\delta = G''/G'$) (Ronda et al., 2017). The mechanical properties of dough are determined using frequency sweep, by implementing a frequency range usually from 0.1 to 100 Hz with a constant stress selected within the LVR. Often, the G′ and G″ moduli versus frequency, specifically in the range of 1–10 Hz, follow a linear scale. Generally a significant correlation can be found between frequency dependence of viscoelastic moduli of gluten-free dough and the other viscoelastic properties (Ronda et al., 2017). Creep-recovery tests are helpful to draw a correlation with results of empirical measurements. The occurrence of creep and recovery is integrated with reorientation of bonds in the visco-elastic material. In creep relaxation measurements, the stress is made constant and the deformation is analyzed. Whereas in stress relaxation measurements, deformation is made constant and the reaction of force is analyzed. These tests are commonly performed on gluten-free doughs to understand their visco-elastic properties inside and outside the LVR. In creep and recovery tests, the shear strain is generally recorded with regard to compliance, or strain/stress ratio (Pa^{-1}) (Ronda et al., 2017). The extensional techniques involve the methods which are used to evaluate the rheological properties of dough when subjected to extension, where the dough is stretched to two opposite direction by compression between lubricating surfaces or with bubble inflation. It includes simple uniaxial and biaxial extension (Cauvain, 2012).

The water absorption capacity of flour indicates the quality and tendency to obtain the visco-elastic dough. Flour hydration is crucial in food processing unit, as it influences the functional properties and quality of the baked products (Berton et al., 2002). Visco-elastic moduli, RVA and Mixolab parameters of gluten-free dough show correlation with specific volume and crumb parameters of resulting product. Mixolab parameters correlate with TPA parameters of bread. Hardness measured by TPA shows strong relation with the parameters characterizing hydration upon mixing and set-back upon cooling (Masure et al., 2016).

8 Influence of Different Additives on Dough Handling Properties

Commercially available gluten-free breads made from rice, buckwheat, corn and millet compete with their gluten-containing counterparts with respect to their functionality, quality and acceptability (Naqash et al., 2017). The development of gluten-free bread is considered to be a technological challenge due to the inability of gluten-free flours to convert into visco-elastic mass upon kneading with water, keeping in account that there is no single ingredient or additive that can completely substitute gluten. Gluten-free flours need more amount of water than wheat flour to obtain desired crumb structure. Hence, gluten-free dough is less elastic and difficult to handle just like cake batter (Capriles & Arêas, 2014). Gluten-free doughs are, therefore, prepared using machines and the breads developed thereof are largely a starchy material where the degree of gelatinization has a profound influence on the final product quality (Mir et al., 2016). Frequently observed defects of gluten-free breads are improper gas retention and expansion upon leavening, which results in lower crumb softness and bread volume (Mariotti et al., 2009). The absence of gluten leads to the formation of a less viscous dough instead of a visco-elastic mass. This is because the dough formed from gluten-free flour has decreased cohesiveness and elasticity when compared to wheat flour dough (Matos & Rosell, 2015).

Baking industries look forward to ameliorate the dough handling properties by applying extensive approaches suggested by food researchers. Incorporation of various additives has been observed to alter the dough and bread properties, without employing any complex equipment, intensive labour, or longer processing time. These gluten mimicking ingredients are added directly into the flour with other ingredients before mixing into the dough (Tebben et al., 2018). Traditionally most gluten-free products were manufactured from a blend of native and modified flour of rice, maize, buckwheat, soy, sorghum, etc. and starches of rice, maize, potato, cassava, and bean. Korus et al. (2015) in their experiment with gluten-free bread preparation, blended acorn flour with potato and corn starch and an improved dough elasticity and strength was observed. With the addition of acorn flour the elastic (G′) and viscous (G″) moduli increased and phase shift tangent decreased in gluten-free dough. The gluten-free bread exhibited reduced retrogradation tendency and improved sensory acceptance. Quinoa and buckwheat were also supplemented to get an enhanced dough elasticity and dough structure to a gluten-free bread prepared from rice flour (Turkut et al., 2016). In recent years, there has been an increasing trend of the development of gluten-free breads by the incorporation of modified starches, hydrocolloids, dietary fibers, proteins, enzymes and emulsifiers. The use of these ingredients, either alone or in combination has been successfully made to substitute gluten and to enhance the structure, mouthfeel, shelf-life and consumer acceptability of gluten-free breads (Matos & Rosell, 2015).

8.1 Hydrocolloids

Hydrocolloids are complex non-digestible polysaccharides which consist of polar or charged functional groups, making them water soluble. They are used as functional ingredients in various food formulations for improving the consistency, texture, gelling effect, flavour, and shelf life. Hydrocolloids are hydrophilic polymers with high molecular weight which act as structuring agents by interacting with water and forming a gel network that mimics the visco-elastic properties of gluten (Ronda et al., 2017). Most commonly used hydrocolloids in gluten-free bread preparation are hydroxypropyl methylcellulose (HPMC), carboxymethyl cellulose (CMC), carrageenan, alginate, xanthan gumcc and guar gum (Table 1).

Table 1 Dough handling properties of hydrocolloids incorporated gluten-free breads

Gluten-free flour formulation	Hydrocolloids used	Effect on dough handling properties	References
Rice flour and corn starch	Pectin, CMC, agarose and xanthan gum	Farinograph curve, dough elasticity and dough strength increased	Lazaridou et al. (2007)
Cassava flour and corn starch	Guar gum, xanthan gum and HPMC	Elasticity of dough increased	Lorenzo et al. (2009)
Rice flour	Xanthan gum, locust bean gum, guar gum and HPMC	Elastic (G′) and viscous (G″) moduli of dough increased	Demirkesen et al. (2010)
Chestnut flour	Arabic gum, CMC, guar gum and tragacanth gum	Elasticity, apparent viscosity, storage and loss moduli of dough increased	Moreira et al. (2011)
Rice flour, corn flour and soy flour	CMC and xanthan gum	Amylopectin retrogradation decreased	Sciarini et al. (2012)
Chestnut flour and chia flour	Guar gum, HPMC, and tragacanth gum	Apparent viscosity at constant shear rate and storage and loss moduli at constant angular frequency reduced, gelatinization temperatures decreased, dough elasticity improved	Moreira et al. (2013)
Rice flour, buckwheat flour, corn starch, tapioca starch and potato starch	HPMC	Improved leavening properties, increased viscosity due to high dietary fiber	Mariotti et al. (2013)
Rice flour, corn flour and corn starch	Cress seed gum and xanthan gum	Improved creep recovery, elastic (G′) and viscous (G″) moduli of dough increased	Naji-Tabasi and Mohebbi (2015)
Rice flour	HPMC	Elastic (G′) and viscous (G″) moduli of dough increased	Mancebo et al. (2015)
Rice flour and corn starch	Sodium carboxymethyl cellulose	Water absorption capacity and dough stability increased	Nicolae et al. (2016)

8.2 Dietary Fiber

Dietary fibers are the cell wall polysaccharide of plants that resist enzymatic digestion in small intestine but partially or completely fermented in large intestine of the human gastrointestinal tract. Based on solubility, dietary fibers can be classified into soluble and insoluble forms. Soluble dietary fibers are galactomannan, β-glucan, psyllium, pectin, inulin, and resistant starch; while insoluble fibers include cellulose, hemicellulose, lignin and chitosan. The influence of the addition of insoluble fibers from maize, wheat, barley, oats, bamboo, potato, pea, resistant starch and the soluble fibers like inulin, polydextrose, nutriose or β-glucan on the rheology of gluten-free dough and the quality of breads prepared thereof has been extensively

Table 2 Dough handling properties of dietary fibers incorporated gluten-free breads

Gluten-free flour formulation	Dietary fibers used	Effect on dough handling properties	References
Potato starch and white rice flour	Inulin and oats β-glucan	Dough elasticity increased	Hager et al. (2011)
Corn starch, rice flour, rice starch and rice protein	Psyllium and sugar beet fiber	Dough performance during leavening improved, workability of the dough improved, water binding capacity of dough increased	Cappa et al. (2013)
Corn flour and potato flour	Linseed mucilage	Elasticity of dough increased and viscosity of dough decreased	Korus et al. (2015)
Maize flour and starch	Psyllium fiber, pea fiber and oats bran	Water absorption and viscosity of dough increased	Aprodu and Banu (2015)
Rice flour and barley flour	Oats β-glucan concentrate	Viscosity of dough increased	Ronda et al. (2015)
Rice flour and cassava starch	Apple pomace	Viscous (G″) moduli of dough increased	Rocha Parra et al. (2015)
Rice flour, corn starch, brown rice flour and pre-gelatinized corn starch	Maize arabinoxylans	Water absorption and rheology of dough improved, peak viscosity and retrogradation decreased	Ayala-Soto et al. (2017)

studied by researchers (Table 2). The role of dietary fibers in gluten-free dough rheology depends upon the molecular weight, solubility and particle size of fibers (Ronda et al., 2017). For example, inulin, a non-digestible polysaccharide with prebiotic properties was observed to develop even and finely grained crumb texture to gluten-free breads (Korus et al., 2006).

8.3 *Proteins and Enzymes*

Proteins from varied sources like egg, milk and legumes can be incorporated to the dough mix to augment both functional and nutritional profile of gluten-free products. Adding proteins to gluten-free breads increases elastic modulus (G′) in which a major role is played by cross-linking enzymes that link the protein networks together and improve the gelation property. Formation of these protein networks also affects the pasting properties of the gluten-free dough. It decreases the peak viscosity, final viscosity and setback viscosity chiefly because of the starch dilution (Marco & Rosell, 2008). The non-gluten proteins from egg, milk, and legumes like soy bean and pea and the dairy ingredients like whey, caseinate, dry milk and skim milk powder have been added to different gluten-free formulations with the aim of improving the dough structure.

Enzymes like glycosyltranferase, transglutaminase, lactase, glucose oxidase, protease and cyclodextrin are also used in the preparation of gluten-free breads. They enhance the dough handling properties, rheological characteristics and shelf-life of gluten-free formulations (Padalino et al., 2016). The functionality of proteins and starch in gluten-free flours is often modified by adding enzymes that aid the formation of protein networks and improve baking characteristics. For example, the addition of transglutaminase to gluten-free dough has been seen to improve the protein functionality and enhanced crosslinking (Collar, 2019). Table 3 shows the list of proteins and enzymes that have been used in the preparation of gluten-free breads.

Table 3 Dough handling properties of gluten-free breads incorporated with proteins and enzymes

Gluten-free flour formulation	Proteins and enzymes used	Effect on dough handling properties	References
Rice flour	Soy bean and pea protein isolates	Water absorption capacity increased, elastic (G′) and viscous (G″) moduli of dough decreased	Marco and Rosell (2008)
Corn starch, Amaranth flour and psyllium flour	Pea isolate	Physical properties of dough improved by forming film-like structures upon kneading	Mariotti et al. (2009)
Potato starch and corn starch	Albumin, collagen, pea, lupine and soy protein	Enthalpy of retrograded amylopectin decreased	Ziobro et al. (2013)
Rice starch	Albumin, calcium caseinate, soy and pea protein isolates	Dough deformation reduced, viscosity profile increased, elastic (G′) and viscous (G″) moduli of dough increased	Ronda et al. (2014)
Rice flour	Ovalbumin and soy milk	Dough volume increased, gas cell retention improved	Nozawa et al. (2016)
Rice flour	Rice bran protein concentrate	Elasticity and shear resistance increased	Phongthai et al. (2016)
Buckwheat flour, rice flour and chickpea flour	Green mussel protein hydrolysate	Leavening and swelling-emulsion properties improved	Vijaykrishnaraj et al. (2016)
Corn starch, potato starch and tapioca starch	Caseinate and soy protein isolates	Dough strength increased, pasting viscosity increased, dough stickiness decreased	Villanueva et al. (2018)
Marama flour and cassava flour	Marama bean protein	Dough extensibility and elasticity improved	Nyembwe et al. (2018)
Rice flour	Transglutaminase	Gas-holding capacity of dough improved	Gujral and Rosell (2004)
Buckwheat flour and brown rice flour	Transglutaminase	Dough pseudoplasticity improved, water holding capacity increased	Renzetti et al. (2008)
Rice flour	Amylase, cyclodextrin, glycosyltransferase crosslinking enzymes and protease	Gas retention and textural properties of dough improved	Renzetti and Rosell (2016)

8.4 Emulsifiers

Emulsifiers are one of the chief additives used to stabilize gas bubbles during proofing and to improve the crumb texture of gluten-free breads. Emulsifier strengthen the dough, soften the crumb and delay the staling of gluten-free breads (Collar, 2019). Onyango et al. (2009) demonstrated the use of emulsifiers like diacetyl tartaric acid esters of mono- and di-glycerides on dough rheology and texture of gluten-free sorghum bread. The study revealed that the incorporation of emulsifier made the final product with high specific volume and least crumb firmness and staling rate. Lorenzo et al. (2009) demonstrated in their study that dough elasticity increased by the addition of margarine to the gluten-free breads prepared from the blend of corn and cassava starch. Emulsifiers like soy lecithin, glycerol monostearate (GMS), calcium stearoyl lactylate (CSL), sodium stearoyl lactylate (SSL) and diacetyl tartrate ester of monoglyceride (DATEM) are commonly used in gluten-free bread preparation.

9 Technological Approaches Used to Improve Dough Handling Properties of Gluten-Free Breads

Apart from addition of functional ingredients to gluten-free breads, some modifications in processing techniques can also be made to improve the dough handling properties of gluten-free formulations which may include sourdough fermentation, dry heat treatment, heat moisture treatment and high hydrostatic pressure treatment.

9.1 Sourdough Fermentation

Sourdough fermentation is a method in which gluten-free ingredients are combined and fermented by yeast and lactic acid bacteria (LAB). The techniques have been found to yield better results in various gluten-free formulations by improving texture, flavour, taste, nutritional value, and shelf-life. LABs like *Weissella cibaria* (Wolter et al., 2014), *Lactobacillus plantarum* (Moore et al., 2008) and *Lactococcus lactis* (Coda et al., 2010) produce compounds like exopolysaccharides, organic acids and enzymes during sourdough fermentation, which play a major role in improving dough rheology and bread texture. Wolter et al. (2014) demonstrated that dough strength increased after sourdough fermentation in quinoa and teff flour based gluten-free breads. Sourdough fermentation in the dough made with the blend of gluten-free flours from cassava, sweet potato and sorghum exhibited increased elastic (G′) and viscous (G″) moduli (Monthe et al., 2019). This fermentation is used as an alternative or to minimize the use of hydrocolloids in gluten-free breads (Capriles & Arêas, 2014).

9.2 Dry Heat Treatment

Dry heat treatment is a feasible method to improve the quality of gluten-free flour formulations that are weak enough to form a visco-elastic dough. During the process of heat treatment, protein denaturation and partial gelatinization of starch takes place giving rise to dough expansion. Marston et al. (2016) observed that heating the sorghum flour at 125 °C for 30 min in a convection oven produced the breads with a higher specific volume. This was attributed to the increased water absorption capacity and dough viscosity resulting from high time/temperature treatment. Dry heat treatment along with extrusion cooking of gluten-free flours from rye, oats and sorghum was studied by Torbica et al. (2019). This treatment produced less elastic gluten-free breads with more granular structure caused by the higher degree of starch crystallinity.

9.3 Heat Moisture Treatment

Heat moisture treatment (HMT) is a hydrothermal treatment which involves subjecting a gluten-free flour or starch at low moisture level heat treatment at a temperature above glass transition, but below gelatinization (Brody, 2006). The parameters generally taken are in a range of 10–30% moisture, and 90–120 °C temperature for a time period ranging from 15 min to 16 h (Zavareze & Dias, 2011). During HMT, the starch undergoes slight thermal degradation, which increases the apparent amylose content while the soluble fraction of the non-starch polysaccharides decreases. Amadou et al. (2014) studied the effect of HMT and *Lactobacillus paracasei* Fn032 fermentation on the physicochemical properties of foxtail millet flour. The total starch and protein content after fermentation and HMT increased. The physicochemical properties and nutrient profile of the gluten-free breads also improved.

9.4 High Hydrostatic Pressure Treatment

High hydrostatic pressure treatment is an efficient non-thermal treatment which has been widely used to improve the dough structure formation of gluten-free breads. Vallons et al. (2010) in their study demonstrated that high-pressure treatment (above 600 MPa) applied on sorghum dough could alter the structure of starch and protein and cause pressure-induced gelatinization of starch. This increases the dough viscosity and enhances the baking properties of gluten-free sorghum bread. A comparative study was conducted by Angioloni and Collar (2012) to know the effect of high-pressure treatment (350 MPa, 10 min) on nutritional and functional properties of gluten-containing breads and gluten-free breads made by blending finger millet and sorghum flours. High-pressure treatment resulted in gluten-free breads with increased dynamic moduli values, higher antiradical activities and better sensory properties when compared to gluten-containing counterparts. High hydrostatic pressure treatment on gluten-free flours has been also found to increase the elastic (G′) and viscous (G″) moduli of dough (Hüttner et al., 2009; Vallons et al., 2011).

10 Conclusion

The determination of dough properties is a necessity for the estimation of dough processing, dough handling behaviour and quality of final baked product. The balance among three dough handling properties; extensibility, elasticity and tenacity determine the strength and quality of dough in bread making. Since baking without gluten represents a technological challenge, researchers adopted a range of additives and technological approaches to develop gluten-free breads with organoleptic properties comparable to gluten-containing counterparts. The additives used in gluten-free breads mimic the viscoelastic properties of gluten and improves the dough structure, mouth-feel, shelf life and acceptability of final baked products. Mostly the dough handling properties of gluten-free breads are governed by the quantity, nature and properties of additives incorporated in the flour formulation. As per the literature, no raw materials that can completely substitute gluten have been identified till date. However, combinations of gluten-free ingredients coupled with novel processing technologies have been observed to produce gluten-free breads of acceptable quality. Empirical measurements are often used to know the effect of the quality of raw ingredients upon the rheology of dough during bread making. The empirical and fundamental measurements are necessary for the computation of dough properties of gluten-free breads. The interaction of additives with one another and/or with gluten-free flours varies for different gluten-free flour formulations. This changes the rheology and dough handling properties of gluten-free breads made from varied gluten-free cereals. The relationship of dough handling properties with structure, processing and baking techniques is yet to be explored in detail for various gluten-free formulations. In the future, studies should focus to analyze the use of newer gluten substitutes and/or technologies to develop the products worthy enough to replace wheat breads with desirable dough handling properties.

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Structural Aspects of Gluten Free Breads

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1 Introduction

Gluten, a chief structural protein complex of cereals especially wheat, is a predominant bread making asset. The functionality of gluten is mainly ascribed to prolamins that makes sure that extensibility and viscosity of the dough system, and glutenins that result in elasticity and dough strength (Xu et al., 2007). Gluten function, however, becomes apparent on hydrating flour with water which in turn leads to extensive dough, with better crumb structure and gas holding capacities in baked bread. Excessive intake, however, can maximize the prevalence of serious health issues such as celiac disease which is an autoimmune enteropathy characterized by lifelong intolerance to gluten or other gluten-associated allergies (Tsatsaragkou et al., 2015). Concern over these diseases has crafted the need for diets with reduced or no gluten. However, complete elimination of gluten has detrimental effect on bread making process and results in product of poor technical qualities exhibits low specific volume, poor crumb and crust characteristics, and inferior flavour and mouth feel as well as high staling rate (Naqash et al., 2017). Such defects are a consequence of in sufficient retention and expansion of gas produced during fermentation

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yielding product of reduced loaf volume with dry crumbling crumb (Tsatsaragkou et al., 2015). In addition, the dough produced without gluten is liquid in consistency resembling liquid cake batter which lacks cohesiveness and elasticity. Therefore, in order to overcome these challenges extensive research has been undertaken to enhance the rheological, textural and structural properties of GF breads. This can be done by incorporating a range of functional ingredients and additives like non-gluten proteins, enzymes, and other hydrocolloids (Roman et al., 2019) that function by acting as water-binders and film-forming agents, structure enhancers, thickeners, taste-giving ingredients and surface-active substances (Bender & Schönlechner, 2020). Recently, numerous gluten-free formulations are being developed using non-gluten components like hydrocolloids and starches to mimic the viscoelastic characteristics of gluten and to enhance the bread structure. Most gluten-free breads prepared with rice still have weaker physical and textural quality parameters than those prepared with conventional wheat breads. Thus, supplementation of gluten-free rice bread formulation using hydrocolloids or other additives is mostly needed. Recently, successful application of various processing aids have prone to meet the challenges of gluten free breads with improved batter consistency and stability and at same time catering needs of gluten- free consumers. Among these, high hydrostatic pressure or other non-conventional baking processes and sourdough fermentation have come into interest for better textural and sensory outcomes. This chapter provides an overview of various nutritional additives and other technological approaches that allows improvement in structural, textural and nutritional value of gluten free breads that would not only benefit future research but also results in advanced commercial applications.

2 Structure of Gluten Free Breads

In contrast to gluten containing breads, gluten free (GF) baking is technological challenge due to inability of GF flour to form a 3-D protein starch network that contribute essentially to formation of viscoelastic dough capable of retaining gases and allowing dough expansion for formation of soft, light and palatable baked product. In addition GF formulations require considerably higher amount of water for complete starch or starch rich ingredients gelatinization so as to enhance the viscosity and dough gas retention capacity. Since, hydration of gluten free flour results in runny batter that lacks cohesiveness and elasticity and is thus difficult to handle (Bender & Schönlechner, 2020). Further deficiencies in gas retention results in bread with numerous post baking quality shortcomings such as cracked crust, low specific volume, a dry, crumbly and gritty texture, lack of cell structure, poor mouth feel and quick staling of bread. In order to counter these problems several alternative formulations and processing aids are being used. Recently, to mimic gluten viscoelastic characteristics and to further enhance the final bread quality various non-gluten components have been used in conjugation with GF formulations (Salehi, 2019). Such components can mainly function by acting as water- binding and film forming ingredients, structural formers, taste enhancers and emulsifiers (Bender & Schönlechner, 2020). The incorporation of ingredients with increased water absorbing capacity could result in dough with enhanced viscosity and visco-elastic properties which in turn results in product with high specific volume as well as with soft and fluffy crumb (Tsatsaragkou et al., 2015). Similarly addition of emulsifiers stabilize or strengthen dough system by forming complex with amylose component of starch thus restricting its leaching and prevent swelling of starch during baking. It further decreases starch retrogradation process, responsible for crumb hardening (Wronkowska et al., 2013). Hen egg white (EW) and soy protein isolate (SPI) are mostly used functional ingredients in GF bread formulation. They consist of high levels of the essential amino i.e., acids methionine and lysine. SPI improves water absorption capacity and affects batter rheology. EW proteins possess high foaming capacity and stabilizes gas cells during bread preparation. Indeed, as proteins diffuse to and adsorb at the air/water interface, reduces surface tension and adsorbed proteins with improved surface activity form a coherent viscoelastic film around the gas cells (Masure et al., 2019). Corn starch and starch from tubers like tapioca and potato are mostly used in gluten free bread preparation. Due to their functional characteristics, water retention capacity, thickening and stabilizing efficiency, numerous hydrocolloids are mostly used in the preparation of gluten-free breads to enhance their structural characteristics and their acceptability.

3 Improving Gluten Free Bread Structure Using Additives

Despite the fact that GF products do not resemble their gluten counterparts owing to lack of continuous three-dimensional protein-starch matrix that influences dough rheology and overall bread quality, it has become a prerequisite to adopt several approaches for altering gluten network structure and in turn ensure the quality acceptance by people consuming GF products. An ample range of functional ingredients and processing methods have being adopted to imitate gluten viscoelastic properties and consequently the overall final quality of GF product as described in subsequent sections.

3.1 Addition of Hydrocolloids

Numerous studies have showed the potential application of food hydrocolloids in leavened baked products to imitate visco-elastic properties of gluten on account of their hydrophilic and structure binding nature (Anton & Artfield, 2008). They form gel network by interacting with water that serves to increase viscoelastic behaviour and the cohesiveness of the batter thereby strengthen and stabilize the expanding gas cells during proofing for improved gas retention and to a subsequently improve bread volume and crumb firmness.

Among hydrocolloids, HPMC has been found to be very effective in commercial GF product formulations and is used often with other hydrocolloids (like guar gum, fibers or gum, locust bean) to enhance the texture of crumb. Breads prepared exclusively with HPMC are categorized by a drier, crumblier texture. As, HPMC increases bread volume and stabilizes crumb bubble structure.

Liu et al. (2018) ascribed to the thermo reversible gel characteristics of this polymer, that are weakened upon cooling and simultaneously strengthened during baking. Zannini and others (2012) reported the gelling characteristics of hydrocolloids and their effect on crumb porosity, loaf volume, and structure. This might be due to the rise in relative crystallinity by upholding a structural reorientation of the starch matrix into an ordered structure upon the addition of resistant starch. Also, addition of hydrocolloids such as starches to bread can promote in gas retention and the gas bubble expansion during proofing and baking, and improves the structural architecture and mechanical strength of gluten-free breads. Sabanis and Tzia (2011) reported the use of cellulose derivatives viz. HPMC, xanthan, guar-gum and k-carrageenan on textural and structural properties of GF bread. The researchers revealed that HPMC treatment resulted in bread with softer crumb and maximum specific volume. However bread formulated with xanthan exhibited firmer crumb, lowest specific volume and stabilized structure as these hydrocolloids promoted network formation, stabilized gas cells and increased batter viscosity. Further, the microscopic examination of bread crumb shows the formation of aerated and continuous structure, leading to the formation of gel network of HPMC during thermal treatment (Sabanis & Tzia, 2011).

3.2 Addition of Dietary Fibres (DF)

The fortification of DF's in GF formulations have been found to have improve their nutritional and functional benefits resulting in their gel forming and water binding capacity, fat mimetic property and positively imparting the texture of product (Tsatsaragkou et al., 2015). Soluble fibres result in increased gas holding capacity since they get readily dissolved in dough aqueous phase, enclose flour particles and starch granules resulting in homogenous internal structure capable to incorporate a greater volume of smaller bubbles. Coarser insoluble fibres build rupture points in structure of dough resulting in easier gas exchange (Martinez et al., 2014). Different cereals fibers (corn flour, rice flour, and HPMC) have also been examined for their effect on quality of GF formulations. Oat dietary fibres gave rise to bread with higher loaf volume and soft crumb followed by maize than non fiber gluten-free bread. Fibre enrichment improved crust appearance with fine crumb firmness, the dark appearance of the crust and crumb can be due to maillard reaction. The functionality of the dietary fibers is mostly due to their water-holding property, binding effect, rheological behaviour, and bulking capacity.

Prebiotics including inulin, oligofructose and resistant starch are the extensively studied soluble dietary fibers for GF bread preparation. They not only influence host health and well-beings but also contribute to improved gluten-free bread characteristics. GF bread with increasing amount of inulin-type fructans (ITFs) (4%, 8%, 10%, and 12%) resulted in highest loaf volume with softer crumb due to increased water-retention capacity (Capriles & Areas, 2013). The authors suggested that ITFs like other hydrocolloids through their interaction with water result in gel structure that enhance viscosity of batter and toughen the boundaries of expanding cell which in turn resulted in more CO_2 retention and enhanced loaf volume similarly as other hydrocolloids do. RS addition improves bread elasticity and porosity without increasing crumb firmness (Tsatsaragkou et al., 2014a, 2014b) due to significant decrease in cell density. Addition of modified inulin to formulation from gluten-free bread with a desirable structure, textural and sensory parameters, that can be considered as a gluten-free bread enhances. Also, the addition of inulin into gluten-free formulation is responsible for higher loaf volume and reduced crumb hardness. This might be related to the significant reduction in cell density and a significant rise in crumb porosity.

3.3 Addition of Proteins

Along with enhancing nutritional properties, incorporation of protein based ingredients to gluten-free breads improves their structural and textural characteristics (Matos et al., 2014). Mainly used for building structural complex that imitate a few of gluten characteristics, proteins improve baking and rheological characteristics of dough along with enhanced shelf-life and sensory characteristics of GF breads. Dairy products, egg, legume, and cereals are the most commonly used protein sources. However, before formulating, recommending, or consuming protein-enriched GF breads it is essential to take lactose intolerance or allergy issue into concern.

Addition of dairy proteins increase specific volume and enhance the taste, texture, GF bread crust color. Since, preferred brown color of the bread crust relies on caramelization and Maillard browning reactions that rely on milk components such as protein and lactose (Houben et al., 2012). Further Van Riemsdijk et al. (2011) observed the impact of whey protein supplementation on bread and dough characteristics and reported the mesoscopic structure in batter created by whey proteins confers dough like characteristics as well as strain hardening. The mesoscopic protein particle networks tend to imitate gluten characteristics, these mesoscopically structured whey protein dispersion act as a substitute for gluten in the formulation of a dough and a leavened bread.

Similarly, eggs result in good crumb shape and structure by forming a cohesive film essential for foam stability and helps in gas retention during baking (Houben et al., 2012). Ziobro et al. (2013) investigated the effect of various protein sources (pea, collagen, albumen, soy and lupine) on GF dough mixes and reported that rise in specific volume when adding lupine and albumen. Compared to control, crumb hardness and chewiness also decreased. Further, reduction in amylopectin retrogradation was also observed, that indicated reduction in bread staling. However, incorporating casein and albumin protein isolates in rice based dough along with transglutaminase resulted in reinforced protein networks and generated bread with enhanced texture of crumb and improved specific volume (Storck et al., 2013). Same effects were observed by Ziobro et al. (2016). Authors found that replacing gums (guar gum and pectin) with selected protein isolates proteins resulted in changes in the crumb structure. Addition of protein results in relatively high volume and a homogeneous and softer crumb structure. Gas cells in batter system are appropriately stabilized, probably due to the presence of proteins, until the crumb structure (at least partly) set, thereby inhibiting major batter collapse. The stabilization of gas cells in batter system leads to breads with high specific volume and an acceptable/desirable initial crumb structure.

3.4 Application of Enzymes

Enzymes act as dough conditioners to enhance the handling and rheological batter properties and as well to improve final baking quality. Since, GF flours are deprived of characteristic functionality of network formation, enzymatic application may stabilize batter, increase specific volume, soften crumb, improve crust color and maintain bread freshness. According to (Gujral & Rosell, 2004a) addition of 1% TG to rice based-GF bread formula promoted protein network formation that improved dough gas holding capacity yielding bread with increased loaf volume and better crumb texture. Same results were also observed by (Mohammadi et al., 2015) however, increasing TG concentration deteriorative quality of GF bread with increased crumb hardness and chewiness. TG (protein-glutamine γ-glutamyltransferase) catalyzes acyl-transfer reactions, introducing newer inter- and intra-molecular covalent crosslinkage between L-glutamine and L-lysine amino acid residues. TG also converts of soluble proteins into insoluble protein polymers with relatively high molecular weight. This protein network can enhance dough and bread characteristics (such as crumb hardness and chewiness).

Proteases mainly hydrolyze peptide bonds present in proteins and therefore improve machinability and dough extensibility. Renzetti and Arendt (2009) evaluated that bread prepared made from brown rice flour exhibited soft crumb structure and increased loaf volume. The rise in specific volume following protease treatment may be due to the change in the dough viscosity due to protein degradation. Hatta et al. (2015) found that reduction of α- and β-subunits of rice glutelins is vital to enhance quality and texture of bread. Protease degradation resulted in improved gas holding and textural properties. The specific volume was found to increase by 30–60% while as crumb hardness got reduced by 10–30% 10–30% in comparison to untreated bread.

Gujral and Rosell (2004b) revealed that white rice flour by incorporating of glucose oxidase resulted in the formation of bread with enhanced quality reflected from the viscoelastic behavior of dough which is mainly attributed to the protein cross-link formation. In addition it showed affirmative impact on loaf volume and crumb softness. It also have been found that the incorporation of glucose oxidase to wheat flour results in higher volume and improves crumb grain properties, the enhancement in the specific volume lowers the firmness of crumb. Renzetti and Arendt (2009) reported other significant impact of glucose oxidase on GF maize and sorghum breads. Both breads had superior specific volume and decreased collapsing at the top, however no significant effect was found in batter properties and bread quality made from buckwheat flour (Renzetti & Arendt, 2009). α-amylase resulted in crumb softening, increased loaf volume, better crumb and crust colour well as improved flavour in GF bread. Further, enzyme hydrolyze the cleavage of α-1,4 glyosidic starch linkages and also bonds between non-reducing and reducing ends, resulting in closed α-, ß-, or γ-cyclodextrin molecules with 6,7, or 8 glucose units respectively. The resulting cyclodextrins because of their polar surfaces and inner hydrophobic cavity are capable of forming complex with fatty acids and proteins improving the batter characteristics (Gujral et al., 2003a). Gujral et al. (2003b) observed that addition of CGT enhanced loaf volume, reduced crumb hardness and staling rate. However, α-amylase addition enhanced volume of bread, but resulted in a sticky crumb texture. The results thus highlighted the obvious antifirming potential of CGT on rice bread crumb compared to α-amylase (Gujral et al., 2003b).

3.5 Use of Emulsifiers

They are commonly used additives that lower surface tension of an emulsion resulting from two immiscible phases. Because of their surface tension lowering property the dough's gas retention capacity gets increased, further the bread staleness is reduced by their interaction with starch molecules retarding starch retrogradation and enhancing water absorption capacity. Onyango et al. (2009) observed that addition of different emulsifiers (glycerol monostearate, diacetyl tartaric acid esters of mono and di-glycerides, calcium stearoyl-2-lactylate and sodium stearoyl-2-lactylate) positively influenced the texture of GF sorghum bread as implied by strengthening and stabilizing dough system through interaction with gluten network, crumb softening, and reduced staling rate. However, the results varied with the nature and quantity of emulsifier used in the formulations, with better results observed for application of 2.4% w/w emulsifier in flour.

Schoenlechner et al. (2013) observed that breads containing emulsifier (combination of diacetyl tartaric acid esters of mono and di-glycerides and distilled monoglycerides) and enzyme obtained from wheat/proso millet composite flour achieved significant improvement in relative elasticity, crumb structure, dough strength, loaf volume, and crumb pore no. The main property of emulsifiers is their amphiphilic nature, which permits them to migrate and interface between 2 immiscible phases, forming dispersions. Emulsifier also acts as anti-staling agent, decreasing the crumb's firming rate. Furthermore, emulsifier addition increased millet proportion in wheat/prosomillet composite flour.

4 Technological Approaches for Improvements in Gluten Free Breads

Recently many processing approaches are being laid to combat the challenges of producing gluten free breads that are mostly related with insufficient gas retention and expansion during fermentation, which results in reduced loaf volume, crumb hardening and faster bread staling rate. However special attention is emphasised on GF processing aids including high hydrostatic pressure, sourdough fermentation, extrusion cooking or non-conventional baking process for improved technological, sensory and nutritional attributes in GF products.

4.1 Sour Dough Fermentation

This technique is being used for centuries and is still attaining considerable attention. Therefore is still considered as a novel processing aid in GF formulations. Basically, sourdough is an amalgamation water, flour, salt, flour, which is fermented by yeasts and lactic acid bacteria (LAB). The sourdough supplementation has different positive impact on texture, appearance, nutritional parameters and shelf stability of GF breads which mostly results from the metabolic activity of LAB (Capriles & Areas, 2015). While yeast is involved in CO_2 production during fermentation process that results in softer crumb texture of bread, the major dough acidification is caused by acetic acid and lactic acid production by LAB. These by-products mainly affect structure building parameters like arabinoxylans and starch modifying dough rheology by swelling and solubilising gluten proteins. Further, dough acidification also activates some endogenous flour enzymes like protease and amylase which softens crumb. In addition acetic and lactic acid ratio is important, as it influences bread texture and aroma (Arendt et al., 2007). Further, sourdough LAB fermentation has affirmative influence on bread staleness (Arendt et al., 2007).

Since, many LAB can synthesis a long chain sugar polymer called exopolysaccharides, either from extracellular sucrose glycan sucrases synthesis, or intracellularly by glycosyl transferases from sugar nucleotide precursors that improves dough rheological behaviour, shelf life and texture of convenient GF foods (Naqash et al., 2017). In this context Galle et al. (2012) found that exopolysaccharides formed during in situ sourdough fermentation resulted in significant reduction in elasticity as well as dough strength, with dextran exhibiting highest effect on GF sorghum bread quality. Furthermore, controlled sourdough bread with organic acid resulted in bread crumb hardening. However, exopolysaccharides formed in sourdough fermentation shield their impact and resulted in bread with softer crumb in both stored and fresh conditions. These techno functional characteristics of exopolypeptides are related to their water-binding capability. Such properties along with their associated health benefits (prebiotic effect) have aroused their interest in the research field (Arendt et al., 2011). Novotni et al. (2012) found that

GF sourdough batter fermented with *Lactobacillus fermentum* resulted in bread with improved texture and volume characteristics with consequent delay in staling rate. Also a considerably reduction in glycemic index from 68 to 54 g per 100 g was reported with LAB sourdough fermentation.

4.2 High Pressure Assisted Structure Formation

It is a non-thermal treatment that involves application of high pressure which causes protein polymerization and starch gelatinization creating new structures and textures (Vallons et al., 2011). Huttner et al. (2010) examined the impact of HP process on quality of oat bread. The authors found that HP application caused pre-gelatinisation of starch which resulted in increased oat batter elasticity, which in turn enhanced gas retention, improved volume and texture of the resulting bread. Further application of 200 MPa showed reduced staling of oat bread. Thus, application of HP could be extended for studying other freshly baked GF breads. In case of composite cereal matrices (wheat, millet, and sorghum),high pressure caused dough structure rearrangements, apparently by altering protein folding/unfolding and aggregation/disaggregation, assisting the use of HP-treated flours as gluten replacers.

4.3 Hydrothermal Treatments

4.3.1 Extrusion Cooking

Extrusion processing is an important hydrothermal treatment involving application of mechanical strain and heat to flour-water mixture for modify flour functionality by solubilisation of dietary fibres, gelatinization of starch, denaturation of proteins and formation of Millard reaction products. Thus, extruded flours represent alternatives to pregelatinized starch and hydrocolloids for GF formulations (Martinez et al., 2014). Positive impacts of extruded wheat bran, flour. Rice flour and cassava flour on texture and sensory characteristics of bread with and without gluten has been demonstrated (Ortolan et al., 2015). Pedrosa Silva Clerici et al. (2009) proposed extrusion application to rice flour for starch gelatinization promotion and found that varying extrusion temperature and the lactic acid concentration resulted in acidic extruded rice flour which on blending with rice flour (10%) resulted in GF bread with similar crumb and crust texture as that of wheat bread. Extruded rice flour enhanced crumb structure, specific volume, reduced initial hardness as well as delayed GF bread staleness (Martínez et al., 2013).

4.3.2 Dry Heat Treatment

Dry heating is a feasible technique of recuperating bread and cake quality particularly prepared from weak and below par wheat flour. Heat results in protein denaturation and starch granule's partial gelatinization (Neill et al., 2012). In bread formulation, heat treatment has been found to enhance flour resistance, stiffness and viscosity (Gelinas et al., 2001). These parameters cause enhancement of dough elasticity and positively influence oven spring and loaf volume. Marston et al. (2016) investigated the effect of dry heat method on sorghum flour used in preparation of cakes and GF bread. Heating flour for 30 min at 125 °C resulted in bread with enhanced specific volume with more cell number per slice area and cakes with higher specific volume along with utmost cells per slice area. Increased heat treatment caused raise in batter viscosity and water holding capacity which in turn enhanced the volume and structure strength of sorghum flour. The change in viscosity is related with capability of cake to form spongy texture and diminish shrinkage while baking (Martson et al., 2016).

4.3.3 Moist Heat Treatment

Amadou et al. (2014) found that fermentation and moist heat treatment of foxtail millet flour not only presented a possible way of enhancing its physicochemical properties but also contributes to improved nutritional value. Considerable enhancement in starch fractions, total starch, and protein was observed after *Lactobacillus paracasei* Fn032 fermentation and moist heat method. Moist-heat treatment resulted in higher thermal decomposition as indicated by Differential scanning calorimetry (DSC). Nevertheless, moist heat treatment significantly decreased the enthalpy. However both methods greatly increased the slowly digestible starch and resistant starch contents.

4.4 Non-conventional Baking Technologies

An appropriate heating method can have a distinctive influence on product quality such as texture, color and flavour. Non-conventional baking is an increasingly persuaded field in baking because of their attractive advantages such as low time consuming and cost effect processing. These technologies consist of microwave and infrared heating, jet-impingement or a combination of them (hybrid heating) (Chhanwal et al., 2019). Infrared and microwave involve low processing cost and time. Baking with microwave can result in reduced loaf volume, firmer and gummy bread texture, and high staleness rate. Although infrared heating improves bread sensory perception, its low penetrating power makes it difficult for use in baking alone. Another special method of forced convection heating is Jet impingement. The method allows forced hot air currents to invade on bread surface that result in very high and uniform heating. However, the method results in thick crust formation and considerably more energy requirement. Among all these methods, hybrid heating is perhaps the most successful method of baking (Chhanwal et al., 2019). This method not only reduces processing cost but also increases quality of bread. However, the only hybrid method used in GF baking is hybrid infrared (IR)-microwave heating. Most of these studies reported a product with characterized higher moisture loss and firmer crumb. Also, decreased starch gelatinization or digestibility, lower starch granule disintegration and increased flavour loss has been repeatedly reported from hybrid heat generation and absence of crust resulting from hybrid heating. Since microwave results in volumetric heating, it is incapable to ignore major defects while baking, thus combination of this technology along with other rapid heating methods like IR may result in desirable quality of bread (Chhanwal et al., 2019).

Ohmic heating (OH), other non-conventional method of heating having advantages compared with other heating methods since it involves rapid uniform heating. This technique basically involves volumetric heating principle instead of traditional heat transfer by convection, conduction or radiation (Bender et al., 2019). In this method an alternating electric current passes through material that is used as an electrical resistance which causes dissipation of electrical energy into heat. Until now only few studies are available that have applied this technique in formation of GF breads. Since volumetric heating neglects the moisture or temperature differences within the batter or dough, thus can highlight the fundamental mechanisms or interactions that are pronounced less during conventional heating. The technique is basically used to study crumb firming mechanism being mostly affect by moisture difference within bread crumb. Since application of this technology is primarily restricted to study purposes with promising results. Goullieux and Pain (2005) studied industrial implementation of OH by Japan for preparation of "panko" bread crumbs. The technique was found to be energy efficient reducing cost of heating and resulted in simplified production process. The system involves that in a chamber between two electrodes dough is placed. The volumetric heating causes development of crustless bread, which is particularly desired in manufacture of panko. Since crust formation is an important sensory attribute in bread formation, it is a major drawback when using OH. Apart from the above mentioned studies, only few investigations that have focused on bread making using OH was carried out with wheat bread-baking. Very recently, Bender et al. (2019) used this approach for enhancing the GF bread quality. As starch gelatinization mainly controls the gas retention, it results in poor gas-holding ability in GF bread dough. However, the fast method of heating stabilizes the structure of crumb at an initial stage of baking before CO2 is released while heating. In depth, the authors tested the behaviour of different parameters (holding time, power input) while OH on the functional characteristics and breads digestibility. An elevated initial power of 2–8 kW was obligatory for complete expansion of dough. Afterwards two downhill power steps of 1 kW followed by 0.3 kW were applied to bake the surface of bread fully. Compared to conventional baking all these parameters resulted in larger loaf volume with a much fine pore structure. However, starch digestibility of bread was somewhat decreased with higher resistant starch being produced during baking. It was concluded that compared to conventional baking process, OH resulted in various benefits in terms of quality of product, cost-efficiency and processing time and might thus be a promising conventional baking alternative for GF bread preparation. Although microwave baking also involves volumetric heating principle, but it results in significant bread quality defects which have not been seen by OH. Thus, the advantages of combining OH with other surface heating techniques may be a potential upcoming approach for formulation of high quality GF breads.

5 Instrumental Analysis of GF Bread

The GF market is experiencing a notable growth primarily due to increased consumer awareness on health issues associated with gluten such as celiac disease or other gluten associated disorders (wheat allergy) and non-gluten associated disorders. Concern over these diseases has created need for GF foods. Generally GF breads are characterized by crumbling texture, low specific volume, poor colour, and unsatisfactory taste with small shelf-life due to lack of gluten. Introduction of new functional ingredients and processing aids help in formulating food products that are acceptably safer, convenient, and affordable with improved health benefits. However, instrumental techniques can provide an insight of structure–function relationships of food components that are necessary for designing quality GF batter/bread.

5.1 Larger Deformation Measurements

Larger dough deformation measurements are mainly performed when the stress exceeds the yield value of a material. Such deformation is much suitable since it provides good correlation with bread making and can be related to eating quality of bread. Kieffer dough extensibility rig has been used to measure the extensibility of dough and gluten where dough strips is elongated under constant deformation rate. These measurements are only applicable for semi solid materials that allow stretching and proper moulding and include uniaxial extension tests, texture profile analysis (TPA), and penetration resistance. However, GF dough is more problematic for larger deformation measurements since they are more liquid like and cannot form cohesive mass. Hardness, elasticity, cohesiveness, gumminess, stickiness, springiness and resilience are important texture parameters in bakery products having strong association with consumers' perception of bread freshness. These parameters are usually measured by texture analyzer. Firmness or hardness is the most undesirable properties of the gluten-free rice breads as they are devoid of gluten. In wheat breads gluten retards the movement of water molecules, while its absence enhances water movement from crumb to crust that results in firmer crumb structure.

5.2 DSC (Differential Scanning Calorimetry) and XRD (X-ray Diffraction) Measurements

In starch based food processing operations, gelatinization of starch is an essential phenomenon that provides the unique functional characteristics of these products. Understanding kinetics of starch gelatinization is important for designing and optimizing of some process parameters like bread cooking time (Hager et al., 2014). DSC endotherm and XRD pattern can be used to measure quantitatively the staling rate. Usually in freshly baked bread starch appears in amorphous but gradually restructure to crystalline state upon storage. X-Ray diffraction patterns can reflect the starch re-crystallization data and can be correlated with DSC for measuring increase in intensity of crystallinity during storage period of bread. Kadan et al. (2001) found a three times higher melting enthalpy (DH) values of rice bread compared to whole-wheat bread, signifying a strong affinity to retrograde. Addition of hydrocolloids increased the melting enthalpy, indicating higher amylopectin recrystallization during storage of bread. Sciarini et al. (2012) also reported higher moisture content and DH value in DSC studies of GF bread made with hydrocolloids. Generally, during storage, the intensity of crystalline refractions increases as evidenced by enhance in the X-ray diffraction peak intensity. Kadan et al. (2001) ascribed that retrograded rice starch develops 2 peaks between 16.7° and 17.0° in rice bread when stored for 7 days. in addition, the quantity of water and its redistribution while bread storage influences the type of starch crystallites developed in gluten-free breads (Osella et al., 2005). Optimization of a gluten-free recipe by addition of xanthan gum, guargum, chestnut flour, and DATEM resulted in reduced peak intensities (Demirkesen et al., 2014).

5.3 Bread Crumb Structure at Macro and Micro Scale

Confocal laser scanning microscope, Scanning electron microscopy, and image analysis are commonly used for analyzing the microstructure of GF breads. Scanning electron microscopy determines the flour particles size and also the shape and size of the starch granules. The microstructure of bread altered with different types of pseudocereals. Alvarez-Jubete et al. (2010) studied that in bread made from potato starch, smallest flour particle size was detected, followed by buckwheat, rice, and quinoa flours and amaranth. Furthermore, they also reported that the starch crystals size of quinoa flours and amaranth was considerably smaller (<2 mm) however, potato starch granules were considerably larger than the rest of the flours. Besides amaranth, buckwheat, and quinoa starch granules were found to be polygonal, rice starch granules were uneven in shape while potato starch granules were found to be oval shaped.

Confocal laser scanning microscopy of the gluten-free bread samples prepared from pseudocereals (quinoa, buckwheat and amaranth) and rice also showed major variation in their microstructure. Contrary to the control bread samples containing rice flour where starch granules fused together and lost their native structure, the gluten-free breads with pseudocereals had extra starch granules that still hold their integrity because of partial gelatinisation. Moreover, the development of fat globules-starch granules complexes was prevalent in the pseudocereal having gluten-free breads as compared to gluten-free control, results in a more homogenous matrix of fat protein and starch with fewer gas vacuoles and smoother surface starch (Alvarez-Jubete et al., 2010).

Similarly, image analysis is another valuable method for crumb microstructure study. It provides a quantitative examination of numerous physical and structural parameters including cell to total area ratio, total cells, cell number and mean cell area with area less than 4 mm^2. Analysis of crumb grain proved that the mean cell area of optimized GF bread (1.05 mm^2) was equivalent to that of wheat bread (1.01 mm^2). Besides, the gas cell walls of GF bread was thicker (22 cells/cm^2) than those in control white bread (32 cells/cm^2) (McCarthy et al., 2005). Further, enrichment of low protein containing powder in GF bread recipe formed the major gas cells to total area ratio (Gallagher et al., 2003).

6 Conclusion

On part of unique viscoelastic characteristics of gluten, the formation of GF bread with similar structural characteristics and quality as compared to wheat bread is a technological challenge. No raw materials or other additives can as such completely replace gluten. However combination of certain functional ingredients and processing aids could result in gluten free bread with satisfying quality especially for celiac patients. Various functional ingredients like hydrocolloids, proteins, enzymes, dietary fibers are added to enhance nutritional and functional GF bread characteristics. Furthermore, improving structure characteristics in GF bread via sourdough fermentation, high pressure processing, sourdough fermentation and non-conventional techniques enhances protein and starch functionality are promising approaches for improving bread properties and batter consistency and stability. Moreover, wide investigation is still required in this area along with other partial baking methods that can diminish the extra costs and aids gluten free production due to ingredient addition.

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Nutritional Quality of Gluten-Free Breads

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1 Introduction

Bread is one of the convenient, easy to prepare and acceptable bakery product by worldwide population. Bread, mostly available in the market is from the wheat source with good compactable gluten network (Okafor et al., 2012). The presence of crumb matrix of gluten network developed during baking stage from gluten protein is the most important character in bread acceptable by the end use consumers (Dewettinck et al., 2008). However, ingestion of gluten rich diet triggers an inherited immune-mediated enteropathy called celiac disease or gluten intolerance in some genetically susceptible individuals (Cureton & Fasano, 2009). It is one of the most common lifelong disorders worldwide with an estimated mean prevalence of 1% of the general population, affecting the children equally, making it one of the most common chronic disorders in the young people (Elliot, 2018). Consumption of gluten rich diets leads to the severe damage to intestinal mucosa and impairs its functionality (Alvarez-Jubete et al., 2010). Various novel approaches for treating celiac patients have been explored, however the only existing method to treat the celiac patients is consumption of gluten free diet- means the strict avoidance of proteins from the grains like wheat, rye, barely and possibly oats containing the gluten or prolamins. Among the gluten free products, bread is most commonly consumed by celiac patients. But the manufacturing of gluten free bread is most

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challenging for the manufacturers due to lack of alternative ingredients that can mimic the functionality of wheat protein (Roman et al., 2019). Various researches have been done to develop gluten free bread from non-wheat sources to alternative as bread for gluten intolerant people. The gluten free bread has been developed from gluten free sources such as rice, maize, pseudocereals, sorghum and legumes with good sensory and nutritional constituents (Melini & Melini, 2019; Wu et al., 2015; Missbach et al., 2015; Cornicelli et al., 2018; Roman et al., 2019; Houben et al., 2012; Rai et al., 2018). The structure network of the gluten free breads were developed by incorporation of additional ingredients as a substitute for gluten proteins. The protein network ingredients used in gluten free breads were eggs, hydrocolloids and pulse protein isolates (Rai et al., 2018). The nutritional compositions especially proteins of gluten free breads are low as compared to white bread, but novel approach of multigrain bread from non-gluten sources can mitigate the lower protein content of gluten free breads. Nutrient dense ingredients from fruit, vegetables, pulses, eggs and milk have been incorporated to improve the nutritional quality and acceptability of gluten free breads for celiac patients.

Nutritional quality of gluten free products were reported lower in protein, fiber and carbohydrates but relatively higher in total and saturated fat as compared to gluten rich products and lower sensory attributes (Missbach et al., 2015). The consumer adhered to gluten free diet showed symptoms of unbalanced intake of carbohydrates, proteins, fats as well as certain intake of certain essential nutrients and vitamins (Hager et al., 2011). The fat content in gluten free diet was higher as compared to recommended level of fat and thus may result in diseases associated with higher fat levels (Melini & Melini, 2019) and lower intake of proteins (Shepherd & Gibson, 2013) resulting a diet with unbalanced of nutrients. The low nutritional quality of gluten free products requires nutritional improvement from non-cereal sources and focused to increase their organoleptic properties. The gluten free breads are one of the main targeted gluten free products with wide markets among the celiac consumers and focused on improvising their structural and nutritional property. Gluten free breads were evaluated for nutritional parameters such as protein, fat, fiber, ash, carbohydrates and energy values with their quality which was significantly lower than gluten containing breads. So the nutritional profile of gluten free bread raised the concern related to health issues of people suffering with celiac disease. In order to overcome the unbalanced diet in gluten free bread, nutritional ingredients were added. Hager et al. (2011) suggested that the gluten free ingredients that possess the dense nutrients have potential to enhance the nutritional profile of gluten free breads. The dense ingredients added to enrich the gluten free bread were pseudocereals, milk proteins, fruit and vegetables ingredients, pulse flour and their proteins sources (Kupper, 2005; Hager et al., 2011).

2 Protein Quality of Gluten Free Bread

From the nutritional point of view, protein is most fascinating, as it deals with the essential fabric of growth, repair and reproduction and with various other dynamic expressions for vital activities in human body. The protein content in bread besides nutritional quality also play important role in structural integrity of breads. The protein responsible for structural support in bread with main property of viscoelastic behaviour is due to gluten protein. Gluten is known for its structure binding, contributing to the appearance, crumb structure and consumer acceptability of breads (Arendt & Dal Bello, 2011). The protein helps in building the structural network in bread making process and is main character feature in bread quality. The gluten free bread without use of gluten protein source imparts baking problem, low protein quality and unacceptable by consumers (Houben et al., 2012). Various researches have been done to develop gluten free bread from protein rich source such as pseudocereals, legumes, dairy ingredients and eggs that result in structural support and enhancing the sensory quality by increasing the milliard browning and flavor (Deora et al., 2015). The protein enriched ingredients (albumin, collagen, pea, lupine, and soy) in gluten free bread enhance the protein content and amino acid profile of breads as compared to the gluten bread (Ziobro et al., 2016). The protein enriched breads showed enhance quality of bread parameters with good sensory acceptance and balanced composition of amino acid with good quantitiesof lysine. In order to enhance nutritional properties of gluten free bread, the focus turned towards the use of pseudo cereals amaranth, quinoa and buckwheat, as an alternative source of protein in bread (Jnawali & Tanwar, 2016). The gluten free breads enriched with amaranth flour showed increase in protein level of breads and promised source for development of gluten free bread (Gambus et al., 2002). The pseudocereals like amaranth are good source of proteins with their quality of proteins similar to milk proteins and are excellent source for enrichment of proteins in gluten free breads (Matos & Rosell, 2015). Bread from amaranth, quinoa and buckwheat flour showed significant improvements in gluten free bread making with increased protein content to 11.60%, 10.60% and 8.4% from 4.2% in gluten free breads without any fortification (Alvarez-Jubete, 2009). Two fold increase in protein content of gluten free bread was observed by replacing the rice flour and corn starch with 50% flour of amaranth, quinoa and buckwheat flour (Thompson, 2000; Kupper, 2005; Thompson et al., 2005; Pagano, 2006). The teff also been used as enrichment ingredient to enhance the protein quality of gluten free bread with high bioavailability and digestibility due to low amount of prolamine (Parades-lopez, 2018). The mixture of ingredients such as amaranth, teff and quinoa was used to increase the protein content of gluten free bread (Rybicka et al., 2019). Pulses flour and protein concentrates are protein rich ingredients with good amino acid profile enhance protein quality and functional property, bread quality parameters and sensory quality of gluten free breads (Marco & Rosell, 2008). Enrichment with pulse proteins enhances the viscoelastic properties of dough resulting in the formation of high quality gluten free products such as bread (Sofi et al., 2020a, 2020b). Lupine and pea proteins have been used in the formulation of gluten free breads with increase in protein content, bread structure and better sensory parameters (Ziobro et al., 2013). The bread from rice flour with well-maintained structural integrity and good protein content was prepared by addition of hydroxypropylmethylcellulose (HPMC) and transglutaminase (Marco & Rosell, 2008). Surmi is a myofibril protein from flesh of fish with protein content of 20% (Thompson, 2009). The surmi as protein enrichment with rice flour and potato starch for gluten free bread development showed significant results on protein content, baking and textural properties of breads (Gallagher, 2005). Collagen as structural and protein ingredient have been used in development of gluten free breads (Ziobro et al., 2013). Various types of protein sources were used to increase protein content and structure of gluten free products (Sofi et al., 2019, 2020a, 2020b) Protein fortification reduced the deficit amino acids and increased the structural and sensory properties of gluten free breads. The various other types of protein sources such as rice, pea and soy are added either as concentrates or isolates followed by addition of other supplements like polysaccharide hydrocolloids, enzymes or surfactants increased nutritional protein and quality parameters in gluten free breads (Deora et al., 2015). Zein and kaffirin from cereal have been applied for gluten free bread formulation (Pontieri et al., 2013). Positive influence of zein proteins in presence of hydrocolloids have been observed on dough rheology, bread structure and its volume (Andersson et al., 2011). Various authors reported dairy protein as another source of protein supplementation in gluten free bread formulation (Deora et al., 2015; Krupa-Kozak et al., 2013; Van et al., 2011). Incorporation of dairy proteins in gluten free bread positively influences the structure, improves texture and appearance and slows down process of ageing. Caseins which possess the emulsifying properties and stabilize the other components of gluten free dough, are most frequently used dairy proteins. Besides, casein, whey protein isolates and concentrates and skim milk powder are used due to their ability of forming gel like structures and high water binding capacity, respectively (Deora et al., 2015). Albumin of egg protein is considered another source of protein enrichment of gluten free breads due to its ability to stabilize foam that helps in retention of gas and stabilization of crumb (Schoenlechner et al., 2010; Tsatsaragkou et al., 2014).

3 Starch Quality of Gluten Free Breads

Starch is the biopolymer present in cereal and non-cereal products as the concentrated source of physiological energy in the human diet. Starch is the most available form of carbohydrates and mostly concentrated in the endosperm region of the seed. Starch besides nutritional importance plays important role in bread making due to its contribution to gluten dilution, water absorption from gluten due to gelatinization which contributes to bread structure permeable to gas, so that bread doesn't collapse while cooling (Miyazaki et al., 2006).

According to the European Food Safety Authority (EFSA), the intake of total carbohydrates including starch and simple sugars should range between 45% to 60% of total energy for both adults and children (Hager et al., 2011). The gluten containing bread is rich source of starch and has a high glycemic index. Gluten free bread possess lower carbohydrate content as compared to gluten containing bread due to dilution factor of added ingredients like milk or whey powder, soy protein concentrate, egg albumin, rice or lupine protein during gluten free bread making (Allen & Orfila, 2018). The nutritional quality of carbohydrates was increased in the gluten free bread by adding starch from various conventional sources such as tubers, pulses, fruits and vegetables. The energy profile of gluten free bread showed a comparable energy content due to presence of carbohydrates (Cornicelli et al., 2018). The gluten free bread from rice flour, corn starch and buckwheat flour showed increase in carbohydrates with better bread quality (Thompson et al., 2005; Pagano, 2006). The gluten free bread from pseudocereals showed good source of available carbohydrates as compared to breads from other gluten free sources such as rice flour and potato starch (Thompson, 2000; Kupper, 2005). The replacement of rice flour and corn starch with the 50% pseudo cereal flour in bread making led to the significant increase in carbohydrates content (Thompson et al., 2005; Pagano, 2006). Corn starch in combination with hydrocolloids (xanthan, guar, locust bean and traganth gum) in gluten free bread formulation enhanced available carbohydrate content as well as bread volume and loosening of the crumb (Acs et al., 1997). The gluten free bread prepared by adding starch sources from conventional types in bread formulation enhanced energy value of 261 kcal/100 g (Hager et al., 2011).

4 Dietary Fiber Quality of Gluten Free Bread

Dietary fiber is related to total content of polysaccharides and lignin components which are not digested and assimilated in gastrointestinal tract. The various components considered as a dietary fiber are cellulose, hemicellulose, pectin, lignin and resistant starch (Sofi, 2017). The dietary fiber are complex polysaccharides with both physiological and food functional activities due to its physicochemical nature. The dietary fiber is associated with various physiological and health benefits such as prevention against cancer, diabetes, obesity and improve gastrointestinal health (Sofi & Singh, 2017). Dietary fiber is often added as processing aid for improving the quality of processed products (El-Khoury et al., 2018). Gluten free breads are low in total dietary fiber content as compared to the bread formulated from wheat flour and thus research was focused on increase in dietary fiber of gluten free bread with dietary fiber rich ingredients. The gluten free bread fortified with dietary fiber ingredients such as maize fiber by (Sabanis et al., 2009), oat beta-glucan isolate (Lazaridou et al., 2007), rice bran (Phimolsiripol et al., 2012), resistant starch from corn and tapioca sources (Korus et al., 2009) to increase their dietary fiber content with better bread quality.

Fruits and vegetables are good source of dietary fiber with better functionality in terms of quality of the products. Quality of gluten free bread was improved with fruit and vegetable pomaces as source of fiber. Oshea et al. (2014) reported an increase in dietary fiber of gluten free breads with added orange pomace and raised the fiber content of gluten free bread to 3.9% as compared to wheat bread (2. 1%). Green plantain flour is rich and easily available source of dietary fiber (Aurore et al., 2009). Incorporation of green plantain flour in gluten free bread showed increase in dietary fiber content with improved color, resistant starch and bread making quality of bread (Mohamed et al., 2010; Sarawong et al., 2013). On the other hand, white bread is known to contain low resistant starch content (Fuentes-Zaragoza et al., 2011).

The various alternate sources of fiber enrichment in gluten free bread such as beta glucans, inulin, linseed mucilage, apple pomace, oligofructose, bamboo fiber, polydextrose and resistant starches was reported by Sciarini et al. (2017) to increase the bead quality and fiber content. Gluten free bread fortified with multi-seed flour showed increase in fiber content to 3.9 to 14.20 g per 100 g as compared to white bread with an average dietary fiber content of 4 g per 100 g, (Hager et al., 2011). Enrichment of defatted black current and strawberry seed powder increased the dietary fiber of gluten free breads (Korus et al., 2012). Rice flour, corn starch and pseudocereal enrichment showed increase in nutritional quality with double fold increase in dietary fiber content of gluten free bread (Thompson et al., 2005; Pagano, 2006).

5 Vitamins and Mineral Quality of Gluten Free Bread

Vitamins and minerals are essential nutrients that help in normal functioning of various metabolic activities in human body. Vitamins and minerals play role in enzyme functioning, help in bone formation and boost immune system. They also convert food into energy, and repair cellular damage. Like other nutrients, gluten free products are deficient in vitamins and minerals (Saturni et al., 2010) and among the minerals, iron is the most deficient mineral in celiac patients from 12% to 69% (Halfdanarson et al., 2006; Tikkakoski et al., 2007) and in vitamins, vitamin complexes are most deficient one with a range from 8% to 41% (Halfdanarson et al., 2006; Dahele & Ghosh, 2001). Celiac people were reported to the deficient in iron, folate, phosphorous, calcium and vitamin (Ojetti et al., 2005). In order to overcome these nutritional deficiencies, a strict gluten free diet with enriched vitamins and minerals is recommended for the celiac patients. The gluten free bread was improved in calcium, magnesium, iron and zinc content by using pseudocereals in bread making (Thompson et al., 2005; Alvarez-Jubete, 2009). Iron enriched gluten free bread was developed by iron rich sources such as ferric pyrophosphate, ferric pyrophosphate with emulsifiers, electrolytic iron, ferrous gluconate, ferrous lactate and ferrous sulphate to increase the iron content (Kiskini et al., 2007). Supplementation of millet flour and buck wheat flour in gluten free bread increases the mineral content (iron, zinc, calcium, potassium, phosphorous, magnesium and copper) in gluten free bread than bread from wheat flour (Sayed et al., 2016). Calcium and magnesium content was increased with addition of teff, amaranth and quinoa in gluten free breads (Rybicka et al., 2019). The increase in minerals and vitamins for development of gluten free bread was observed with the addition of pseudocereals and millets (Marpalle et al., 2014; Rybicka et al., 2019).

6 Lipid Quality of Gluten Free Bread

The lipids provide essential energy support and cell growth to the body. They help in functioning of various hormones and physiological activities for normal metabolism. Besides the nutritional importance, lipids provides structural support in bread making with proteins and starch in gas cell stabilisation and their impact on bread loaf volume, crumb structure and crumb firmness. Fat content in bread enhances its palatability (Drewnowski et al., 1992). Gluten free bread was reported to be higher in fat content 9.7% as compared to gluten containing bread with fat content of 3.6% (Berti et al., 2004). The increased fat content in gluten free bread restrict its use by obesity and overweight celiac people and hence need of reducing the fat content of gluten free bread is need of the hour. Enriched gluten free ingredients with low fat content can solve the problem of gluten free breads. The white bread was reported 1.87% fat content with lower fat content as compared to gluten free bread from teff, amaranth and rice flour. The gluten free bread from rice flour was lower in fat content as compared to pseudocereal based gluten free bread (Alvarez-Jubete, 2009). The gluten free bread from teff and pseudocereals were rich in oleic acid and linoleic acid (Matos & Rosell, 2015). Fatty acid profile of pseudocereal based gluten free bread was rich in stearic acid, oleic acid, linoleic acid, mono and polyunsaturated fatty acids (Alvarez-Jubete, 2009). Among the pseudocereals, gluten free bread from quinoa flour was reported higher in fat content as compared to other pseudocereals (El-Sohaimy et al., 2019). The fat content in gluten free bread can be reduced by incorporation of fruit and vegetable ingredients, less use of shortenings and reduced use of fat rich gluten free ingredients in bread making.

7 Conclusion

Gluten free bread is a one of the challenging type of gluten free products to satisfy the needs of end use celiac consumers in terms of nutritional quality. The gluten free bread available in market is deficient in protein, fibre and micronutrients and high in fat content and hence reduced acceptability among consumers. The unbalanced nutrient compositions of gluten free breads are one of the targeted approaches to increase their nutritional profile. The balanced composition of gluten free bread could be approached by using non-gluten sources with high nutrient dense ingredients. The nutrient dense ingredients source for gluten free bread making were used are pseudocereals, fruit and vegetable, pulses, animal sources, milk and milk products and egg based products to modify their nutritional quality in relation to their use to celiac consumers. Future aspect of gluten free bread however needs more research on developing the bread with balanced nutrient ingredients to alter their nutritional profile and effective diet for celiac people.

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Glycaemic Response of Gluten Free Bread

K. Devi

1 Introduction

History of gluten free breads dawned in association with the prevalence of Celiac disease. Celiac disease was confirmed in association with gluten sensitivity in accordance with the finding that the severity of the symptoms of celiac diseases were reduced through gluten removed diet by Dike in 1953 (Dicke et al., 1953). The technology of gluten free breads (GFB) are focussed devoid of cereal prolamines in composition contradictory to typical breads so that the dietetic requirements can be attained to alleviate the gastro enteropathic conditions in Celiac disease. However, GFB contains high amount of sugar and lacks of dietary fibre, vitamins and minerals, essential nutrients (Wild et al., 2010). Celiac disease has also been reported for the associative diagnosis of type I diabetes (Cronin & Shanahan, 1997), which is astonishingly diagnosed prior to CD in 90% of celiac cases (Holmes, 2001). Hence gluten free diet including bread and the pathology of celiac disease are found to be associated with alarming glycaemic response and necessitates the importance of gluten free diet consisting of essential nutrients as well and maintaining the glycaemic response not predisposing to diabetes mellitus. This chapter covers the related concepts and factors to be considered in the production of gluten free bread with quality and low glycaemic index.

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2 Concept of Glycaemic Index

Glycaemic index (GI) is a contemporary term, was first used in 1980s (Grant & Wolever, 2011). GI is defined as a measure of the potential of increasing the level of blood glucose upon the consumption of carbohydrates containing foods in certain quantity with reference to white bread or glucose. Therefore GI refers to the absorption of carbohydrates in foods. The classification of foods based on the values of GI in to low GI foods (<55), intermediate GI foods (>55 to <70) and high GI foods (>70) is used in the dietary management of impaired glycaemia associated disease conditions (Dona et al., 2010).

GI estimation is conducted through in vivo and in vitro methods. In vivo method should be involved with human subjects, expensive, require diligent skill in protocol and time taken ethical procedures. In vitro method yields estimated GI, which is the predicted GI of in vivo method as calculated from the following equation (Granfeldt et al., 1992).

$$eGI = 8.198 + 0.862HI$$

Hydrolysis index (HI) is calculated as the ratio of area under curve for the hydrolysis of test sample to that of white bread as a standard for 0 to 180 min as obtained from the hydrolysis curve.

3 Enzymatic Hydrolysis of Starch

Glycaemic index depends on the digestibility or hydrolysis of starch. Chemistry of starch shows that starch is composed of amylose and amylopectin with glycosidase linkages. During hydrolysis, glycosidase linkages are cleaved and hence freed amylose and amylopectin molecules are degraded in to glucose, which is then absorbed in to blood stream to increase glycaemic level. However, enzymatic degradation of starch is rapid in gelatinization of starch than the subsequent starch changes including gelation and retrogradation with the effect of moist heat methods and dextrinization of starch with the effect of dry heat methods. Starch is hydrolysed in to three fractions based on the extent of hydrolysis (Englyst et al., 1996).

Rapidly Digestible Starch (RDS) is referred by its rapid hydrolysis in small intestine and hence can be calculated as the amount of digestible starch that is hydrolysed during the first 30 min of hydrolysis.

Slowly Digestible Starch (SDS) is referred by its slow hydrolysis in small intestine so that the increase in glycaemic response and insulin level (Englyst & Hudson, 1996). It is calculated from amount of starch that is hydrolysed between 30 and 120 min of hydrolysis.

Resistant Starch (RS) is referred by the characteristic of being resistant to hydrolysis in small intestine and pass in to large intestine in the form of intact starch and retrograded starch differed from RDS and SDS that are absorbed in small intestine (Englyst & Cummings, 1990). It can be calculated from the amount of starch remained not hydrolysed even beyond 16 h of hydrolysis.

Generally the amount of hydrolysed starch is estimated at 90 min during hydrolysis irrespective of estimation of the above hydrolysed fractions of starch to determine the digestibility of starch. Since hydrolysis of starch is determinant in glycaemic response, the amount of fractions of starch have a high impact on glycaemic response of foods.

In the processing of bread, cereal starch is likely to get completely gelatinized for enzymatic degradation to the high glycaemic index (Parada & Aguilera, 2011). Enzymatic hydrolysis of starch in bread has been reported to be influenced by several factors including structure of granules, the ratio of amylose and amylopectin in starch granules, the presence of proteins, lipids or minerals, particle size and digestion conditions (Al-Rabadi et al., 2009).

4 Celiac Disease and Diabetes Mellitus/Blood Sugar Level

Celiac disease is globally prevalent around 1% in general populace (Fasano et al., 2003) and increases five to seven folds along with type 1 diabetes (T1DM) (Gillett et al., 2001; Schuppan & Hahn, 2001; Mahmud et al., 2005). Similarly, the research in United States and Europe also reported in general population, but up to 16% of prevalence of celiac disease with the impact of TIDM (Guptar et al., 2009; Mustalahti et al., 2010). In the study in North India, the associative prevalence of celiac disease with TIDM was reported to be 11% (Bhadada et al., 2011). Hence the highest prevalence of celiac disease was established in association with TIDM.

The coexistence of celiac disease and T1DM is attributed to the overlapping genetic and environmental factors. Genetically, genotypes of HLA genes play a role in the concomitant occurrence of celiac disease and TIDM. HLA is major histocompatibility complex, predominantly, class II with DQ peptides particularly DQ2 and DQ8 are found in patients suffering from both CD and TIDM disease conditions due to the production of antigliadin antibodies predisposing to mutual causative effect between TIDM and CD. Patients lack of HLA DQ2 and HLA DQ8 genotypes are not vulnerable to the coexistence of TIDM and CD (Sollid & Jabri, 2005). Environmentally, lack of breast feeding, early introduction of gluten and viral infections are common risk factors for both CD and TIDM (Green & Jabri, 2006).

Celiac disease show the minimum or absence of its typical gastro intestinal inflammatory symptoms in association with TIDM (Fasano & Catassi, 2001), but observed with the symptom of poor glycaemic control (Leeds et al., 2011) that can be controlled with gluten free diet (GFD). Hence the formulation of GFD is an optimum dietary intervention for reducing the severity of coexistence of CD and TIDM and the processing of gluten free bread (GFB) gains importance in the context of intervention.

5 Processing Factors for Low Glycemic Index

Generally the glycaemic response of breads is high due to its easily degradable and absorbable starch in to gastro intestinal territory. In the process of bread making, kneading of dough and fermentation leads to porous structure in breads and baking at 250 °C gelatinizes the starch in breads. These porous structure and gelatinization releases starch for enzymatic degradation and the increased release of glucose in to blood stream.

Foster – Powell table depicts the variability in GI among ninety five types of breads (Foster-Powell et al., 2002) and analysis gives the insight on the parameters controlling glycaemic response of breads through selection of raw ingredients and modification in baking process.

5.1 Amylose: Amylopectin Ratio

Starch consists of amylose and amylopectin molecules; amylose in linear structure is less accessible to hydrolysis by α amylase and amylopectin in branched structure is easily degradable by α amylase. Hence cereal flours or flour mixes containing more amount of amylose can result in bread with low GI (Akerberg et al., 1998).

5.2 Interactive Components with Starch

Starch is likely to be bound with proteins and fats and encapsulated in fibres and become less accessible to breakdown by α amylase resulting in low glycemic response. Therefore the baking mix may be added with either of these interactive components like soluble or insoluble fibres, soy flour for protein and monoacylglycerols for lipids to reduce the GI of bread.

5.3 Incorporation with Intact Grains

Whole grains or unmilled grains holds insoluble fibres that can act as a physical barrier against α amylase (Liljeberg et al., 1992; Holm & Bjorck, 1992) and also gastric emptying is delayed for poor absorption of glucose. Hence flour incorporated with intact grains results in breads with low GI.

5.4 Soluble Fibres

Soluble fibres are soluble in digestive content and increase its viscosity and consequently digestive contents are slowly emptied from stomach and delayed in reaching small intestine, affecting the diffusion and absorption of glucose in to intestinal mucosa cells. Soluble fibres like β glucan, arabinoxylans (Lu et al., 2000, 2004), guar gum, psyllium fibre (Wursch & Pi-Sunyer, 1997) were proven for reducing glycemic index.

5.5 Organic Acids

Generally bread is formulated with sodium propionate and calcium lactate for the production of lactic acid and propionic acid. Even bread is consumed with vinegar. This acidic environment is related to the delay in gastric emptying and to the interaction between starch and proteins (Ostman et al., 2002), which are related to reduction in glycemic response., Sour dough based leavened bread has an effective glycemic response than bread baked with yeast (Adam et al., 2003).

5.6 Baking Settings

Conventionally breads are baked at 200 °C for 45 min. Baking at different conditions were found to reduce glycemic index. Barley bread with high amylose was baked at 120 °C for 20 h and found for increased amount of resistant starch around 10% due to the reaction of annealing for crystalline amylose (Akerberg et al., 1998).

6 Glycaemic Response of Gluten Free Breads

The typical formulation of gluten free breads is nutritionally composed of lack of gluten protein, essential nutrients and dietary fibre (Hager et al., 2011) so that the glycaemic value is higher than gluten bread (Berti et al., 2004; Matos & Rosell, 2011). Gluten is attributed to form protein network around starch granules limiting the hydrolysis by α amylase and consequently a relatively slow glycaemic response from gluten breads (Jenkins et al., 2002). However, approaches have been undertaken for modification in the formulation of gluten free breads with low glycaemic index taking in to account the importance of controlling pathogenic glycaemic response in the mutual occurrence between celiac disease and TIDM.

6.1 Fibre Enrichment

Dietary fibre plays a functional role in the modification of rate of digestion and absorption of carbohydrates, proteins and fats (Capriles & Areas, 2013). Despite many studies on GF breads optimized with the incorporation of fibres for technological quality, few studies focussed along with glycaemic values. Rice bread was formulated with the incorporation of insoluble fibres including oats fibre (OF) and resistant starch (RS) and soluble fibre like inulin (In) at 5% and 10% levels (Sciarini et al., 2017). Insoluble oat fibre and soluble inulin fibre at 5% level were shown for increased GI values while decreased GI values at 10% level. RS was found for decreased GI at both 5% and 10% levels as compared to control bread. This glycaemic effect respective to types and amount of dietary fibre is attributed to the changes in the structure of bread. At 5% level of insoluble fibres, the structure was inhibiting the network between starch and protein and starch is thus freely available for enzymatic action whereas fibres at 10% level were physical and chemical barrier like encapsulating the starch or sugars being restricted to the access of enzymes (Englyst et al., 1996). Soluble insulin was found to form the layer around starch granules restricting the swelling of granules and leaching of amylose to decrease the GI of bread (Vazquez-Gutierrez et al., 2016). In vitro and in vivo glycaemic responses were also decreased at 12% level of addition by 10% and 30% respectively (Capriles & Areas, 2013).

6.2 Processing Conditions

In GF bread making, the processing parameters have been approached for low glycaemic index. Rice bread was formulated with the variation in particle size of rice flour and water content in dough making (de la Hera et al., 2014). Rice flour was processed in to fine flour and coarse flour with the particle size of less than 132 μm and between 132 and 200 μm respectively. Dough was made with the water content at 70%, 90% and 110% levels. The impact of variation in water content and particle size was observed on the degree of hydrolysis of starch by α amylase and the amount of hydrolysed starch fractions were varied correspondingly. Rapidly digestible starch fractions (RDS), slowly digestible starch (SDS) and resistant starch (RS) were in the range between 82.07 and 96.54 g, 0.60 and 11.40 and 0.89 and 1.96 g/100 g respectively similar to the GI among commercial GF breads (Matos & Rosell, 2011). Coarse flour bread and fine flour bread only with 70% of water content showed the greater amount of SDS and RS while other breads showed greater amount of RDS.

Glycaemic index was observed corresponding to the amount of hydrolysed starch fractions as observed from low glycaemic index for breads with high RS and SDS and high glycaemic index for the greater amount of RDS in breads. However the impact of water content alone was reported on glycaemic index while the impact of both water content and particle size were reported on the degree of hydrolysis of starch in breads.

The increase in particle size of flour fractions, approximately more than 150 μm, reduces the surface area of starch granules accessible for hydrolysis by α-amylase (De la Hera et al., 2013), whereas fine fractions with a larger surface area undergone to a relatively higher degree of gelatinization at 90% and 110% of water content to release the amyloses for degradation by α-amylase enzyme (Tahir et al., 2010). Ground corn starch were found for each 100 μm increase in particle size to reduce the accessibility of α-amylase by 26.8 g/kg starch in grains (Blasel et al., 2006).

Glycaemic index was influenced only with the effect of water content as observed from the increased glycaemic index at 90 and 110 levels of water content and decreased glycaemic index at 70% level of water content irrespective of particle size of flour fractions. The effect of adequate water content is attributed to the gelatinization of starch and subsequent enzymatic hydrolysis and absorption of glucose (Roder et al., 2009) contradictory to the inadequate water content leads to low degree of gelatinization along with compact structure of bread for low glycaemic index (Fardet et al., 2006).

7 Conclusion

Gluten free breads with low glycemic index could be processed through the selection of raw ingredients and modification of processing conditions to limit the gelatinization of starch and accessibility of starch to α amylase along with other physiological effects like delayed gastric emptying and diffusion and absorption of glucose in to blood stream. Glycemic response is varied with the amount of Rapidly Digestible Starch (RDS), Slowly Digestible Starch (SDS) and Resistant Starch (RS) with the influence of factors such as structure of starch granules, the ratio of amylose and amylopectin, the presence of proteins, lipids or minerals, particle size and digestion conditions in the hydrolysis of starch of gluten free bread.

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Consumer Adherence to Gluten Free Diet

Tahira Sidiq

1 Introduction

Gluten sensitive enteropathy is the most common autoimmune gastrointestinal disorder in which the body is unable to digest gluten, the protein found in wheat, rye and barley. Gluten sensitive enteropathy is readily recognized when gastrointestinal symptoms are present as; diarrhoea, bloating, flatulence, weight loss and malabsorption. In these cases, the gluten protein is not digested properly by the body and indigested peptides activate the abnormal immune response which damages the exterior line of small intestine, where absorption of food takes place. It also affects the absorption of other essential nutrients including carbohydrates, proteins, fat, vitamins and minerals. The main treatment for these affected consumers is adherence to gluten free diet for life long. Depressive symptoms and perception of disease among consumers may interfere with adherence to gluten free diet (Hall et al., 2013) and (Sainsbury et al., 2013).

The consumer's adherence to gluten free diet (GFD) in youth was related with the gastrointestinal symptoms, age of the child (<10 yrs) and ethnicity of both parents and child. The reduced consumers adherence to GFD in children was related with feeling of "unhappiness" i.e. related with eating meals outside like at school or social gathering than in the home. Parental influence plays an important role towards improved adherence to gluten free diet among child and peer influence in adolescents. It is a challenging and stressful task for consumers to adhere with gluten free diet as it is a lifelong dietary change and lifestyle change. The ability to maintain dietary changes requires support from all domains of life: sociocultural, socioeconomic, psychological, and physiological and emotionally that influences both dietary intake and health related quality of life.

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Acceptance of GFD varied economically as it is too much expensive which limits its availability to some consumers for life long. Expensive GFD becomes a barrier for affected low socioeconomic consumers to diet adherence. Psychological issues cost and easy access becomes a barrier for adherence to gluten free diet. Gluten free diet adherence is associated with patient's capability of food intolerance, food allergy, ability of consumers follow up of GFD outside the home irrespective of type of occasion, mood, stress, perception of patient towards gluten free diet. Many patients were not cooperative with the suggestions/services provided by dieticians or health care members to help them in treating the problem. Clinical dieticians and physician recognize that it is a tough task to comply totally with gluten free diet. There are several reasons why consumers face difficult with GFD; like, diet is restricted especially when eating out, in social gatherings, special occasions and ceremonies. Children & adolescents find it problem to eat different meal from their peers, friends.

Adherence to GFD will results in intestinal villi regeneration after a period 6–24 months. Lifelong GFD protects affected consumers from other related problems as; osteoporosis, diarrhoea, anaemia, flatulence, abdominal pain, low stature and constipation. It also secures the growth and development of the child. It reduces the intestinal tumour and cardiovascular problems. Gluten free diet adherence will increases the likelihood of persons gains in problems related with celiac problem like fatigue, depression and infertility. In ethnically diverse population there is less awareness towards perception of burden of celiac disease among children, its effect on quality of life and adherence to the diet. But parents often perceive its burden greater than child.

Consumer's adherence to gluten free diet is problematic for both parent-children. They required detailed information regarding problem and gluten free diet requirements. In order to get better results or better management we need daily routine examination of affected person related to gluten free diet. There are several reasons for not accepting gluten free diet for life long, including the fact that wheat based food items are staple diet in many countries. The reliable tools for assessing the consumers adherence to gluten free diet are; Celiac Dietary Adherence Test (CDAT; Leffler et al., 2009), Biagi gluten free diet score (Biagi et al., 2009) adapted to consumers adherence to gluten free diet (Casellas et al., 2008).

Researchers found that consumers adherence to gluten free diet was influenced by perceptions of the gluten free diet. Many schools of thoughts suggest theories about the factors influencing the adherence to gluten free diet.

1. One of the influential factors for non-adherence to gluten free diet is to follow the diet outside the home.
2. Another factor is the changes in emotional state such as mood and stress (Leffler et al., 2008).
3. As per Hall et al. (2009), the two factors such as socioeconomic status and Socio demographic variables such as age, gender, education doesn't appear to be related to adherence levels.

Large number of studies have also found link between Poor adherence to gluten free diet and lower Quality life cycle. (e.g., Casellas et al., 2008; Hauser et al., 2007; Usai et al., 2002).

Aside from the above studies, several other studies reported that strict consumer's adherence to gluten free diet varies between 36% and 96%. Adherence to dietary change is lower than other treatment types in these consumers. It is reported that consumers adherence to gluten free diet is restrictive, difficult to follow & effect on the quality of life (Hall et al., 2009). It was found that smoking affects consumers adherence rate to a gluten free diet and those who have never smoked tended to maintain better adherence to gluten free diet (Dana et al., 2020).

2 Contributing Factors for Consumers Better Adherence to Gluten Free Diet

There are numerous factors which are responsible for the adherence to gluten free diet. The factors may vary from person to person and will depend on the mental setup of the person adhering for gluten free diet. The identified main factors for consumer's better adherence to gluten free diet are as under:

- Consumers should have wide knowledge of celiac disease, its prevention and better adherence to gluten free diet.
- Man is a social animal. One cannot live in isolation. Society plays major role in diet. So maintain better social position of the family in society which helps consumers in accepting the gluten free diet in freely.
- High self-esteem, higher education level and good grading at school plays major role in consumers better adherence to gluten free diet.
- Role of peers is important for one individual in accepting meals. Maintain good peer relationship which directly affects the acceptance of adherence to gluten free diet.
- Gluten free diet should be available at reasonable price and wide availability of products as per ease of consumers. Therefore, consumers will not face any difficulty in adherence to gluten free diet.
- Consumers should adopt habit of reading food labels carefully before purchasing any meal from the market. The labelling of foods should be in a good way and provide accurate knowledge. Labelling of food provide consumers wide knowledge of diet and aware them towards presence of all ingredients in the product. The reading habit will provide clear idea about the product ingredients.
- Consumers get emotional support from family members, friends, dieticians and health care personnel towards adherence to gluten free diet.
- Good contact with dietician and doctor is important for consumers as they influence them in a better way and motivate them to adhere to gluten free diet.
- Dieticians are able to encourage the consumers for following gluten free diet. They play a major role in acceptance of consumers towards gluten free diet.

- One should have varied knowledge of preparing gluten free meals in different ways so that consumers get attract towards diet and their acceptance will enhance by presenting gluten free diet in different ways. Presentation of meal for consumers is an important factor for adherence to gluten free diet.
- Consumers should have capability to follow gluten free diet when they went outside with their friends or relatives or in social gathering.
- They have ability to accept gluten free diet without mental stress or force in any situation.
- Mental fitness is one of the important points among consumers for better adherence to gluten free diet as mental state is related with acceptance of diet.
- Dieticians or public nutritionists should organize seminars on gluten food sources and provide wide knowledge of gluten diet.
- Training should be imparted to workers, pregnant and lactating mothers about the gluten free diet and its implications on different aspects of the health.

3 Comparison of Studies for Consumers Adherence to Gluten Free Diet

Majority of the consumers are not aware about the gluten free diet, thus throws a challenging situation for a consumer who are recommended gluten free diet. Many studies observes that the depression will act a barrier tool for better adherence to gluten free diet (DiMatteo et al., 2000; Gonzalez et al., 2008; Grenard et al., 2011). The long term consumption perception for maintaining the adherence to gluten free diet will change the state of mind of a person to stick for good adherence to gluten free diet. Other studies carried out by the lead dietician shows that adherence to gluten free diet can be dependent on the factors like attitude towards the diet, length of adherence, knowledge about the diet and perception about the gluten free diet. Some studies also reveals that adherence to gluten free diet will also lead to physiological health problems after continuously consuming gluten free diet for a period of six and a half years (Tursi et al., 2009; Zarkadas et al., 2013; Van Hees et al., 2013; Ford et al., 2012).

Different institutions defines the meaning of adherence in different ways by having different set of parameters for the evaluation/assessment and measurement of adherence to gluten free diet. In one study the adherence are evaluated on the parameters like strict, partially and nonadherant which are discrete in nature that means either yes or no. while as in another study, strict and partially are considered as adherent and others as nonadherant. An example of adherent is “food usually contain gluten free” which means sometimes it may not but comes under the category of Adherent. In some cases, the definition of adherence was carried out with respect to the histopathology or serological tests. The studies included in this book chapter regarding the measurement of strict adherence rates by the assessment of the experts are ranging from 44% to 90% and via self-reporting, it ranges from 42% to 91%. As already discussed in the above few lines, varying nature of the measurement and definition of the gluten free adherence, it was very hectic to compare adherence levels. The rate of complete non adherence rate may deviate from 0% to 32% and average is below 5% in maximum studies. Children population were found to have highest non-adherence rate of approximately 44% and the height in ethnic minority group with 66%.

4 Factors Related to Gluten Free Diet Adherence

The factors which are related to the gluten free adherence are as under:

1. *Factors specific to gluten free diet*:

   Factors listed in the Fig. 1 showed the factors like one's adopting time to the gluten free diet, perceptions towards gluten free diet and diet knowledge are the commonly studied factors related to the adherence to gluten free diet. The person

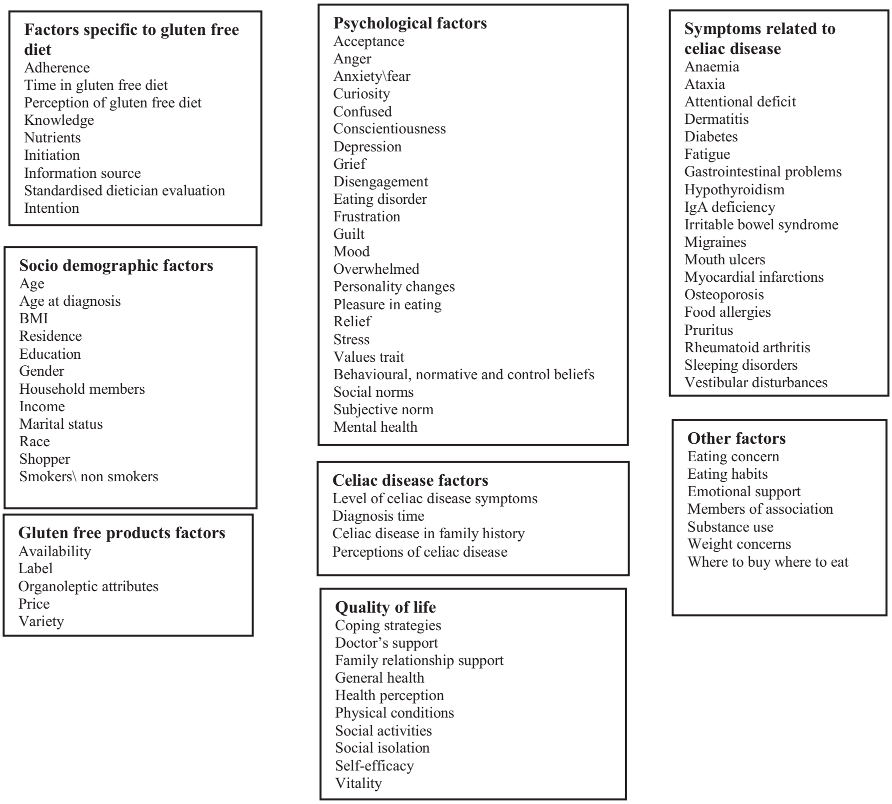

Fig. 1 Factors affecting adherence to gluten free diet

which are on gluten free diet (celiac) may need to know that the quantity of the nutrients available in the diet with the complete information about the other things as well. One should have really intention to follow the gluten free diet. But with respect to the non-celiac adherence to gluten free diet is mostly self-initiated. The reason for this adherence is the perception of better health benefits than those of consuming non gluten free diet. Researchers reported that 44% of the non-celiac participation is self-initiated and only 27% among them has knowledge about the gluten free diet. Comprehending the overall scenario when we compare the celiac with non-celiac adherence, we observed that non-celiac consumers do not possess a good knowledge about the diet and are less keen to consult a health care profession or the dietician (Silvester et al., 2016a, 2016b).

2. *Socio demographic factors*:

   Young consumers who received higher education and are employed have higher probability of adherence to gluten free diet likewise the persons which are higher qualified but seeking for their initial employment is having the probability for consuming gluten free diet but is not adherent to it continuously. It has also shown that the young people are much more intend to infract from the gluten free diet in their lateral part of the life and are not satisfied by the food they consume. It reflects that education plays an important role in predicting the factors for analysing gluten free adherence and younger men reorganization for chronic disease is very difficult (Kautto et al., 2016). Among the followers of self-initiated gluten free diet it has been found that the females of the age group between 30 and 40 years are very strict to gluten free diet. In conclusion, the young population struggle to adhere gluten free diet in particular men population (both cases celiac or non-celiac) and women are more adherent to gluten free diet.

3. *Factors associated with gluten free products*:

   The factors associated with gluten free products draws a broader spectrum of the problems. The problems faced while adherence to gluten free products are as:

   (i) High cost of gluten free diet products
   (ii) Low availability of gluten free products
   (iii) Poor labelling of gluten free products.

   The consumers which are consuming gluten free diet faces above problems very often. The cost factor plays a major role in adherence as the cost of the gluten free products are very huge as compared to gluten containing diet, thus are not very satisfied with the cost of products with gluten free which internally affects the adherence level among the consumers. The second point which makes hindrance in adherence to gluten free diet is the low availability of the products containing gluten. The availability of gluten free product is not easily made to the consumers. It has been studied that nearly 67% of the consumers who consumes gluten free diet find it very difficult to add gluten free diet in their diet schedule. Moreover, the third factor is poor labelling of gluten free products in order to find the gluten free diet. As most of the consumers who are following the gluten free diet are very careful in reading the food labels in order to avoid serious health issues. Therefore, efficient labelling will increase the adherence to the gluten free diet and understanding these labels will increase the ingesting gluten risks which will result the poor adherence of gluten free diet. The ineffective/poor labelling of food items will lead to serious health consequences like heart problem, gastrointestinal problems. Which in turn make impact on adherence of gluten free diet. What appears to be a consistent finding from this work is that there is convergence in showing a relationship between attentions to, and labelling of GF product and adherence to a GFD.

4. *Psychological factors*:

   Mental health is one of the main functions of the food. Mental distress affects the consumption pattern of meal. When the consumers are mentally well, adherence of gluten free diet will be better. Mental health problems like depression, anxiety, distress are the most commonly related with consumers who have celiac disease. Several studies showed that depression and anxiety have negative affect on adherence to gluten free diet (Ford et al., 2012; Mahadev et al., 2015).

5. *Factors related to celiac disease and other symptoms*:

   The consumer with presence of symptoms impacts and interferes with the better adherence or adoption to gluten free diet. They did not fully adopt a gluten free diet. The researcher namely Tursi found that consumers with additional intestinal symptoms were highly disapproving towards gluten free diet (Tursi et al., 2009). These symptoms have been identified as relevant in association with gluten free diet.

6. *Quality of life and social factors*:

   Cultural norms and social standards are important for better living as man is a social animal. Standard of one's life, its position in the society as per cultural rules, its norms, expectations, standards and concerns plays an important role in adherence to gluten free diet. In fact, what to eat, where to eat, how to eat, when to eat becomes a serious task and have dramatic changes on the acceptance of gluten free diet. The consumers feel social isolation when they are adherent to gluten free diet and they prefer to eat at home which has domestic environment as compared in social group (Zarkadas et al., 2013). They develop social isolation and maintain distance between peers and relatives. To overcome consumers from this problem, dietician support, family and peers support are very important for betterment of consumers especially in adherence to gluten free diet. The consumers adopt personal control and coping strategies for better adherence thus found correlation between gluten free diet adherence and self-efficacy and coping strategies.

5 Recommendations

Celiac disease patients should not consume food products that are rich in gluten (wheat, rye and barley). A gluten free diet which contains 20 mg gluten per kilogram can be marked as gluten free. The adherent consumers should follow the below mentioned recommendation for selecting gluten free diet.

- One should purchase only labelled items embedded with "gluten free".
- One should read the food labels with ingredients clearly mentioned on it very seriously so that there is no gluten present in the packed items.
- One should not buy any unpacked cereals which will result in non-adherence of gluten free diet thus we should avoid buying open food items from locals mills/ factories where chances of cross contamination are high.
- One should always check the dates both manufacturing/packaging with best before date before purchasing the food item from any kind of store.
- One should avoid trying new food items before having knowledge about the food item.
- One should avoid listening to other over choosing food items adhering to gluten free diet. Instead he should consult a marked dietician which will give him proper knowledge about the product.

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Understanding the Role of Additives in Gluten-Free Breads

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1 Introduction

The preference of people for gluten-free (GF) breads has increased drastically over the last few years. The main reason for such an inclination may be the pathologies arising out of gluten protein. The gluten related pathologies usually include gluten allergy, celiac disease and other forms of gluten sensitivity. Gluten-free bread making is a challenging task as the ingredients used could not mimic wheat gluten functionality. Doughs derived from gluten-free flours exhibit poor rheological properties and the resultant breads are characterized by textural and nutritional defects. The cohesiveness and elasticity of gluten-free dough is lesser than wheat dough. It is sticky and difficult to handle unlike wheat dough. However, large number of additives are being used to counter the technological and other kinds of defects in the production of gluten-free breads. Most commonly used additives are hydrocolloids, enzymes, emulsifiers, dietary fibre, proteins, starch, salts, acids and minerals. Incorporation of additives in dough improve the organoleptic properties by imitating some of the functions of wheat gluten. These agents help to retain carbon dioxide gas released from yeast fermentation during proving and accomplish binding of starch granules thereby improving dough cohesiveness.

Hydrocolloids or gums such as guar gum, xanthan gum, agar gum, tragacanth gum carboxyl-methyl-cellulose (CMC) and hydroxyl-propyl-methyl-cellulose (HPMC) are extensively used additives in gluten-free breads to improve dough

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handling properties and enhance quality and shelf life of bread. They mimic gluten functionality in gluten-free batter system through stabilization of emulsions, foams or suspensions by increasing viscosity, and preventing phenomenon of coalescence and flocculation. The quality and shelf life of GF bread is also determined by the nature and quantity of hydrocolloid used. Similarly, the use of surface active agents such as egg white, lipoproteins prevent coalescence of gas bubbles by forming a protective film around them in gluten-free batter system. Enzyme technology is explored widely in wheat bread preparation to increase functionality of proteins, enhance quality of bread and improve dough handling. The effect of enzymes on functionality of protein in gluten-free systems has also been evaluated to promote network formation by protein and therefore to enhance characteristics of bread making. Pre-gelatinized starch ensures structure stability, improves color, aroma and nutritional quality of GF breads. Recent studies have revealed the nutritional inadequacies of GF breads. These are found deficient in micronutrients like vitamins, folates, minerals and dietary fibre. GF breads therefore, need to be fortified with vitamins like B and D, and minerals (calcium, iron, zinc and magnesium). Simple carbohydrates, saturated fats, lipids, and sodium are found in excess in GF breads which too is considered a kind of nutritional inadequacy. GF breads lack dietary fibre and proteins due to abundant use of starch and refined flours in them. Fortification of GF breads with alternative flours such as flours from pulses gluten-free cereals like millet, rice, sunflour etc., bran or dietary fibre, nuts, pseudocereals or any oil seed is therefore recommended.

2 Hydrocolloids (Gums)

Hydrocolloids and gums exhibit a special role in order to improve the appearance and texture of GF products (Mir et al., 2016; Moreira et al., 2014). They have properties like water holding capacity, thickening, Stabilization of dough networks by increasing the viscoelastic behaviour of the GF dough. In some cases, only GF flours are unable to develop a firm arrangement due to which hydrocolloids may be added in order to obtain appropriate baked products (BeMiller, 2008). Houben et al. (2012) observed that use of various gums such as carboxy methyl cellulose, xanthan, guar gum, pectin, locust bean gum, methylcellulose etc., are responsible for improving the sensory and structural characteristics of the baked goods. Xanthan and carboxymethyl cellulose gums can be incorporated with corn starch for the development of bread which could be consumed by phenylketonuria patients. Moreover, staling property in bread was also found to be reduced due to these additives. Combination of xanthan and guar gum resulted in higher viscosity of cake batter whereas batter containing HPMC showed reduced viscosity. The viscoelastic properties of batter can also be improved by amalgamation of amylolytic enzymes and emulsifiers with hydrocolloids (Akbarian et al., 2015). Guar gum was found to be one of the additives added into the bread due to its high viscous nature at low concentration (Table 1). However, it leads to the retention of water and prevent the

Table 1 Effect of different additives on rheological properties of dough

S. no	Type of additive	Ingredient used	Dough properties	References
1.	Fibre	β-glucan	Enhanced dough elasticity	Hager et al. (2011)
		Carob flour	Enhanced elastic character and strength of dough structure	Tsatsaragkou et al. (2014a)
		Psyllium and pea fibre	Improved dough cohesion and modified dough structure	Aprodu and Banu (2015)
2.	Enzymes	Microbial transglutaminase	Improved dynamic rheological properties of dough by increasing viscous and elastic modulus	Gujral and Rosell (2004a)
		Glucose oxidase	Increased elastic and viscous modulus of dough	Gujral and Rosell (2004b)
3.	Sourdough	Rice flour and corn starch sourdough	Improved viscoelastic characteristics of dough	Ucok and Hayta (2015)
		Lactobacillus fermented sourdough prepared with pearl millet flour	Enhanced elasticity and reduced stiffness of dough	Nami et al. (2019)
4.	Fruits and vegetables	Acorn flour	Improved strength of dough structure and increased values of storage and loss modulus	Korus et al. (2015)
		Defatted strawberry and blackcurrant seed	Improved dough structure by modifying viscoelastic properties.	Korus et al. (2012)
5.	Hydrocolloids	Exopolysaccharides	Improved viscosity of the batter	Raymundo et al. (2014)
		Guar gum	Improved viscosity, and reduces the stickiness of the dough	Sworn (2000)
6.	Starch	Resistant starch/modified starch	Increase in bread volume	Witczak et al. (2010)
		Cassava flour	Simulate the viscoelastic properties	Lazaridou et al. (2007)
7.	Pseudocereals	Buckwheat flour	Improved flavour and rheological property	Torbica et al. (2012)
8.	Non wheat flour	Corn flour	Increased bread volume and viscosity	Chau et al. (2006)
		Germinated chickpea	Improved structure of bread	Ouazib et al. (2016)

(continued)

Table 1 (continued)

S. no	Type of additive	Ingredient used	Dough properties	References
9.	Acids and salts	Orthophosphoric acid, disodium diphosphate pyrophosphate (Strong acids)	Firm crumb and higher expansion volume	Scheffers (2018)
		Ascorbic acid, Tartaric acid (Weak acids)	Firm crumb	Roman et al. (2019)
10.	Emulsifiers	Sodium stearoyl-2-lactylate (SSL) Diacetyl tartaric acid esters of monoglycerides (DATEM)	Increased resistance of dough Increased elastic modulus of dough thus providing solid elastic behaviour	Sciarini et al. (2012)

syneresis under refrigerated condition (Thombare et al., 2016). Furthermore, guar gum also showed positive effect on dough consistency and hardness. Addition of xanthan and guar gum mixture decreases the rate of retrogradation, hardness, and controls the change in appearance of the bread during storage. It was reported that modified cellulose could effectively reduce the hardness and improve the bread firmness. Incorporation of locust bean gum resulted in reduced proofing time, increased loaf volume, more extensibility, with softer crumb. Many of these gums were able to control the moisture retention in bread during storage and also retarded the staling phenomenon. A high molecular weight cress seed gum, showed improved dough rheology with better quality of bread. The blend of cress seed and guar gum added into the rice and wheat flour leads to stable dough, higher retention of water, and improved viscosity (Salehi, 2019). Bread incorporated with HPMC resulted in less elasticity as compared to the bread obtained with the blend of HPMC and xanthan gum whereas the water holding capacity was found to be improved in both the combinations (Dizlek & Ozer, 2016). Xanthan gum and carboxy methyl cellulose (CMC) exhibited more aeration which resulted in softer crumb and produces web like structure in bread. It was found that xanthan gum stabilises the starch and reduces the retrogradation for longer shelf life of the product. Utilization of psyllium husk powder was employed for the development of bread and biscuits due to their rich source of fibre which encompasses the well retention of water capacity in developed products (Raymundo et al., 2014). Addition of exopolysaccharides from various strains showed improvement in dough development and better retention of gases due to higher viscosity of the batter and may retain water during baking. Lacaze et al. (2007) reported that incorporation of dextran with linear chain leads to more efficient rise in volume of the dough. Brea gum (BG) is one of the hydrocolloids obtained from an exudate of *Cercidium austral* (green chanar tree). Incorporation of BG act as a barrier for gas diffusion which leads to reduction in vapour loss and leads to higher moisture content in bread and same features were also reported by using hydroxyl propyl methyl cellulose, Moreover, it also decreases the hardness of the bread crumb (Barcenas & Rosell, 2005). Mohammadi et al. (2015) found that guar gum (GG) has similar structure to gluten; hence it was added into the rice flour for bread preparation which increases the water absorption, viscosity, and reduces the stickiness of the dough leading to production of stiff batter. Additionally, GG resulted in higher cohesiveness, adhesiveness and less chewiness in GF bread at low concentration with firm and soft crumb texture (Table 2). Commonly, xanthan gum (XG) is used for the formulation of GF bread due to its high viscous pseudoplastic behaviour and is mainly hydrated in cool water for maintaining the shear thinning behaviour (Sworn, 2000). *Lepidium sativum* seed gum is one of the hydrocolloids necessary for texture and physicochemical changes in bread crumb, and it shows comparable behaviour to xanthan gum (Naji et al., 2012). Incorporation of these seeds attributed to the better retention of moisture through hydrogen bonds, provided firmness to interface during proofing which in turn improved the volume of the bread (Gavilighi et al., 2006). Sciarini et al. (2010) observed that use of alginate and gelatin leads to lighter colour of crust whereas XG and CMC resulted in dark colour of crust (caramelization), and light colour of crumb (Maillard reaction) which could be due to the different reaction of these hydrocolloids with water. Jahromi et al. (2012) suggested that gums may also stabilise aeration of cells by forming thick layer on the surface to avoid the coalescence of individual gas pores which resulted in stable structure. Inclusion of guar gum with flaxseed resulted in reduced hardness with higher volume due to the binding of mucilage from the flaxseed which may provide elasticity to the Gluten-free bread (Garden-Robinson, 1994). Tsatsaragkou et al. (2014a) found that the use of carob germ flour for the preparation of gluten-free bread showed significant effect on its viscous and elastic nature of the dough which thereby influences the quality of baked goods. Moore et al. (2004) reported that addition of xanthan gum and konjac gum resulted in intact structure due to the reduced springiness, cohesiveness, and resilience, and resulted in improved shelf life of the GF bread. Demirkesen et al. (2010) observed that the mixture of xanthan and guar gum resulted in maximum increase in viscoelasticity and minimum hardness in GF bread. Mixture of corn and cassava starches with hydroxypropylmethyl cellulose (HPMC) replaced as gluten mimic and the dough was analysed through differential scanning calorimetry by Kobylanski et al. (2004). A combination of pectin, agarose, CMC, and xanthan gum showed significant effect on rheological property of dough and increases its strength. Resistance to deformation in GF dough due to the presence of hydrocolloids followed the order of xanthan> CMC> pectin> agarose (Lazaridou et al., 2007). Mahmoud et al. (2013) deliberately observed the effect of diverse quantity of hydrocolloids incorporated into the rice, potato, and corn flour leading to the effective entrapment of air bubbles into the baked products thereby maintain the stability of the dough. Addition of carboxymethyl cellulose (CMC) is responsible for the enlargement of gas entrapment cell, and lead to the improved crumb porosity and appearance of bread. Hydrocolloids such as pectin, inulin, gum arabic, β-glucan, guar gum, chitosan, carrageenan, and resistant starches etc. have healthy and functional effects after their incorporation. Moreover, they are also related to bowel function, decrease the risk of osteoporosis (Bosscher et al., 2006), and prevention of heart diseases, type 2 diabetes and colon cancer (Hu et al., 2011).

Table 2 Effect of various additives on rheological property of gluten-free breads

S. no	Gluten-free bread	Additive/ingredient	Properties	References
1.	Bread	Cricket powder	Decreased hardness, increased chewiness and water holding capacity	da Rosa Machado and Thys (2019)
2.	Refined flour bread with starch	Sorghum, chia seeds, and flaxseed	Better retention of carbon dioxide and presented an intense colour to the bread	Maidana et al. (2020)
3	Rice flour bread	Guar gum	Increases the water absorption, viscosity, and reduces the stickiness of the dough	Mohammadi et al. (2015)
4.	Flaxseed bread	Guar gum	Better crust and softer crumb	Ozkoc and Seyhun (2015)
5.	Bread	Corn flour	Improved elasticity with hydration properties	Chau et al. (2006)
6.	Bread	Rice flour and buckwheat flour	Reduced retrogradation of starch	Wronkowska et al. (2013)
7.	Maize based gluten-free bread	*Glucose oxidase* mixed with oat bran	Improved crumb firmness	Aprodu and Banu (2015)
8.	Bread	*Transglutaminase*	Improved bread quality by promoting a protein network Improved specific volume, crumb porosity and staling	Gujral and Rosell (2004a), Dluzewska et al. (2015)
9.	Corn and sorghum based bread	*Glucose oxidase*	Increased specific volume of bread	Renzetti and Arendt (2009a)
10.	Brown rice bread	*Protease*	Significantly increased specific volume and decreased crumb hardness and chewiness	Renzetti and Arendt (2009b)
11.	Bread	Pomegranate seed powder	Increased specific volume and springiness. Decreased crumb hardness and chewiness	Bourekoua et al. (2018a)
12.	Bread	Defatted strawberry and blackcurrant seed	Decreased hardness and enhanced chewiness and gumminess	Korus et al. (2012)
13.	Bread	Concentrated raisin juice	Decreased hardness and increased crust color Reduction in staling rate	Sabanis et al. (2008)

(continued)

Table 2 (continued)

S. no	Gluten-free bread	Additive/ingredient	Properties	References
14.	Bread	Organic calcium supplements such as calcium citrate and calcium caseinate	Increased specific volume of bread Increased softness and elasticity of crumb	Krupa-Kozak et al. (2011)
15.	Rice flour based Bread	Sourdough fermented with lactic acid and acetic acid bacteria	Increased cohesiveness and decreased hardness of the bread crumb	Jitrakbumrung and Therdthai (2014)
16.	Bread	Freeze-dried red potatoes	Increased bread volume, decreased hardness and significantly improved cohesiveness	Gumul et al. (2017)
17.	White rice flour bread	Distilled monoglycerides and lecithin	Increased specific volume of bread and reduced staling rate of the crumb	Nunes et al. (2009)
18.	Bread	Sodium stearoyl-2-lactylate (SSL) and diacetyl tartaric acid esters of monoglycerides (DATEM)	Decreased crumb firmness and reduced staling rate of bread	Onyango et al. (2009)
19.	Bread	Cyclodextrin-glycosyltransferase (CGT)	Increased bread specific volume and improved bread crumb texture	Basso et al. (2015)

3 Emulsifiers

Emulsifiers are substances having both hydrophilic and hydrophobic characteristics, and are greatly explored in several bread formulations (Table 1). Emulsifiers are composed of substances that have very different chemical structures so they are expected to show different effects on properties of both dough and bread (Eduardo et al., 2014). Emulsifiers interact with the hydrophobic regions of proteins and thus results in strengthening of dough. The emulsifier-protein complex allows better retention of CO_2 during ovenspring by improving the strength of the dough. Emulsifiers interact with starch molecules by suppressing the water movement and thus assist in retardation of starch retrogradation, and also enhance the stabilization of gas cell in the dough by creating lamellar films which surrounds the gas cells (Sroan & MacRitchie, 2009). Emulsifiers can be non-ionic, anionic or amphoteric in nature and based on their reactivity. Emulsifiers like sodium stearoyl-2-lactylate (SSL) and diacetyl tartaric acid esters of monoglycerides (DATEM) are anionic emulsifiers that are commonly used as additives in gluten-free bread formulation and have proven to be beneficial in improving texture and softness of bread crumb and crust, and enhancing loaf volume (Table 2). Nunes et al. (2009) studied the quality and rheological properties of gluten-free breads with the addition of emulsifiers such as lecithin (LC), DATEM and SSL or distilled monoglycerides (DM). The specific volume of bread was found to be increased with the addition of different emulsifiers, and higher volumes were observed with addition of DM whereas lower volumes were obtained with DATEM addition to breads. The addition of DM to gluten-free breads significantly reduced staling rate of the crumb and the shelf life of bread enhanced with the addition of high concentration (0.5%) of SSL and DM. Cell size and distribution in crumb structure was largely influenced by the addition of DATEM and LC. They have concluded that use of emulsifiers as an additive in optimum concentration in gluten-free bread formulation had positive effect on the bread quality. Lopez-Tenorio et al. (2015) reported that there was no effect observed on the dough rheological properties with the addition of emulsifiers such as DATEM and SSL. Onyango et al. (2009) studied the effect of emulsifiers such as DATEM, SSL and glycerol monostearate on the properties of gluten-free bread. They observed that with the increase in emulsifier concentration from 0.4% to 2.4% firmness of crumb decreased. With the increasing emulsifier concentration, the staling rate of bread was also decreased as compared to the control bread. The ability of emulsifiers in delaying firming of crumb is largely attributed to their hydrophilic-lipophillic index and ionization potential that influences their interactions with intact starch granules and proteins. Sciarini et al. (2012) investigated the role of emulsifiers (DATEM and SSL) on the properties of dough and bread in gluten-free bread formulation prepared with rice flour, cassava starch and soy flour. They reported that addition of SSL increased resistance of dough to deformation but decreased in case of DATEM. The elastic modulus (G') for all gluten-free dough was found to be higher than viscous modulus (G'') which indicated the solid-elastic behaviour of dough. In rice-flour based breads, addition of DATEM emulsifier improved bread specific volume and organoleptic properties (Demirkesen et al., 2010). Thus, emulsifiers have the potential to modify, improve dough properties and overall bread quality and can be explored as an additive in gluten-free bread making.

4 Fibre

Gluten-free (GF) products are based primarily on starches and refined flours (Foste et al., 2020). The carbohydrate content of such products is therefore high and they even have additional sugar and fat content. Due to the increased percentage of starch in it, the amount of dietary fibres and proteins lacks in it and this leads to imbalanced energy value and enhanced glycemic response. Thus, fortification of gluten-free bread (GFB) with alternate flour sources has been recommended such as flours from pulses, GF cereals like millet, rice, sunflour etc., bran or dietary fibre, nuts, pseudocereals or any oil seed (Thompson, 2000).

The quality of bread is impaired in terms of loaf volume, texture, crumb color and overall appearance when fibres are added for its preparation (Foste et al., 2020). This happens due to the gluten dilution in wheat dough which lowers retention of gas and reduces loaf volume. Additionally, a research done by Pomeranz et al. (1977) suggested that for the preparation of wheat dough enriched with high fibre, modifications in water absorption and time of mixing should be considered. The formation of structure in gluten-free dough basically depends upon the interaction of flour or starch with water absorbing hydrocolloids unlike in wheat dough. For forming gluten-free dough, requirement of water is slightly higher as compared to wheat dough that resembles more like cake batter. Due to the absence of the proteins responsible for network formation, it is difficult to determine optimum water addition to flour. Lately, the cereal bran properties and the structural role of dietary fibre addition to gluten-free bread were assessed (Tsatsaragkou et al., 2016). The application of flours enriched in fibre portions from legumes or pseudocereals appears to be effective for nutritional fortification of gluten-free bread (Foste et al., 2015, 2020).

4.1 Utilisation of Dietary Fibre in Gluten-Free Breads

The increasing number of people suffering from celiac disease and the growing demand for healthier and nutritive foods has raised a new market that consists of cereal products prepared from ingredients other than wheat. In this category the bread acquires a special position as it is globally an important product in human nutrition (Huttner & Arendt, 2010). Due to the vital nutritional attribute and the acceptability by many celiac disease patients, oats appears to be outstanding grain for gluten-free bread preparation. Replacement of gluten in gluten-free flours is a difficult task as a protein network is not developed by gluten-free doughs. The incorporation of β-glucan in GFB preparation resulted in addition of higher water level. Hager et al. (2011) on assessment of rheological property revealed that the incorporation of β-glucan from oat resulted in enhancement of dough elasticity (Table 1). Addition of inulin and β-glucan also had an effect on bread baking characteristics such as volume and crust colour. Inulin addition has increased loaf specific volume whereas β-glucan decreased the specific volume. The incorporation of oat produced dark colour crust and had a negative effect on crumb hardness, whereas addition of β-glucan resulted in a light color crust of gluten-free bread and also produced bread with softer crumb texture. The incorporation of inulin with low degree of polymerization aids in the reduction of crumb staling, this is attributed to the inulin ability to form soft gel (Ziobro et al., 2013). Phimolsiripol et al. (2012) developed gluten-free bread with better color with the addition of rice bran using increased amount of soluble dietary fibre. Sabanis et al. (2009) produced gluten-free breads with the incorporation of oat, maize, barley and wheat fibres, and reported that addition of dietary fibre from barley enhanced the color and provided better volume in breads as compared to other fibres. It was also reported that the addition of maize and oat fibre in formulations of gluten-free bread produced breads with higher loaf volume and softer crumb when compared to non-fibre control gluten-free bread. The water absorbing capacity of the gluten-free bread enhanced with the incorporation of dietary fibres. Several studies have revealed that addition of high fibre to gluten-free breads resulted in higher moisture in crumb and crust, due to the high water binding ability of dietary fibres.

4.2 Utilisation of Carob Flour

The incorporation of fibres influences rheological properties of dough, as they have the ability to alter water absorption of dough which normally increases with addition of fibres. Increased amount of fibre degrades the fermentation capability of dough as dilution in gluten network occurs, which results in reduction of gas retention capacity of dough. When gluten-free breads are concerned, viscosity of dough becomes important attribute affecting the product quality and this is ultimately related to loaf volume in the baking process. Tsatsaragkou et al. (2014a) studied the effect of addition of carob flour on the rheological properties of gluten-free breads (Table 1). Carob germ after the separation of locust bean gum is regarded as underutilized product in food industries but it can be explored as a safe ingredient in gluten-free bread formulation for enhancing nutritional value. Carob germ is rich in protein and dietary fibres. They have studied the rheological properties of gluten-free doughs prepared from rice flour with addition of varying amounts of carob flour. Tests such as dynamic oscillatory and creep tests were performed to know about the rheological behaviour of doughs which is required for the high quality bread production. They have evaluated the effect of addition of carob and water in dough rheological properties. The results showed that an increase in water content reduces the dough resistance to deformation and therefore the strength of dough enhances while addition of carob flour enhanced the elastic character and strength of dough structure. Different stages of baking process are significantly influenced by the rheological properties of dough and it, therefore plays a vital role in the production of high quality baked goods.

4.3 Soluble and Insoluble Fibres

The nutritional attributes of dietary fibres has been studied extensively. These dietary fibres have several health promoting effects such as helping in controlling blood glucose and cholesterol, protect against various cardiovascular diseases, promotes health growth of gut and protection against colon cancer. These health positive effects, have prompted the researchers to extensively study the incorporation of dietary fibres into breads. Martinez et al. (2014) conducted a research to study the effect of different microstructural features of soluble and insoluble fibres on dough rheology and bread making of gluten-free bread. Soluble fibres such as nutriose and polydextrose, and insoluble fibres such as oat;bamboo, fine;coarse and potato;pea were studied. They have reported that soluble fibres reduce dough consistency, promote increase in volume during fermentation and thus produce breads having higher specific volumes, reducing hardness and produce breads with greater cell density when compared to control breads. The breads prepared with incorporation of fine fibres too provide higher specific volume and reduced hardness than control breads. In the aqueous phase, soluble fibres with hydrocolloid promote the formation of a film and provide coating to starch granules and flour particles, providing more stability to structure whereas insoluble fibres do not support structure. The dough developed with insoluble fibres showed to have increased consistency especially those containing potato fibre and slightly less consistency obtained with pea flour. The doughs prepared with pea, potato and coarse bamboo fibres provide higher elasticity as compared to control dough. The effect of fibres on consistency and elasticity of dough could be attributed to their effect of dough internal structure. Insoluble fibres remain unchanged after the formation of dough and acquire more round structure in the presence of starch granules and leading to more irregular and larger structure than the control dough. On the contrary, soluble fibres dissolve in aqueous medium and provide lubrication to the final dough by enveloping the starch granules and thus the consistency and elasticity of dough reduces.

5 Enzymes

The use of enzymes for the quality improvement of food products has been the area of interest of several researchers. The enzymes when extracted or obtained from animals, microbial or plant sources are considered as purely natural, non-toxic and are very much preferred by consumers as compared to chemicals used for food preparations (James & Simpson, 1996; Huttner & Arendt, 2010). Enzyme technology is explored widely in wheat bread preparation to increase functionality of proteins, enhance quality of bread and improve dough handling (Table 1). From the past few years, the effect of enzymes on functionality of protein in gluten-free systems has also been evaluated to promote network formation by protein and therefore to enhance characteristics of bread making.

5.1 Transglutaminase

Enzyme transglutaminase catalyzes reactions involving transfer of acyl group which results in cross linking of proteins could be an option to enhance gluten-free breads structure (Moore et al., 2006). Protein molecules when react with transglutaminase, formation of lysine cross links occurs. Trasnglutaminase can be used in the food industry as an agent to enhance firmness, water holding capacity and elasticity of food with mild enzymatic reactions (Li et al., 2013; Dluzewska et al., 2015). Several researchers have reported that modification of protein functionality can be done by transglutaminase. Moore et al. (2006) studied the transglutaminase application on a basic gluten-free recipe, consisting of xanthan gum, dried yeast and different gluten-free grain flour such as rice flour, corn flour and potato flour and various protein sources too were used for each recipe viz. skim milk powder (SMP), soya flour (SF) and egg powder (EP). It was observed that addition of transglutaminase to three different breads had different effect on specific loaf volume and breads with egg powder reported a higher specific loaf volume as compared to the other two. Within the gluten-free systems, transglutaminase formed cross-links containing skim milk and egg powder. However, the requirement of level of addition for the formation of network is proportional to the source of protein available to the enzyme. The specific protein structure and disposition of glutamine and lysyl residues are both responsible for the rate of protein cross-linking by transglutaminase (Gerrard, 2002). They have concluded that the formation of gluten network due to the presence of transglutaminase may help in improving crumb characteristics, loaf volume, appearance and overall acceptability of gluten-free breads. According to Gujral and Rosell (2004a), transglutaminase addition to a gluten-free recipe prepared with rice flour and hydroxypropyl methyl cellulose (HPMC), led to the modification of the visco-elastic characteristics of the rice batter (Table 1) and resulted in the improvement of gluten-free bread quality by promoting formation of protein network.

5.2 Glucose Oxidase

Glucose oxidase is preferred for improving overall bread quality over other chemical oxidising agents. Glucose oxidase has varying effects on bread quality in gluten-free baking. Gujral and Rosell (2004b) studied the effect of addition of glucose oxidase on gluten-free bread prepared with rice flour and reported that bread quality was improved by enhancing loaf volume and reduced crumb hardness. Glucose oxidase resulted in the crosslinking of protein present in rice which in turn causes alteration of viscous and elastic behaviour of the gluten-free rice dough (Table 1). Renzetti and Arendt (2009a) reported that application of glucose oxidase improved quality of breads prepared with sorghum and corn by increasing bread specific volume and decreasing rupturing at the surface. These positive effects could be attributed to polymerization of protein that enhances elastic behaviour of corn and sorghum batters. They have concluded that protein polymerization could be beneficial for providing elastic like behaviour to gluten-free dough and thus bread making performance can be improved.

5.3 Proteases

These are widely used in making of wheat bread for improving the extensibility and machinability of the dough and also for the reduction of time of mixing due to decreased resistance to mixing (Huttner & Arendt, 2010). In gluten-free systems, proteases have proved to be beneficial in improving functional and foaming properties of cereal proteins. According to Renzetti and Arendt (2009b), performance of bread making prepared with brown rice flour improved due to hydrolysis of soluble protein of protein matrix in which starch granules are embedded by proteases that resulted in enhanced bread volume and reduced crumb hardness and chewiness

(Table 2). Furthermore, breads produced from gluten-free rice flour dough, treated with protease, resulted in the improvement of bread quality in terms of higher loaf volume, good crumb appearance and softer crumb texture as compared to the control bread (Kawamura-Konishi et al., 2013). Breads prepared after treatment with protease enzyme showed low staling rate. Thus, protease proved to be beneficial for improving overall bread quality. Protease is found to improve the overall quality of gluten-free bread prepared with treated oat flour. The addition of 0.001% and 0.01% concentration of protease significantly improved quality of oat bread by increasing specific volume, reducing chewiness and crumb hardness. The improvement in bread making performance was due to the increase in softness of batter and elasticity that were achieved by the addition of protease enzyme (Renzetti et al., 2010). Sciarini et al. (2012) investigated the role of two enzymes, glucose oxidase and α-amylase in GF breads and they have observed that addition of glucose oxidase to rice flour bread resulted in reduced crumb firmness, and also the specific volume of bread was found to be similar to the control bread. The lowest concentration of α-amylase resulted in increase in specific bread volume which was because of the hydrolysis of the starch that leached out due to gelatinization during baking, thus causing reduction in dough resistance. Addition of glucose oxidase produced the bread with higher cell number of reduced size and α-amylase resulted in good quality crumb structure. Basso et al. (2015) observed that addition of cyclodextrin-glycosyltransferase (CGT) enzyme from Bacillus firmus strain 37 in GF bread making resulted in breads with increased specific volume and improved crumb texture, and the sensory properties of corn and pinion flours based bread prepared with CGT were also improved. According to Palabiyik et al. (2016), addition of fungal amylase whose substrate was mainly starch clearly affected the pasting and textural properties of bread whereas enzymes having fibre and protein as substrates did not affect these properties at all. Hatta et al. (2015) established that for the improvement of texture and quality of bread, degradation of α and β subunits of glutelin fraction of rice protein is required. They observed that textural and gas holding properties of rice bread, prepared with protease, were found to be improved.

6 Minerals

The gluten-free diet and products are characterised by a low nutritional, vitamins and mineral content. Among different deficiencies linked with gluten-free diet, deficiency of calcium and iron are very well known. The manufacturing of food products fortified with iron and calcium especially dairy products, snacks and beverages has been well established in the food industries. However, only limited gluten-free products are available in the market enriched with essential minerals as compared to wheat based products. The development of mineral fortified products could prove to be beneficial in improving the nutritional attributes of gluten-free products.

6.1 Calcium

Calcium, being an essential nutrient, is required in some amounts but there are diets which are deficient in calcium and thus the supplementation of calcium becomes necessary. Krupa-Kozak et al. (2011) investigated the effect of individual and combined supplementation of organic calcium supplements on the sensory properties and technological attributes of gluten-free bread (Table 2). They have studied the individual and combined effect of addition of two organic supplements of calcium i.e., calcium citrate and calcium caseinate to the gluten-free bread formulation. The fortification of calcium to gluten-free bread affected all characteristics. The supplementation of calcium citrate in gluten-free bread showed significant increase in specific volume of breads. The incorporation of calcium caseinate is not solely responsible for influencing change in the specific volume of bread. It is highly soluble in water and gets dispersed quickly in an aqueous mixture; therefore it can compete with starch granules for water in the process of baking. Due to the presence of calcium caseinate, less water is available for starch which results in reduction of starch swelling and it could be the possible explanation for the poor specific volume of breads prepared with calcium caseinate.

The calcium caseinate effect on the specific volume of bread can be improved significantly with the combination of calcium citrate. The rise in specific volume of fortified breads containing both calcium supplements is directly proportional to the increasing amount of calcium citrate. Increased amount of calcium with higher calcium citrate amount could be responsible for influencing the textural attributes of bread as calcium ions have the ability to form cross links between free carboxyl groups of the chain of pectin which leads to improved structure of the cell wall. Addition of both salts of calcium produced bread with fine crust colour. They have observed that the crumb of breads fortified with calcium were soft and even more elastic as compared with unfortified control bread. Palatability of breads supplemented with calcium was found to be significantly higher in comparison to unfortified bread. The sensory qualities were enhanced with increasing amount of calcium citrate and calcium caseinate such as aroma, taste as well as springiness. The supplementation of calcium caseinate (containing 92.8% protein in dry matter) to gluten-free bread, increased the content of total proteins in fortified breads. Bread prepared with the addition of calcium caseinate was two times richer in proteins than control unfortified bread and bread prepared with calcium citrate supplementation.

6.2 Iron

Iron deficiency is very much prevalent in large number of population. Gluten-free products fortified with iron supplements are rare, but it has been established that developing such products would be beneficial in the improvement of diet quality

(Kupper, 2005; Kiskini et al., 2012) and can be supported by the fact that most of the patients suffering from celiac disease have iron deficiency. Iron may led to the adverse changes occurring to the flavour, texture and colour of the food, thus becoming one of the difficult minerals to add. Additionally, for a fortification programme to become successful, it is required that both the fortificant and carrier agent should be accepted by the target people (Bovell-Benjamin & Guinard, 2003).

Kiskini et al. (2012) conducted a research to evaluate the effect of fortification of iron on sensory and physical quality of gluten-free bread. Breads were developed with fortification of different forms of iron. Significant differences were observed in both bread samples fortified and unfortified control sample in colour and firmness of crust, and sensory properties were also affected such as metallic taste, smell of moisture, pore number and stickiness. All bread samples fortified with iron showed higher moisture content to a slight extent in both crust and crumb as compared to unfortified breads. Presence of high moisture in the finished product can result in undesirable quality alterations at the time of storage like occurrence of rancidity due to interaction between fat and iron. Gluten-free bread developed with iron fortification gives rise to a stiffer and firmer crust except for bread sample prepared with electrolytic iron fortification. The textural properties of gluten-free bread developed with iron fortification affected prominently when fortified with ferric pyrophosphate and Sodium iron EDTA that had a stiff or firm crust and a more firm crumb. They have mentioned that textural characteristics of crust were more affected with the addition of iron as compared to crumb textural attributes. Color is a physical property which can be easily and strongly influenced by fortification of iron. The color of all the bread samples prepared with fortification of different forms of iron was affected significantly to a greater extent. A dark color of crumb and crust was observed whereas a greenish color was noticed for both crumb and crust. Fortification of iron has also affected the sensory attributes in breads samples. Gluten-free bread developed with ferric pyrophosphate fortification showed more air cells, and that fortified with ferrous sulphate showed the most intense metallic taste. Thus, the results obtained from this research can be beneficial to know gluten-free bread fortification with different forms of iron.

7 Proteins

Gluten is the wheat storage protein that constitutes around 80 to 85 parts of the total proteins. Viscoelastic properties of dough owe unique behaviour of the protein. Gluten is composed of gliadins and glutenins. A monomeric protein, gliadin attributes to the extensibility and viscosity of the dough, whereas polymeric protein, glutenin is responsible for cohesive and elastic behaviour in dough (Wieser, 2007). This leads to the distinctive structure to the dough, batter after processing such as baking and results in development of the desired texture (Veraverbeke & Delcour, 2002). But in some cases, individuals are allergic to gluten due to which they demand for gluten-free (GF) products. In such circumstances, it becomes important to exploit some other sources of protein which possibly will be helpful to celiac patients (Moreno et al., 2014). Therefore, substitution of gluten from different sources or modification of protein is one of the technological challenges to simulate the viscoelastic nature of protein in gluten-free commodities (Taylor & Rosell, 2016). Later on, various researches was done for improving the rheological and sensory characteristics of goods through the addition of modified proteins (Aprodu et al., 2016). Ziobro et al. (2016) mentioned that inclusion of various protein isolates such as collagen, pea, soya, albumin, lupine etc. resulted in improved structure and quality of the gluten-free bread. Protein such as zein and caroubin could also be used as an additive for improving the quality in bread. Qi et al. (2011) used soy protein extractions (pH 5.4) which acted as possible gluten protein substitute for providing elasticity, extensibility, and stickiness to the dough due to their viscoelastic properties. β-conglycinin concentrate (β-CC) was one of the soy protein extracts that resulted in improvement in the colour of the crumb of the bread with lowest amount of protein (Krupa-Kozak et al., 2013). Bread produced with β-conglycinin concentrate (β-CC) resulted in higher volume and softer crumb compared to gluten, due to higher retention of carbon dioxide during proofing (Houben et al., 2012). Addition of βCC also resulted in crumb porosity of the bread due to its high hydration property as compared to gluten bread (Espinosa-Ramirez et al., 2018). Many animal and dairy proteins are being successfully used for the replacement of gluten to improve the sensorial and textural appearance in many of the gluten-free formulations (Buresova et al., 2017). Many studies have also shown incorporation of insect powders, especially cricket powder in cereal resulting in improved characteristics of baked products such as bread, cake, and muffin. The protein content and water holding capacity of cricket powder was found to be better in comparison to lentils (Buckwheat) which could play an essential role for maintaining the juiciness, texture and appearance of the baked goods (da Rosa Machado & Thys, 2019). Inclusion of these protein results in decreased hardness and increased chewiness of the gluten-free bread which would be desirable for slicing and reduces the disintegration during mastication. Moreover, it also showed improved springiness which is responsible for elasticity in gluten-free bread (Onyango et al., 2011; Cornejo & Rosell, 2015). Table 3 shows the effect of various ingredients on nutritional composition of gluten-free breads. The use of protein from other sources such as albumin, collagen, pea, lupine and soy isolates and concentrates were also used for the development of gluten-free breads, and contributed to improved porosity and physicochemical change in properties such as solubility, and hydration capacity (Pareyt et al., 2011). The nutritional value of the bread observed to be higher in terms of protein due to the addition of cricket powder. Pulses can be incorporated in GF products in diverse forms such as dehusked/ husked flour, protein hydrolysates/protein isolates, malted/ fermented flour, and blends of two or else more than two pulses which could impart changes in its nutritional, physicochemical, and functional properties. Thus, formulated bread resulted in increasing value of all nutrients especially protein, fibre and mineral content when compared to wheat based bread (Indrani et al., 2011). Incorporation of lentil and beans flour into the wheat flour, showed an increase in water holding capacity of dough which is responsible for increased level of protein.

Table 3 Effect of various additives/ingredients on nutritional composition of the gluten-free bread

S. no	Ingredients	Nutritional quality	References
1.	Cricket powder	Increase in protein, fibre content with increase in concentration of cricket powder	da Rosa Machado and Thys (2019)
2.	Psyllium husk powder	Increased protein, and fibre content	Raymundo et al. (2014)
3.	Buckwheat flour with cassava starch, corn starch and rice flour	Increased fibre and taste of the bread	Sanchez et al. (2002)
4.	Replacement of potato starch with pseudocereal	Increase in protein, fibre, iron, and phytonutrients	Alvarez-Jubete et al. (2010)
5.	Quinoa and amaranth	Increase in protein and fat content Increase in antioxidant property	Alvarez-Jubete et al. (2009)
6.	Colocasia flour	Increase in fibre, starch, minerals along with some phytochemicals	Arici et al. (2016)
7.	Pomegranate seed powder	Increased antioxidant activity	Bourekoua et al. (2018a)
8.	Defatted strawberry and blackcurrant seeds	Increased protein, dietary fibre and polyphenolic content Increase in color of crumb	Korus et al. (2012)
9.	Concentrated raisin juice and dried raisin juice	Increased vitamin and mineral content Enhanced shelf life of bread	Sabanis et al. (2008)
10.	Organic calcium supplements such as calcium citrate and calcium caseinate	Enhanced protein and mineral content	Krupa-Kozak et al. (2011)
11.	Acorn flour	Increased protein, mineral and dietary fibre content	Korus et al. (2015)
12.	Freeze-dried red potatoes	Improved level of protein, minerals and insoluble fibre content Improved appearance, crust colour, crumb elasticity, thickness and taste	Gumul et al. (2017)
13.	Moringa oleifera leaf powder	Increased total phenolic content and antioxidant activity	Bourekoua et al. (2018b)
14.	Dairy proteins (Milk protein, skim milk)	Increase in protein and calcium content	Krupa-Kozak et al. (2013)

Moreover, it was found that addition of lupine flour in different amounts leads to increase in water absorption capacity which could be due to the higher water retention ability of flour, and it also showed the highest overall acceptability score as compared to wheat bread during sensory evaluation (Guemes-Vera et al., 2008). Replacement of whole wheat flour with blend mix of chickpea, barley, soybean, and fenugreek at different concentrations could be used for the development of gluten-free bread due to their increased water absorption capacity and reduced extensibility and viscosity (Baik & Han, 2012). Eggs are also used for the better structure and crumb of the Gluten-free bread (GFB) due to its higher emulsifying and foaming ability during baking. Inclusion of dairy proteins leads to the improvement of crust, colour, crumb, and sensorial parameters of the GFB due to amadori rearrangement and caramelization (Houben et al., 2012). Dairy proteins such as milk proteins or skimmed milk could be effective for enhancing colour of the bread due to Maillard reactions with very less amount. But the people who are lactose intolerant, incorporation of these dairy proteins need to be avoided. Carob germ and corn protein can be used with starch for improving the viscoelastic and cohesive property of the dough which could be helpful in holding the gas, resulting in soft crumb of the GFB (Smith et al., 2012).

8 Salts and Acids

Sodium bicarbonate and carbonates are the most common additives used in baking as a leavening agent. These salts have a capability to produce carbon dioxide when react with some acids. When a high temperature is applied during heating or baking it results in dissociation of salts with acid. Inclusion of acids such as orthophosphoric acid, disodium diphosphate pyrophosphate, and acid phosphate ensures the production of carbon dioxide which would further expand and results in more firm crumb and higher expansion volume ratio of the bread (Table 1). The addition of these additives should be in acceptable range otherwise it shows a negative influence on appearance and taste of the bread (Scheffers, 2018). The use of lactic acid, ascorbic acid, malic acid, tartaric acid, and citric acid are found to be weak acids which could be added as an additive for the formulation of gluten-free breads. Glucono delta-lactone has also been added into the baked products which hydrolyses to form gluconic acid to promote the acidification of the dough. It also act as a preservative and helps in production of carbon dioxide. The use of ascorbic acid in bread making influences the strength of the gluten bread. Therefore, addition of ascorbic acid would not be suggested in case of GF breads as there will not be any gluten formation (Roman et al., 2019).

9 Fruits and Vegetables

Several studies have emphasized on the research of compounds and ingredients of natural origin like fruits, leaf extract, seeds, hull and other different parts of plants. Incorporation of these substances is increasing in food products as these are rich source of antioxidants and could be used in formulations of bread to improve the nutritional quality of finished product (Bourekoua et al., 2018a). Vegetables and their leaves have been explored for the preparation of gluten-free bread and their ability to enhance bread quality has been studied. The leaves of *Moringa oleifera* are rich in phytonutrients and a good source of carotenoids. The incorporation of *Moringa oleifera* leaf powder into gluten-free bread formulation has proved to be beneficial for improving bread quality when added upto 2.5%. The crumb hardness and chewiness found to be decreased with its addition. It was also observed that the total phenolic content and antioxidant activity of bread increased with the addition of *Moringa oleifera* leaf powder (Bourekoua et al., 2018b).

9.1 Pomegranate Seed Powder

Pomegranate is an old fruit and is popularly consumed fresh or as a beverage and is rich in total polyphenolic content containing hydrolysable tannins that have excellent antioxidant capacity. Pomegranate seed which is rich in punicic acid and by-product of pomegranate peels have also been used for the preparation of wheat bread for improving the baking characteristics (Sulieman et al., 2016). Pomegranate seed can also prove to be beneficial in gluten-free baking systems due to their antioxidant activity. Bourekoua et al. (2018a) conducted a study to determine the effect of pomegranate seed powder addition on the sensory, physical and antioxidant properties of gluten-free bread. The pomegranate seed powder was added at varying levels for the formulation of gluten-free bread. They have reported that springiness and specific volume of gluten-free bread enhanced, and there was a reduction in chewiness and crumb hardness with the increase in pomegranate seed powder. The colour analysis revealed that the yellowness and lightness of crust and crumb colour decreased with the addition of pomegranate seed powder whereas redness was increased. The total phenolic content also increased from 46% to 181% with the addition of pomegranate seed powder when compared with the bread without seed powder. The antioxidant activity was observed to be significantly higher in bread prepared with pomegranate seed powder and it increased with increase in its percentage. The sensory analysis data revealed that breads prepared with addition of pomegranate seed powder received higher overall acceptability scores for texture, appearance and taste.

9.2 Defatted Strawberry and Blackcurrant Seeds

Pomace obtained from different fruits could be used as a potential ingredient for preparing various food products due to its significant content of protein, bioactive compounds, minerals and dietary fibre (Korus et al., 2012). Korus et al. (2012) investigated the functionality of defatted strawberry (DS-ST) and blackcurrant seeds (DS-BC) in gluten-free bread and reported that 15% addition of DS-BC or DS-ST in gluten-free bread formulation results in modification of dough viscoelastic properties and caused reduction in values of flow indices and consistency coefficients. The textural analysis data revealed that the hardness of bread decreased with supplementation of DS-BC or DS-ST (Table 1) when compared to control bread and the effect of DS-ST was more in comparison to DS-BC. Supplementation of bread with DS-BC and DS-ST also had positive effects on colour parameters of crumb by decreasing the lightness and yellowness, and enhancing the redness of the product. It has also enhanced nutritional quality of gluten-free bread with the increase in protein, dietary fibre and polyphenolic content.

9.3 Raisin Juice, Potato and Acorn

The concentrated raisin juice addition has been established since many years in the bakery industry as natural sweetener supplement and to enhance shelf life, volume and color of baked products. Sabanis et al. (2008) conducted a research to investigate the effect of different raisin juice preparations on some selected characteristics of gluten-free bread. As discussed earlier, gluten-free breads are usually poor in color, and baking characteristics and are not shelf stable even for a short period of time. Therefore, this research was conducted to solve these issues with the addition of raisin juice which is a natural sweetener, contains zero preservatives, and has low caloric value compared to sucrose, and in addition to that it contains several vital vitamins and minerals which are important for celiac patients. The study revealed that addition of 3% concentrated raisin juice in gluten-free bread formulation contributes to a great extent in improving loaf volume, hardness and color of bread. Dried raisin juice increased bread loaf volume and improved color of gluten-free bread in comparison to control bread and also enhanced the shelf life of the former due to its moisture absorbing characteristics. Data from sensory analysis showed higher overall acceptability score for raisin juice containing breads which is attributed to its fruity flavour. The color analysis showed darkening of the crust and crumb color due to the addition of 3% raisin juice which is a desirable characteristic in gluten-free breads in comparison to wheat breads they tend to have lighter color. Gumul et al. (2017) reported that with the 5% level of addition of freeze dried red potatoes resulted in increase in content of insoluble fibre and protein with high biological value. They have also observed that, it has improved physical characteristics of bread such as high bread volume, good chewiness and decreased hardness. Addition of freeze dried red potatoes also enhanced organoleptic properties in terms of appearance, crumb elasticity, crust colour, taste and smell. Korus et al. (2015) reported that addition of acorn flour as an additive in corn starch and potato starch gluten-free dough resulted in increase in both elastic and viscous modulus, and a reduction in phase shift tangent that indicates firming of structure of dough. They have also observed that addition of a corn flour in limited amounts resulted in increase in bread volume and improved characteristics of crumb. Furthermore, the rate of staling slows down as retrogradation of starch gets diminished resulting in improved sensory properties. They concluded that debittered acorn flour could be used as a potential additive in gluten-free bread making for improving its quality.

10 Starch

Rice starch is commonly used for the people who are allergic to wheat especially celiac disease patients. Aoki et al. (2020) observed that dough viscosity was lower with rice sample containing high amylose content and vice versa. The optimum viscosity for bread making ranged from 185 BU to 247 BU for various rice varieties. Maize, rice, sorghum are always considered gluten-free flour thereby could be used for the development of products for coeliac patients (Alvarez-jubete et al., 2010). Major sources of starch such as rice flour, corn, wheat, and potato have been consistently used for improving the bread rheology, structure, and quality. The small particles of starch agglomerates together into a bigger size and form a starch-hydrocolloid matrix for the retention of appropriate amount of water and such a matrix is responsible for improving the viscoelastic behaviour of the dough, and reduces the hardness of bread (Salehi, 2019). Rice is usually used for production of GF products due to its hypoallergenic and digestibility property. On the other hand, it has a smaller amount of protein and easily digestible starch (Rosell et al., 2014). Buckwheat, quinoa, sorghum, teff, and oats flour was also used for the GF bread and was evaluated for the glycaemic index (GI) and glycaemic load (GL). Quinoa bread indicated maximum GI followed by buckwheat, teff, sorghum and oats whereas sorghum bread showed highest GL followed by quinoa, oats, and teff (Wolter et al., 2013). However, low GI values of bread is associated with less hydration of flour due to controlled gelatinization of starch and less susceptibility to enzymatic hydrolysis (de la Hera et al., 2014). Table 1 shows addition of starch in the form of resistant starch, physically/chemically modified starch etc. into the gluten-free products could bring a great change in rheological properties of the products. Majorly, maltodextrin with varied dextrose equivalent (DE) value was used as an ingredient for maintaining the quality and stability of baked goods. It influences the bread volume due to the heating of starch at higher temperature (Witczak et al., 2010).

Pre-gelatinized cassava starch is suitable for the production of GF bread results in sticky and gummy dough ensuring the stable structure and holds the sufficient amount of carbon-dioxide, and improves the colour, aroma, and nutrient quality of GF bread (Taylor et al., 2006). Pongjaruvat et al. (2014) reported that the use of pregelatinised tapioca starch in rice flour based bread acted as forming agent with positive effect on bread crumb and dough volume. Resistant starch utilization leads to the improvement of nutritional composition of the GF bread, and it also improves the elasticity in baked products (Tsatsaragkou et al., 2014b). Quinoa and amaranth mostly consists of insoluble polysaccharides including homogalacturonans and rhamnogalactouronan linked with arabinose side chain. It also possesses highly branched xyloglucans and cellolose (Lamothe et al., 2015). Buckwheat contains higher amount of dietary fibre than other pseudocereals with less soluble to more insoluble dietary fibre ratio (Dziedzic et al., 2012). Table 3 shows combination of cassava starch, corn starch and rice flour used for the development of GF breads which resulted in better nutritional quality, appearance, taste and soft crumb (Sanchez et al., 2002). Incorporation of suitable amount of minor ingredients such as chestnut, and sorghum improves the nutritional attributes of GF breads otherwise higher percentage of these ingredients results in undesirable taste and appearance of the product (Capriles & Areas, 2014). Tsatsaragkou et al. (2014a) reported that the limited replacement of rice flour with carob flour prominently improved the quality of bread but did not affect the structural and textural parameter by its addition. Miyazaki et al. (2004) determined that maltodextrin with higher DE value is more effective in reducing the retrogradation as compared to maltodextrin with lower DE. Ziobro et al. (2012) formulated GF bread by using chemically modified starch such as hydroxypropyl distarch phosphate and acetylated distarch adipate to obtain elastic crumb with increased loaf volume. Christa et al. (2009) observed that buckwheat flour starch resulted in better water holding capacity as compared to wheat and potato starch which could be significantly responsible for the enhancement in specific volume of the loaf. Giuberti et al. (2016) found that higher fibre content is related to the decrease in starch hydrolysis during baking and thereby limiting the amylose release, interfere with the digestibility and leading to lower viscosity. An enormous source of flours have been used as a source of starches such wheat, rice, corn, cassava to simulate the viscoelastic properties of gluten in GF bread and other products which contribute in improving the structure, taste, mouthfeel, over all acceptability, and shelf life of such products (Lazaridou et al., 2007). The staling of bread is associated with change in colour, crust becomes softer, and fragrance get reduced. Usually, the retrogradation of starch is significantly associated with the spoilage of bread due to variation in the amylopectin over the period of time. However, it has been proposed that bread stiffening could be due to starch-gluten interfaces, where gluten is crosslinked by gelatinised starch (Martin et al., 1991).

11 Pseudocereals

In addition to proteins, starch, hydrocolloids, a variety of pseudocereals could also be used for the development of products for coeliacs and also add-on the nutritional value to the products (Alvarez-jubete et al., 2010). Use of quinoa and amaranth for bread development resulted in firm, and intact structure with increased cohesiveness and adhesiveness. Utilization of pseudocereal shows significant effect on loaf volume, crust/crumb appearance, and bake loss (Alvarez-jubete et al., 2009). Buckwheat is one of the mostly used pseudocereals, containing rutin as a major phytochemical for maintaining the antioxidant property. Torbica et al. (2012) reported that combination of rice flour and buckwheat flour was effectively incorporated in GF flour which resulted in improved flavour and rheological property (Table 1). Alvarez-Jubete et al. (2010) observed that the nutritional components such as protein, vitamins, fibre could be increased by replacing potato starch with pseudocereal flour. This could also be responsible for softer crumb, increased bread volume, and retention of polyphenolic content. GF dough is less elastic and requires less mixing time, proofing, baking as compared to wheat dough (Zannini et al., 2012). Hence, it is necessary to add gums, stabilizing agent, foaming agents, and pre-gelatinized starch for the retention of gas during baking. Pseudocereals are good source of dietary fibres, which can be used to regulate the blood sugar level, obesity (Zhou et al., 2019). Wronkowska et al. (2013) formulated GF bread by incorporating buckwheat into the rice flour and evaluated for its sensory attributes. It was reported that presence of buckwheat flour reduces the retrogadation of starch and further could extend the shelf life due to its anti-staling property. Various gluten-free extruded products were also optimized by a significant number of scientists in which Yalla and Manthey (2006) suggested that incorporation of buckwheat flour resulted in increased hydration level of pasta along with well-structured regularity as could be seen only in case of gluten rich products. Alvarez-Jubete et al. (2010) found that replacement of potato starch with pseudocereal consequences are increased value of some nutrients such as protein, fibre, iron, phytonutrients and increased bread volume with a softer crumb. Additionally, bran portion of quinoa and amaranth could be a better source for improving the protein and fat content in GF products. Alencar et al. (2015) studied the influence of amaranth flour, quinoa flour and sweetener on gluten-free bread which resulted in the bread with improved water activity, firmness as compared to the control bread.

The textural and nutritional characteristics of corn and oats bread observed to be comparable with the one which was produced from barley flour. Addition of dietary fibre by partially replacing pseudocereal could significantly bring changes in colour, appearance, texture, and quality of the GF products (Arslan et al., 2019).

12 Non-wheat Flours

Proso millet is one of the grains mostly used for the development of GF products. Millet grains are typically de-hulled to direct its taste and biological value in a definite way for human nutrition (Bora et al., 2019). The replacement of 10% rice flour with proso millet bran showed a significant effect on the superiority of gluten-free bread related to its higher fibre and phenolic content, and improved the bread volume and softer crumb (Mustac et al., 2020). The specific volume of the rice loaf was found to be higher when it was treated with protease due to the low starch damage. Additionally, the dough viscosities of various samples of rice were analysed and reported that rice with higher amylose contents showed lower value than rice with lower amylose contents under similar conditions and vice versa (Aoki et al., 2020). ‘Mizuhochikara’ rice is one of the commercially available rice varieties in Japan which is recognized to be suitable for the development of gluten-free rice bread. Colocasia flour could also be considered as an alternative flour for the formulation of GF bread owing to improved nutritional value such as fibre, starch, minerals along with some phytochemicals (Arici et al., 2016). Bee pollen powder is rich in phytochemicals, fibres, and minerals which could be an alternative for the preparation GF bread with improved sensorial characteristics. The functional GF breads which were fortified with bee pollen resulted in increased moisture level and total ash content as well as biologically active components with enhanced antioxidant activity (Conte et al., 2020). Utilization of corn flour (Table 1) increased the surface area of the breads due to complex lattice formation which led to a significant increase in the number of free water binding places for the enhancement of hydration properties. Bread obtained from corn dough resulted in solid like behaviour since elastic values was higher than viscous values for same bread (Chau et al., 2006). Apart from lentils and pseudocereals, some more sources may be utilized for the formulation of products for celiac patients including nuts (chestnut, walnut, almonds, cashew nut, and hazelnuts), seeds (pumpkin seeds, flax seeds, chia seeds), and tubers such as arrowroot, tapioca, taro, potato (Green et al., 2008). Pulses can also be included in bread in various forms such as dehusked or husked flour, germinated or fermented flour, and single or in combination. The inclusion of pulses to wheat (whole/refined) flour for bread making influences the nutritional (protein, fat, dietary fiber, mineral), textural, and functional properties (Indrani et al., 2011). Phytonutrients such as phytin, tannin, ferulin, vallinin can be reduced from the pulses by soaking, cooking, steaming etc. thereby contributing to improved sensory characteristics (Aremu et al., 2016). Bread with added germinated chickpea improved its specific volume, structure, and texture. Moreover, it may also contribute in improving the hydrolytic enzymatic activity (Ouazib et al., 2016). Incorporation of processed pulse (fermented) flour in breads subsequently enhances its nutritional percentage of dietary fiber, protein, mineral, fat, and antioxidant contents. Simultaneously, it also reduces the phytic acid content and tannins when compared to non-fermented pulses (Chinma et al., 2016). Paraskevopoulou et al. (2010) reported that incorporation of lupin protein isolates resulted in extending the shelf life of bread by reducing the staling effect. This could be possibly due to the interaction between starch and protein which helps in avoiding the movement of moisture content from crumb to crust. The blend of milk protein isolate and rice starch was used for the preparation of gluten-free bread which resulted in increased volume, enhanced appearance and overall acceptability. It also showed softer crust and improved crumb appearance (Gallagher et al., 2003). A significant increase in bread volume was reported when the combination of amaranth flour and soybean flour was used. Replacement of soybean flour from lupin resulted in better specific volume of the bread as compared to the bread obtained from amaranth flour and soybean flour. Amaranth bread was noted to be much softer than wheat bread. Moreover, firmness and resilience in bread was influenced by the blend of soybean lupin and navy beans (Liu et al., 2019). Bread produced out of amaranth, soybean and lupin flours showed improved nutritional quality in terms of protein, dietary fibre for the better health benefits. There are many more flours which can be used for the development of GF products such as legume flour (Minarro et al., 2012), tigernut flour (Demirkesen et al., 2013), Carob germ flour (Tsatsaragkou et al., 2013), and apple flour (Averbeck & Petrasch, 2013) for increasing dietary fibre, mineral and protein contents to enhance their nutritional value. Incorporation of guar gum into the flaxseed flour could also be used as an alternative for the preparation of GF bread with better crust and softer crumb (Ozkoc & Seyhun, 2015).

13 Sourdough

Sourdough is a combined mixture of flour and water fermented with yeasts and lactic acid bacteria which are responsible for aroma, acid production and leavening of sourdough. Application of sourdough has been in practice from years for the production of wheat and rye breads, and is well established (Huttner & Arendt, 2010). The positive effects associated with the sourdough are lowering of pH due to production of acids by lactic acid bacteria during fermentation, ensuring more gas retention, activation of proteases endogenously present in flour, water binding of starch granules, phytate complex solubilisation by endogenous phytases, pentosan swelling and prevention of spoilage (Katina et al., 2005). Similarly, the potential of sourdough for the development of gluten-free bread gains attention of researchers.

Vogelmann et al. (2009) investigated a variety of lactic acid bacteria and yeasts to assess their adaptability to sourdoughs prepared from cereals containing gluten like wheat, barley and rye, gluten-free cereals like rice, millet, oat and maize, pseudocereals like quinoa, amaranth, buckwheat. It was observed that each substrate when mixed with same starter culture and fermented under similar conditions showed different activity as some strains were substrate specific. Similarly, apart from other factors responsible for sourdough, the ingredient of gluten-free flour predicts the microbiota of the resultant sourdough. Thus, there is a need to design and develop fermentation conditions and starter mixtures specific for sourdoughs obtained from gluten-free flours. Despite of all this, the research dealing with the sourdough effect on gluten-free bread quality exhibits positive effects of some lactic acid bacteria which are specifically used for rye and wheat sourdoughs. Clarke et al. (2002), observed increase in loaf volume when added with gluten-free sourdough prepared with Lactobacillus plantarum, Lactobacillus brevis or a commercially available starter culture designed specifically for wheat dough. The crumb structure of gluten-free bread, developed from sorghum flour was enhanced by sourdough fermentation (Schober et al., 2007). Furthermore, research conducted by Moore et al. (2008), obtained softer gluten-free bread using Lactobacillus plantarum for sourdough starter culture, and it was also observed to inhibit mould growth. Sourdough fermentation has been proved to be beneficial in improving the nutritional attributes of gluten-free bread. It increases the mineral uptake and amount of bioactive components, reduces the glycemic reponse value and also the level of anti-nutritional factors.

13.1 Chia, Flaxseed and Millet

Maidana et al. (2020) developed gluten-free breads with chia and flaxseed sourdoughs fermented by selected lactic acid bacteria. Inoculants from fermented sorghum, based on techno-functional properties, such as Weissella cibaria CH28 along with Lactobacillus fermentum FUA3165 and Lactobacillus plantarum FUA3171, for the fermentation of flaxseed and chia sourdoughs were used for preparation of gluten-free bread from sorghum. Sorghum based breads prepared with different percentages of oilseed sourdoughs (fermented by lactobacilli and W. cibaria) showed that the breads started with 30% and 40% sourdoughs improved specific volume and appearance of bread as compared to 100% bread. Sensorial property analysis revealed that gluten-free breads prepared with 40% replacement were highly acceptable by the panel members.

Pearl millet sourdough: Pearl millet has been cultivated to serve a number of purposes such as food, forage and feed. It has not been explored much in the food industries for food products, and thus has been in the category of under-utilised crop despite of all the essential nutrients it contains. It is an excellent source of zinc and iron providing a minimum cost solution for the issue of mineral deficiency, and therefore it can be used for the production of foods rich in nutrients. Recently, a study has been conducted by Nami et al. (2019) to explore the efficiency of pearl millet for preparation of sourdoughs fermented with combination of four Lactobacillus species to improve the gluten-free bread quality. They have studied the effect of sourdough on the physicochemical properties, consumer acceptance and shelf stability of bread prepared from flour of pearl millet. Fermentation carried out by single and multiple species, cause reduction in the dough pH and raised the level of titratable acidity. They have reported that incorporation of sourdough proved beneficial in enhancing the elasticity and reducing the stiffness of the dough. Breads prepared with fermented sourdough with L. brevis showed great effect on specific volume, loaf height, moisture content and porosity. Storage study of breads revealed that the crumb moisture content decreased whereas moisture content of crust increased during storage. Moisture of breads made with Sourdough was better retained compared to that of conventional breads, and the development of mold was suppressed for a longer period of time. The sourdough based bread received highest sensory scores, and was more palatable than other conventional or chemically modified breads. Thus, they have recommended the use of pearl millet sourdough for the production of high quality bread.

14 Conclusion

The role of additives among ingredients in gluten-free bread making is of paramount importance. Gluten-free breads prepared without using additives are characterized by various defects including textural and nutritional. There is no single wheat gluten replacer used in gluten-free breads. However, the additives are being used in combination to mimic the functionality of gluten in commercial breads. Use of additives especially hydrocolloids, enzymes, emulsifiers, dietary fibre, proteins, starch, salts, acids and minerals besides improving organoleptic and structural properties of GF breads address to their nutritional inadequacies.

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Role of Starch in Gluten-Free Breads

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1 Starch

Starch is the chief carbohydrate present in plant parts such as leaves, flowers, fruits, seeds, stems and roots. Plants use starch as a major source of carbon and energy stored within starch is synthesized in the amyloplast and chloroplast of green leaves. Primary sources of starches are cereals (40–90% on dry weight basis), tubers (65–85% on dry weight basis), roots (30–70% on dry weight basis), legumes (25–50% on dry weight basis) and certain fruits such as mango (70% on dry weight basis) (Alcázar-Alay & Meireles, 2015). Starch plays an important role in the development of food products such as sauces, soups, bakery and confectionery products. It acts as a viscosifier, thickener, stabilizer, gelling, water retention and emulsifying agent in different food products. It is also used to replace lipids in various food products after modifications. Modified starches mimic the functions of fat by binding with water and improve the texture and mouth feel of the products. It gives slippery mouth feel in high moisture foods such as salad dressings, sauces, margarine and meat emulsion (Fellows, 2017). Higher availability, lower cost and good film forming property of starch calls for the replacement of gelatine (Poeloengasih et al., 2018). Starches have a significant role in the gluten-free breads. The main sources of starches used in the gluten-free breads are corn, tapioca, potato, rice, buck wheat and quinoa (Horstmann et al., 2016, 2017). The functions of starch in the gluten-free dough depend upon the factors such as composition, degree of damaged starch, amylose-amylopectin ratio, degree of hydration, gelatinization properties, pasting properties and the interaction between the starch and other ingredients in the formulation. Starch is primarily added in the gluten-free bread dough to enhance viscosity, thickening and water retention properties. It also improves the gas holding capacity of gluten-free bread doughs resulting in higher volume of breads.

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1.1 Flour as a Source of Starch

Flour is a fine and soft powder from plant origin (Witczak et al., 2016). Starch containing flour has a significant role in the replacement of wheat flour for the development of gluten-free breads. Starch is a major constituent of flour from different cereals and thus controls most of the properties of dough. Flour also contains non-starch components such as proteins, lipids, vitamins, minerals and enzymes. The non-starch components depend on the flour origin and processing methods. Presence of non starch components enhances the nutritional value of flour. Milling process and sieving techniques play a considerable role in the quantity of non starch components (Kang et al., 2019). Effective milling and sieving operations reduce the amount of non starch components in the flour (Heshe et al., 2016; Kumar, 2015). These non starch components of the flour bind with water molecules and affect the rheological properties of dough. Proteins influence the rheology and water binding properties of the dough. Lipids improve the texture and flavour of the finished product. Vitamins and minerals increase the nutritional profile of the gluten-free breads and while enzyme help in the formation of simple sugars for the fermentation process.

2 Starch Composition

2.1 Amylose and Amylopectin

The starch molecule is composed of two main building units that is amylose and amylopectin. α-D glucopyranosyl units are the monomeric units of starch. In amylose fraction, glucose units are linked by the α-1, 4 glycosidic linkages and in amylopectin fraction glucose units are linked by both α-1, 4 and α-1, 6 glycosidic linkages (Alcázar-Alay & Meireles, 2015). Amylopectin units are composed of a large number of short chains in a branched type and amylose units contain linear or slightly branched chains. Amylose and amylopectin constitute 98–99% of the dry weight of starch and the remaining percentage is occupied by protein, lipid and minerals (phosphorus, calcium, potassium, iron, sodium, zinc, sulphur, copper and magnesium) (Horstmann et al., 2017). Starch contains both amorphous and crystalline regions. Hence, it shows semicrystalline properties. The crystalline region of starch is formed by the single helices and double helices of amylose and amylopectin units, respectively. Sequential packing of double helices formed by the flexible A-chains with 6 to 12 degree of polymerisation of amylopectin units form crystalline sites in the starch granules (Sudheesh et al., 2019a). Major commercial sources of starch such as corn, wheat, rice and potato contain 70% to 80% amylopectin and 20% to 30% amylose units while waxy corn starch contains only about 2.5% amylose and rest is occupied by amylopectin (Horstmann et al., 2017). Amylose-amylopectin ratio of starch depends upon its botanical origin, climatic conditions of plant and the time of harvesting. The ratio has a considerable role in the gelation property of starches (Sudheesh et al., 2020b). Amylose-amylopectin ratio should be high in the starch used for bread making. Gelatinization properties of starch depend upon the amylose-amylopectin ratio which also influences the dough rheological properties. Hence, it has a considerable role in the structure of baked products. Higher amylose content provides good pasting and gelation properties and it decreases the digestibility of starch. Higher pasting property improves the volume of breads. Amylose content also influences the swelling and solubility of the starch. Depending upon the proportion of amylose, starch can be classified into waxy starch which contains less than 15% of amylose and non-waxy starch which contain more than 15% amylose (Alcázar-Alay & Meireles, 2015).

2.2 Damaged Starch

Damaged starch is the type of starch, which is formed by the disruption of starch molecules during the process of extraction and refining. The damaged content of starch varies from 0.5% to 7.5%. The factors affecting the damaged starch content are the starch source and the milling condition and techniques. Granular morphology of starch granules change by the damages occurring during the processing. Damaged granules have a significant role in the alteration of rheological and functional properties of the starch (Horstmann et al., 2017). Damaged starch granules are structurally weaker and adsorb more water as compared to native starch granules thus having more swelling capacity. As a result, damaged starch is considered to be more hygroscopic in nature and is less resistant to enzymes. This is because morphological damages facilitate the penetration of enzymes causing higher degradation of starch. Damaged starch also performs an important role in dough. The enzyme amylase acts on the damaged starch easily and converts it to simple sugars. The latter is utilized by yeast to produce more carbon dioxide to generate gas cells within the dough. It increases the raising of dough and helps in development of products with higher volume. With such attributes, damaged starch has also a role to play in gluten-free breads (Van Der Maarel et al., 2002; Devi et al., 2009).

2.3 Enzymes

The major enzyme present in the starch is amylase. Amylase can be classified as α-amylase and β-amylase. α-amylase is an endogenous enzymes which cleaves α-1, 4 glycosidic linkages present in the inner part of amylose and amylopectin units. β-amylase acts on the α-1, 4 glycosidic linkages present in the outer part of amylose and amylopectin units. Amylase has an essential role in the preparation of baked products. It produces simple sugars by acting on the starch molecules (Horstmann et al., 2017) which is the only substrate consumed by the yeast during bread making.

In addition, amylase enzyme retards the retrogradation capacity of amylose chains. Retrogradation is the reassociation of starch molecules, which leads to recrystallisation of amylose and formation of double helices by the amylopectin chains. Retrogradation causes the formation of hard and opaque texture and leads to staling of the baked products (Van Der Maarel et al., 2002; Giannone et al., 2016). The refined starch incorporated gluten-free breads stale faster than conventional breads.

2.4 Protein

The protein content of starch depends upon the source of starch and the isolation procedure used for its extraction. Common starch sources like rice (0.04%), wheat (0.19%), corn (0.37%), sorghum (0.25–0.28%), buck wheat (1.15–3.96%), waxy corn (0.20%), potato (0.08%), oats (0.02–0.09%) and tapioca (0.03%) contain different amount of protein. Starch can be isolated by various methods such as alkali method, water method and enzymatic methods. The protein content depends on the effectiveness of isolation methods. It has an important role in the starch properties. It influences the physico-chemical and functional properties such as swelling, solubility, pasting and gelatinization properties of starches (Horstmann et al., 2017). The protein adhering to the surface of starch granules undergoes denatuaration during the heating. The denatured layer of protein surrounds the starch granules and restricts the water penetration and amylose leaching. It decreases the swelling and solubility of starch granules. Pasting viscosity also decreased by the lower swelling and solubility index (Noora et al., 2019; Maniglia & Tapia-Blácido, 2016). Higher granular stability and lower swelling property of starch granules increases the gelatinization temperature. Hence, the lower protein content improves the functional properties of starch and aids in the development of gluten-free breads.

2.5 Lipids

Like other non-starch components, the lipid content of starch also depends on the source of starch, amylose content, structure of grain endosperm and type of isolation procedure used (Horstmann et al., 2017). Most of the commercial starches contain lipid <1%. Lipids exist in the starch granules as amylose–lipid complexes. These generally align with the core part of the amylose helix. So, lipid is mainly linked with the amylose units of the starch molecules. Surface active lipids such as triglycerides link with amylose units by the ionic or hydrogen bonds. Lipid content has a considerable role in the functional properties such as swelling capacity, solubility, gelatinization, pasting and retrogradation. Lipid molecules strongly bind with amylose units which restricts the swelling capacity of starch granules. Interactions between the starch chains and lipid molecules reduce the amylose leaching resulting in lower solubility. Pasting properties of starch are decreased with the decrease in amylose leaching and swelling properties. Amylose–lipid complex increases the gelatinization properties of starch. It also decreases the retrogradation properties of starch paste (Copeland et al., 2009). This property can be accredited to the lower amylose leaching due to the interactions between the amylose chains and lipid units.

2.6 Minerals

The major minerals present in the starch are calcium, phosphorus, iron, potassium, magnesium and sodium. Common starch sources like wheat (0.16%), corn (0.07%), sorghum (0.10–0.14%), buck wheat (0.23%), waxy corn (0.07%), potato (0.33%) and oats (0.13–0.20%) contain various amount of minerals (Horstmann et al., 2017). The mineral content of starch generally improves the nutritional profile of gluten-free breads. Phosphorus in starch granules occurs mainly in three forms viz.; starch mono phosphate, phospholipids and inorganic phosphorus. Starch mono phosphate is linked with amylopectin units of starch granules and phospholipids are linked with amylose units (Alcázar-Alay & Meireles, 2015). Starch mono phosphate increases the viscosity and light transmittance of starch paste and phospholipids decrease viscosity and paste clarity. This implies that the nature of phosphorus has a significant role in the pasting and light transmittance properties of starch. Starches from the cereals such as rice and wheat contain a higher amount of phosphorus in the form of phospholipids thereby producing low clarity paste. The higher amount of starch mono phosphate in the potato starch forms a paste with high clarity (Alcázar-Alay & Meireles, 2015). Starch mono phosphate improves the textural property of gluten-free breads by increasing the pasting viscosity.

3 Starch Morphology

Morphological properties of starch depend upon its botanical origin. Generally, the size of starch granules ranges from 0.1 μm to 100 μm (Alcázar-Alay & Meireles, 2015). Starch granules have different shapes such as oval, spherical, lenticular and angular while their size distribution can be uni, bi or polymodal. Amyloplasts are double layered organelles in plant cells which contain individual or grouped starch granules. Potato starch granules are oval in shape with a smooth surface. Potato starch includes both small and big granules, the size of which range between 4 to 10 μm and 10 to 87 μm respectively. Tapioca starch granules are agglomerated and their size varies from 7–25 μm. The shape of tapioca starch granules is polygonal and oval with plain surface. Corn starch has a polyhedral shape with size varying from 3 to 21 μm. Rice starch granules are agglomerated like tapioca starch. The shape of rice starch granules is polygonal and its size varying from 2 to 7 μm (Horstmann et al., 2017; Wani et al., 2012). Most of the starch granules exhibit central line called maltese cross when observed under the polarized light (Pérez et al., 2009). Each granule contains one or two maltese crosses. Birefringence is the ability of starch granules to doubly refract polarised light. Birefringence pattern of starch granules is related to the crystalline properties of starch granules. Strong birefringence pattern of starch granules indicates higher crystalline properties (Alcázar-Alay & Meireles, 2015). The size of starch granules has an important role in the pasting properties and enzymatic degradation. Higher size of starch granules gives starch paste of higher viscosity. The enzymatic degradation rate of starch granules with smaller size is higher as compared to those with a larger size. Higher rate of enzyme action is attributed to the larger surface area of smaller sized granules. Starch granules with a smaller size show higher bulk density. Higher bulk density of starch granules provides rough surface to the finished products while lower bulk density leads to smooth texture of the products (Sudheesh et al., 2019a). Starch granules with higher size generally exhibit higher viscosity and it enhances the textural property of gluten-free breads.

4 Starch Digestibility

The digestibility of starch depends on the factors such as granular size and morphology, swelling properties, amylopectin chain length, amylose content, simple sugar content, relative crystallinity, amylose-lipid complexes and the interaction between starch chains (Sudheesh et al., 2019b, 2019c). Based on digestibility, starch can be classified as rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). RDS increases and maintains the higher blood glucose level. SDS undergoes complete digestion in the small intestine and it slowly increases the blood glucose level. SDS reduces the risk of diabetes and cardiovascular diseases (Sudheesh et al., 2019c). RS doesn't undergo any digestion in our body as it is resistant to amylase enzyme. The lower surface area of amylose units reduces the action of amylase enzyme on its chain. Hence, starch with higher amylose content has lower digestibility. Higher extent of gelatinization of starch increases the RDS content in the baked products. This is because gelatinized starch has higher digestibility while retrogradated and recrystallised starch has lower digestibility. Cooling of gelatinized starch increases the SDS and RS content and decreases the RDS content (Horstmann et al., 2017). Modified starches with higher amylose content can be used as an additive in low digestibility gluten-free breads (Horstmann et al., 2017). Amylose also plays an important role in the rheological properties of starch paste. It increases the pasting viscosity of the starch. Starch with higher pasting viscosity tightens the dough and improves the volume of the gluten-free breads. Hence, it facilitates the development of gluten-free breads.

4.1 Role of RS in the Gluten-Free Breads

RS is the type of starch that remains indigestible after 120 h of the digestion period (Sudheesh et al., 2019b, 2019c). It can be classified into four major groups that is; RS1, RS2, RS3 and RS4. Resistant starch type 1 (RS1) is the starch present in the whole cells and tissues, which are in physically inaccessible form. Resistant starch type 2 (RS2) is the native, non gelatinized starch which cannot be digested by the human amylase. Resistant starch type 3 (RS3) is the retrogradated starch. When starch gel is stored at low temperature (4 to 21 °C), it undergoes retrogradation (reassociation of starch chains) forming RS3. Resistant starch type 4 (RS4) is the chemically modified starch. It does not undergo digestion due to the presence of chemical functional groups such as carbonyl, carboxyl, acetyl, carboxy methyl, hydroxy propyl, hydroxy ethyl and structural changes occurred by the modifications. RS acts as food for beneficial micro flora inhabitating our intestine (prebiotics). It also regulates mineral absorption, fat accumulation and cholesterol deposition (Liu et al., 2015). RS maintains the low glycemic condition and regulates blood glucose level. It increases satiety values (the degree at which food gives a human the sense of food gratification) and improves digestion. When RS reaches the bowel, it undergoes fermentation and produces short chain fatty acids like butyric acid. This reduces the pH and stimulates the growth of beneficial bacteria inhabitating the intestine. Lower pH also assists in the elimination of harmful microorganisms from the colon (Witczak et al., 2016).

RS has a significant role in the development of baked products. Retrogradated acetylated starch, that is RS4 changes the quality of wheat dough as well as the breads. It also increases the water absorption of flour, development of dough and yield of breads. As per an earlier study, the incorporation of modified pea starch increased the RS content of the breads and decreased the amylopectin retrogradation (Witczak et al., 2016). Addition of RS improved the total dietary fibre (up to 89%), insoluble dietary fibre (up to 137%) and soluble dietary fibre (up to 18%) in gluten-free breads (Korus et al., 2009). RS helps to develop a soft crumb in gluten-free breads. Addition of RS4 in the dough significantly improves the bread volume and elastic properties and, decreases the bread hardness (Witczak et al., 2016). Hence, RS has a significant role in the rheological properties of gluten-free breads.

5 Functional Properties of Starch

The use of starch in various food products depends upon its functional properties. Starch in its native state exhibits various functional properties such as swelling, solubility, emulsification, water retention, pasting, gelatinization and retrogradation. The factors affecting the functional properties of starch are amylose-amylopectin ratio, amylopectin chain length, granule size and morphology, density and, crystalline properties. The functional properties of starch play a significant role in the structural and rheological characteristics of gluten-free breads. Starch is sparingly soluble in cold water, but readily soluble in hot water. The crystalline properties of the starch decrease its water solubility. Heating of starch in an excess amount of water leads to swelling of granules which finally rupture and form a viscous paste. The hot viscous paste forms gel upon cooling (Horstmann et al., 2017). All these functional attributes of starch have a role to play in different food formulations.

5.1 Swelling and Solubility Index

When water is added to starch, the granules absorb water molecules. While heating the suspension, the hydrogen bonds present in the starch granules are broken due to which it absorbs more water molecules. Further, during heating, as the hydrogen bonds between starch chains are broken, the newer hydrogen bonds between the starch and water molecules are formed. Morphological damages such as holes, cracks and fissures gradually occur in the starch granules due to thermal force generated during the heating. Water molecules generally penetrate the granules through these surface damages and make hydrogen bonds with starch chains. This increases the water retention capacity of starch granules. Starch granules swell due to the penetration of water molecules. Further swelling of starch granules leads to breaking and leaching of chains. The leached chains are soluble in water. In the native state, starches are sparingly soluble in cold water. Lower solubility of starch is attributed to the crystalline properties of starch molecules. The factors affecting the swelling capacity and solubility of starch are; its morphology, amylose-amylopectin ratio, crystalline properties, interaction between the starch chains and the number of amylose–lipid complexes. Swelling capacity and solubility have an important role in the pasting properties, *in vitro* digestibility and gelatinization properties of starch. During the swelling of starch granules, separation of amylose and amylopectin phases and loss of crystallinity is observed (Sudheesh et al., 2019a, 2019c; Witczak et al., 2016). Swelling and solubility index of starch granules influences its pasting properties. The higher swelling and solubility index leads to a higher pasting viscosity of starch, which in turn improves the volume of gluten-free breads.

5.2 Gelatinization Properties

Gelatinization is the swelling of starch granules and the formation of viscous starch paste when heated in an excess amount of water. During gelatinization, starch granules undergo swelling, solubilisation, melting of crystallites and, loss of crystalline properties and birefringence (Witczak et al., 2016). Gelatinization increases the viscosity of the starch containing medium. Onset temperature, peak temperature, conclusion temperature and enthalpy are the gelatinization parameters. Onset and conclusion are the temperatures required to break the weak and strong starch crystallite, respectively. Enthalpy is the energy required to convert starch suspension to viscous starch paste. It measures both the quality and quantity of starch crystallites. Crystalline properties (relative crystallinity and stability of starch crystallite), amylose-amylopectin ratio, amylopectin chain length, amylose-lipid complex, amylose content and swelling index are the factors affecting the gelatinization properties of starch (Sudheesh et al., 2019a, 2019b, 2019c). Gelatinization is the property which determines the use of starch in food products. When starch granules reach peak swelling point, these provide desired thickening to the food formulations. Gelatinization parameters of root and tuber starches are lower than cereal starches (Witczak et al., 2016).

5.3 Pasting Properties

Pasting properties of starch have a significant role in the processing of food products. Starch suspension is converted to viscous starch paste during heating. The starch paste acts as an excellent thickening agent and viscosifier in the dough and improves the structure of gluten-free breads (Witczak et al., 2016). Pasting temperature is the minimum temperature required to cook the starch suspension. It depends on the number of cross-links present in the starch granules and strength of intermolecular forces (Sudheesh et al., 2019c). Peak viscosity is the viscosity of starch at the equilibrium between the swelling of starch granules and polymer leaching (Sudheesh et al., 2019d). Higher peak viscosity leads to more thickening of food products at higher temperature. The decrease in the viscosity of starch paste just following the peak viscosity at higher temperature is called trough viscosity. Thermal and shear force stability of starch granules can be concluded in terms of breakdown viscosity. Higher breakdown viscosity indicates lower stability of starch granules towards heat and shear force. Setback and final viscosity are related to the temperature stability of starch paste where the final viscosity depends upon the aggregation of amylose molecules, the setback viscosity is something related to the retrogradation of the starch. Higher setback viscosity of starch indicates the higher rate of retrogradation. Starch with higher retrogradation properties forms a hard gel (Sudheesh et al., 2019a, 2019c).

Gelatinization and pasting properties have a significant role in the development of gluten-free breads. In general, gluten-free bread doughs contain starch, water and yeast. One of the challenging attempts during the production of baked products is to keep the air and CO_2 inside the dough and prevent the settling of yeast cells and ungelatinized starch granules (Witczak et al., 2016). Yeast cells ferment the simple sugars and produce CO_2 and alcohol. This CO_2 has a significant role in the expansion of dough to improve the bread volume. However, CO_2 produced by the fermentation has a higher chance to escape from the dough. During baking, starch granules undergo gelatinization and form a viscous starch paste (Witczak et al., 2016). This viscous starch paste gives the tight texture to the dough and prevents the settling of yeast cells and ungelatinized starch granules and keeping the air and CO_2 inside the dough. It gives the desired crumb structure of gluten-free breads.

5.4 Retrogradation Properties

Retrogradation is the property of recrystallisation exhibited by the starch paste during low temperature storage. It involves the reassociation of starch chains (amylose and amylopectin units) leached out during gelatinization. During retrogradation, amylose-amylose, amylose-amylopectin and amylopectin–amylopectin interactions take place. It leads to recrystallisation of amylose units and formation of double helices by amylopectin units which finally form a three dimensional network of starch gel (Sudheesh et al., 2019a). During reassociation of starch chains, inter junction zones are formed leading to the formation of an opaque and turbid gel. Retrogradation generally increases the hardness of starch gel.

Retrogradation can be linked to the staling after baking process. Staling is the formation of harder crumb of gluten-free breads during the storage period (Witczak et al., 2016). Reassociation of amylose units occurs within minutes to hours of storage while amylopectin reassociation occurs over hours or days. Hence, amylose-amylopectin ratio of starch has a significant role in the duration of retrogradation of both gluten-containing as well as gluten-free breads. Higher amylose content leads to an increase in the retrogradation property of starch, resulting in higher hardness of gluten-free breads. In general, amylose crystallisation has a higher impact on the hardness of breads when compared to amylopectin. Amylose units make complexes with lipids due to which their leaching is reduced during gelatinization. Lower amylose leaching retards the retrogradation thereby reducing staling/hardness of the gluten-free breads. In this way, the staling effect of gluten-free breads can be prevented or reduced by the addition of lipids, emulsifiers and other ingredients in the dough (Horstmann et al., 2016).

6 Modified Starches in Gluten-Free Breads

6.1 Modification of Starch

Most of the starches show a lower swelling, solubility, thickening ability, thermal stability and poor shear resistance in their native form (Sudheesh et al., 2019b, 2019c). In addition, these are sensitive towards pH variations and all such properties limit the industrial application of starches in their native form. This necessitates the modification of native starches to increase their industrial applications. Modification of starch can be done by physical, chemical and enzymatic methods. Physical modifications can be further classified as thermal and non-thermal modifications. The thermal modifications, include pregelatinization, annealing, heat moisture treatment, microwave irradiation, radiowave treatment and dehydration (drum drying and spray drying). Non-thermal modifications include irradiation, pulsed electric field, cold plasma, high pressure processing and ultrasonication. The physical modifications make use of the parameters such as moisture, temperature, shear force and irradiation to bring about desired changes in the starch. On the other hand, the chemical modifications involve acid hydrolysis, esterification, etherification (hydroxypropylation, carboxy ethylation), alkali treatment and cross-linking (Alcázar-Alay & Meireles, 2015).

Modified starches have an important role in the development of baked products. Modified starches may find more applications as thickeners, viscosifier, texture modifiers and emulsifying agent in gluten-free breads. Modified starches also show lower retrogradation properties due to which the staling effect of breads is reduced and softness is increased.

6.1.1 Physical Modification of Starch and Its Application in the Gluten-Free Breads

Pregelatinization

Pregelatinization involves heating the starch suspension above the onset temperature so that the starch granules undergo complete gelatinization. During gelatinization, starch granules swell due to which the amylose and amylopectin chains leach out to form a viscous paste. Pregelatinization leads to complete fragmentation of starch granules and loss of birefringence and crystallinity. It improves swelling index, solubility and cold water dispersion of starch granules (Alcázar-Alay & Meireles, 2015; Horstmann et al., 2017).

The gelatinized starch is subjected to drying using the techniques like drum and spray drying. In drum drying, heated metallic rotating drums are used to dry the starch suspension. The dried starch cake formed on the surface of drums is removed and sized to small particles to be used in the baked products. Pregelatinized starch is generally soluble in cold water. It swells and forms a thicker paste without heating, thus reducing the time and energy for processing of gluten-free breads (Horstmann et al., 2017). Pregelatinization improves the viscosity and thickening properties of the dough and can be used as a texture modifier in the gluten-free breads. Spray drying of starch is another method followed for drying of pregelatinized starch. In this technique, drying is commenced by passing the atomized starch suspension through the preheated chambers. Spray dried pregelatinized starch shows excellent functional properties in cold water. Pregelatinized cassava starch after spray drying showed higher cold viscosity, swelling and solubility index (Dos Santos et al., 2019). Moreover, spray drying of partially gelatinized corn starch drastically improved its hydration properties at lower temperature (Fu et al., 2012). Pregelatinized cassava starch has been used to improve the texture of gluten-free breads prepared from rice flour. It gave a batter like appearance to the dough and decreased its shear force stability. Pregelatinized starch also enhanced the crumb softness and volume of gluten-free breads (Pongjaruvat et al., 2014; Witczak et al., 2016; Horstmann et al., 2017).

Annealing and Heat Moisture Treatment

Annealing is a technique of hydrothermal modification of starch. Heat and moisture are the two parameters that are controlled during the process of annealing. It is performed above the glass transition temperature and below the onset gelatinization temperature of starch (Sudheesh et al., 2019c). Annealing is generally carried out at a higher (moisture >60%) or medium moisture content (40–50%) (Alcázar-Alay & Meireles, 2015). It reorganizes the granular structure of starch. It also improves the molecular mobility of starch granules. Thermal force generated during annealing increases the interaction between the starch chains. Annealing stabilizes the amorphous regions of starch by reorganizing its molecular chains. Hydration of starch granules converts the glassy state to a static state and increases the mobility of the amorphous region to the crystalline region (Horstmann et al., 2017). Therefore, annealing increases the crystalline properties, thermal stability and gelatinization properties while it decreases the swelling index and solubility of starch molecules. It also decreases the digestibility and increases the SDS and RS content of starch (Sudheesh et al., 2019c, 2020b, 2020d).

Heat moisture treatment includes heating the starch with a limited amount of moisture content (less than 35%) for a duration of 15 min to 16 h. Heat moisture treatment helps to form new crystalline spots thereby increasing the crystalline region of starch granules. It stabilizes the starch granules by increasing the interaction between the starch chains and generally decreases the swelling index, solubility and amylose leaching. It also increases the RS and SDS content and decreases digestibility of starch. Strong interaction between the starch chains have been reported to stabilize the starch structure and decrease the accessibility of enzymes (Alcázar-Alay & Meireles, 2015; Horstmann et al., 2017; Sudheesh et al., 2020c).

Annealed and heat moisture treated starches can be used as an additive in the dough to reduce the staling effect of gluten-free breads. The main aim of hydrothermal modifications is to increase the pasting temperature, viscosity profile, thermal stability, gelatinization temperature range and resistance of starch to acids and shear. Hydrothermally modified starch reduces the crumb hardness and retrogradation enthalpy of gluten-free breads (Witczak et al., 2016).

Other Physical Modifications

Microwave irradiation and radiowave treatment are the other types of thermal modifications that change the physico-chemical and functional properties of starches. These modifications lead to the degradation of starch chains changing their hydration, crystalline and gelatinization properties (Xia et al., 2018; Nawaz et al., 2018;

Shah et al., 2016). Super heated steam also has a considerable role in the starch modification. Super heating of starch improves the textural and functional properties of gluten-free breads in which they are used as additives. It can be produced by heating the starch suspension above gelatinization temperature which makes a starch paste. When cooled, it forms a spreadable starch gel with a creamy texture. Such modified starches can be incorporated in the gluten-free breads as fat replacers (Horstmann et al., 2017).

Non-thermal modifications such as high pressure processing, cold plasma treatment, gamma irradiation and ultrasonication lead to molecular depolymerisation or cross-linking of starch granules (Sudheesh et al., 2019d; Alcázar-Alay & Meireles, 2015). Non-thermal modification also changes the swelling index, solubility, amylose leaching, crystalline properties, gelatinization properties, pasting properties, retrogradation and digestibility of starch granules (Sudheesh et al., 2019b). Therefore, physically modified starches have a significant role in the bakery industries.

6.1.2 Chemical Modification of Starch and Its Application in the Gluten-Free Breads

Chemical modifications involve introduction of new functional groups in the starch chains using different chemical reagents. The functional groups introduced in the starch during modifications have a considerable role in the alteration of its physicochemical and functional properties. These change the hydration, pasting, gelatinization and retrogradation properties of starch. Acid hydrolysis, esterification (acetylation), alkali treatment, etherification (hydroxypropylation and hydroxyethylation) and cross-linking are the types of chemical modifications usually done with starch (Alcázar-Alay & Meireles, 2015). During the chemical modifications, highly reactive functional groups are introduced in the starch to modify its hydration properties, pasting properties, gelatinization properties, granular morphology, thermal stability, shear force stability and retrogradation.

Esterification and Etherification

Esterification is a type of chemical modification, in which ester groups like acetyl, phosphate and citrate are introduced in the starch chains. Acetylation is a type of esterification, performed by the chemical agents like acetic anhydride, succinic anhydride, vinyl acetate and acetic acid. It is generally carried out in the presence of an alkaline catalyst such as sodium hydroxide, calcium hydroxide, potassium hydroxide and sodium carbonate (Alcázar-Alay & Meireles, 2015). Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives (JECFA) defined starch acetate is an ester produced by the addition of acetic anhydride or vinyl acetate to the starch under the alkaline condition, that is responsible for the replacement of hydroxyl groups (second, third and sixth positions of carbon atoms) by the acetyl groups. The FDA (Food and Drug Administration, USA) recommended the amount of acetyl groups in starch acetate might be less than 2.5 g/100 g and E-1420 is the number assigned by the European Union for this additive (Sudheesh et al., 2019c; Huang et al., 2007; Han et al., 2012).

The effectiveness of acetylation on the physico-chemical and functional properties of starch depends on the degree of substitution (DS) and the percentage of acetyl groups. DS is the number of moles of acetyl groups substituted per mole of α-D glucopyranosyl units. DS depends on the type of starch, the concentration of the chemical agent and the reaction conditions such as temperature, pH and time. Amylose-amylopectin ratio, lipid content and intragranular packing are other factors which affects on the acetylation of starch. Starch with high amylose content showed lower DS after acetylation (Singh et al., 2007). Based on the DS, starch acetate can be classified as high (1.5–3), medium (0.2–1.5) and low (0.01–0.2) DS. Low DS starch is broadly used as a stabilizer, thickener, adherents, texturisers and encapsulation agents in various food products (Tian et al., 2018). Maintaining higher pH (8.2–8.5) during the acetylation generally formed low DS starch. The hydrolysis of acetyl groups and development of partially gelatinized layer on the starch granules in the higher pH leads to formation of low DS starch after acetylation. High and medium DS starch showed higher solubility in the organic solvents such as acetone and chloroform. Hence it has important role in the development of thermoplastic and biodegradable materials (Tian et al., 2018).

Hydroxypropylation and hydroxyethylation are types of etherification, which can be performed by using propylene oxide and ethylene oxide respectively. Hydroxy groups of α-D glucopyranosyl units are replaced by the hydroxypropyl groups and hydroxyethyl groups. During the hydroxypropylation, starch molecules are first activated to O-H bond nucleophiles and subsequently form starch-O^- form The alkaline catalyst assists the reaction between starch-O^- and propylene oxide resulting in substitution of hydroxypropyl groups. The DS of etherification is depending on the type of reagent, reaction conditions and starch properties. Etherification mainly occurs in the amorphous region of starch granules. The reaction efficiency is the percentage of reactant reacted or substituted on the starch granules. The remaining reagent is converted to by-products. It depends on the penetration of alkali catalyst and etherifying agent into starch granules and the collision of etherifying agent with starch nucleophile. The elevation of temperature of reaction medium leads to increase in the penetration of alkali catalyst and etherifying agent into starch granules resulting in higher reaction efficiency (Tian et al., 2018). The hydrophilic hyroxypropyl groups introduced in the starch granules during the etherification leads to disruption of amylose and amylopectin chains. The breakage of inter and intra molecular hydrogen bonds in the starch granules improves its hydration capacity, it alter the gelatinization and retrogradation properties of starches. Hydroxypropylation improves the paste consistency, clarity and low temperature storage stability (Chen et al., 2018).

Acetyl, hydroxyethyl and hydroxypropyl groups introduced in the starch chains have negative charges. Hence, such substitutions increase the electrostatic repulsive forces between the starch chains and decrease their stability. On the other hand these modifications increase the swelling index, solubility and pasting properties of starches and decrease the retrogradation properties of starch paste. The functional groups introduced during the etherification and esterification reduce the probability of reassociation of starch chains there by reducing the retrogradation (Alcázar-Alay et al., 2015; Tharanathan, 2005). Lower retrogradation results in lower staling and development of softer crumb in gluten-free breads.

Cross-linking

Cross-linking of starch chains can be done using the agents like sodium tripolyphosphate, sodium tri meta phosphate, orthophosphoric acid, epichlorohydrin and phosphorus oxychloride (Young et al., 2018; Chen et al., 2018). Phosphorus oxychloride is an excellent cross-linking agent in aqueous medium with higher pH (greater than 11) in the presence of neutral salt. Sodium tripolyphosphate is a more efficient reagent if cross-linking is performed at higher temperature with semidry starch or warm temperature with hydrated starch in aqueous medium. Epichlorohydrin has lower solubility and partially decomposes into glycerol. Hence, phosphorus oxychloride and sodium tripolyphosphate are widely used cross-linking agents for starch. Starch phosphates can be grouped into two classes: mono starch phosphates and distarch phosphates. Distarch phosphates are cross-linked starch and showed lower DS as compared with monostarch phosphates (Singh et al., 2007).

During cross-linking, sodium hydroxide is used as a catalyst. Phospho diester bonds introduced during cross-linking increases the interaction between the starch chains and stabilize the starch structure (Young et al., 2018; Sudheesh et al., 2020b). As a result, the pasting viscosity and paste clarity of starch increases and retrogradation decreases. Cross-linked starch paste has higher thermal and shear stability and lower break down viscosity. Hence, it extended the cooking time. Cross-linking also reduced the granules disruption, loss of viscosity and formation of stringy paste during the cooking (Singh et al., 2007). Crystalline and gelatinization properties are considerably increased in the cross-linked starch. The granular stability and presence of phosphate groups in the cross-linked starch improved resistant starch content (Sudheesh et al., 2020c, 2020e). Cross-linked starch can be used as a thickening and stabilizing agent in the food products (Alcázar-Alay & Meireles, 2015). Higher concentration of cross-linked starches, however, increases the hardness of breads up to an unacceptable level (Witczak et al., 2016).

The stabilized cross-linked starches widely used for food processing are acetylated distarch adipate (E1422) and hydroxypropyl distarch phosphate (E1442). Acetylated distarch adipate can be formed by the esterification of acetic anhydride with adipic anhydride. Esterification of sodium trimetaphosphate or phosphorus oxychloride combined with etherification of propylene oxide produced hydroxypropyl distarch phosphate. High amylose corn starch, acetylated distarch adipate and hydroxypropyl distarch phosphate also have an important role in the preparation of gluten-free breads (Witczak et al., 2016). Acetylated distarch adipate and hydroxypropyl distarch phosphate increase the volume of gluten-free breads. Textural parameters like hardness and gumminess of gluten-free breads decrease by the incorporation of acetylated distarch adipate and hydroxypropyl distarch phosphate (Ziobro et al., 2012).

Other Chemical Modifications

Acid Hydrolysis

Acid hydrolysis is a type of chemical modification, which involves the treatment of starch with strong acid such as hydrochloric and sulphuric acid. During the acid hydrolysis, regeneration of hydroxyl groups occurs generating hydronium ions (H_3O^+). These ions randomly attack the α-1, 4 glycosidic linkages and slowly attack the α-1, 6 glycosidic linkages. The oxygen atom present in the α-1, 4-glycosidic linkage undergoes hydronium ion attack and leads to the transfer of one electron from carbon-oxygen bond to the oxygen atom. Finally highly energetic, unstable and reactive carbocation complex will be formed, which act as a Lewis acid and react with water molecules (Lewis base) regenerating the hydroxyl groups (Hoover, 2000). Acid hydrolysis primarily affects on amorphous regions of starch granules. During the acid hydrolysis of amorphous regions, hydronium ions attack on the glycosidic bonds of α-D glucopyranosyl units by changing its conformation (chair to half chair form of α-D glucopyranosyl units) and regenerate hydroxy groups. But, in crystalline regions conversion of chair to half chair form of α-D glucopyranosyl units is difficult and hydronium ions cannot attack easily in the glycosidic bonds. Dense molecular packing also restricts the action of hydronium ions in the crystalline regions. Hence, crystalline regions are less susceptible to acid hydrolysis. Acid hydrolysis depends on the factors such as amylose-amylopectin ratio, granules size and crystalline property. Acid hydrolysis increases the crystalline properties and decreases the molecular weight of starch (Tharanathan, 2005). It also increases the pasting temperature and gelatinization enthalpy of starch by reducing its amylose content.

Oxidation

Oxidation of starch is another method of chemical modification. Oxidised starch can be produced by the treatment of starch with the oxidising agents like sodium hypochlorite, potassium permanganate, nitric acid and peracetic acid. During oxidation, there occurs the formation of carbonyl and carboxyl groups in glucopyranosyl chain by the substitution of the hydroxyl groups. In general, oxidation reduces the pasting properties of starch and increases the light transmittance (Tharanathan, 2005). Oxidation is an exothermic process, so temperature must be controlled during the reaction. The rate of oxidation is higher when conducted in the gelatinised starch dispersion as compared with granular suspension. The oxidation has higher rate at neutral pH, hence, it can be controlled by reducing pH and destroying free chlorine atoms. The extent of starch oxidation is indicated by the carbonyl and carboxyl contents in the oxidised starch. The major factors which affects the oxidation of starch are starch origin, starch molecular structure, packing of crystalline lamellae, size of amorphous lamellae, reaction pH and temperature and, concentration of reactants and catalyst. Most of the oxidised starch showed lower hot paste viscosity. It also showed higher paste clarity, binding and film forming property and, lower retrogradation property. Hence, it can be used as fillings in bakery products and has wide application in the batters and breading. The carbonyl and carboxyl groups introduced in oxidised starch decreases the *in vitro* digestibility by increasing the resistant starch content (Sudheesh et al., 2019c).

Dual Modifications

Dual modifications involve combination of diverse modification techniques such as physical-physical, physical-chemical and chemical-chemical modifications. Physical-physical combination involves combination of annealing with heat moisture treatment, annealing with ultrasonication and microwave with ultrasonication. Effect of dual modifications depends on the preparation procedure (Singh et al., 2007). The combination of annealing and heat moisture treatment improved the resistant starch content and low temperature stability of starch paste (lower retrogradation) (Sudheesh et al., 2020a). Sonication after annealing improved the final viscosity, acid and shear stability of the starch (Babu et al., 2019). Chemical-chemical combination involves combination of acid hydrolysis with oxidation, acid hydrolysis with acetylation, oxidation with acetylation, oxidation with alkali treatment, acetylation with succinylation and cross-linking with hydroxypropylation. Acetylated oxidised starch exhibit clear gel forming capacity that makes its use in confectionary industry (Pietrzyk et al., 2014). It also showed higher hydration property, peak viscosity and paste clarity and, lower pasting temperature and setback viscosity (Ali & Hasnain, 2014). Acetylation on oxidised starch has considerably increased the resistant starch content. Hence, it can be incorporated in the diet of non insulin dependent patients (Sudheesh et al., 2019c). Hydroxypropylation of cross-linked starch improved the thermal and shear force stability (Singh et al., 2007). Physical-chemical combination involves combination of succinylation with annealing, cross-linking with annealing and heat moisture treatment etc. Combination of cross-linking with annealing and heat moisture treatment in various starches changed the physico-chemical properties and *in vitro* digestibility and increased resistant starch (Sudheesh et al., 2020b; Young et al., 2018). The combination of succinylation and annealing improved the hydration, pasting and gelatinization properties of starch (Ariyantoro et al., 2018). Table 1 shows the impact of various starches in native /modified forms on the physico-chemical properties of gluten-free breads.

Dextrins and maltodextrins are formed during the physical, chemical and enzymatic hydrolysis of starch (Gray & Bemiller, 2006; Witczak et al., 2016). Dextrins have a significant role in the preparation of gluten-free breads. These reduce the retrogradation properties of breads and have a higher antistaling effect. In presence

Table 1 Effect of various starches in native /modified forms on the physico-chemical properties of gluten-free breads

Bread/ dough	Basic ingredient	Additive used	Observations	References
Bread	Common buckwheat, tartary buckwheat.	Chia flour	Proteins, insoluble dietary fibres and ash content were increased. Omega-3 fatty acids increased.	Costantini et al. (2014)
Bread	Rice flour, maize starch	Amaranth flours	Ash, protein, fat and carbohydrate content increased.	de la Barca et al. (2010)
Bread	Rice flour.	Chestnut flour	Structure of bread was uniform.	Demirkesen et al. (2013)
Bread	Corn and potato starches	Acorn flour	Protein, minerals and dietary fibre content increased. Bread volume and crumb characteristics of bread improved.	Korus et al. (2015)
Bread	Corn and potato starches	Native and hydrothermally modified bean starch	Crumb hardness of bread decreased while the elastic properties increased.	Krupa et al. (2010)
Bread	Corn and potato starches	Buckwheat flour	Quality of bread improved, specific volume index and loaf size increased. Rising concentration, proteins and micro minerals (copper and manganese) increased.	Witczak et al. (2016)
Bread	Corn starch	Amaranth flour	Bread volume increased and crumb hardness reduced.	Witczak et al. (2016)
Bread	Tapioca starch, corn flour	Soybean flour, whole egg and vegetable fat	Specific volume of bread increased and crumb hardness and elasticity decreased.	Milde et al. (2012)
Bread	Corn starch	Chickpea flour, carob germ flour, pea protein isolate, soya flour	Physico-chemical properties and sensory parameters of bread improved. Carob flour also improved the rheological properties of batters. Chickpea flour and pea isolate improved the properties of breads.	Miñarro et al. (2012)
Bread	Rice flour	Pregelatinized tapioca starch and transglutaminase	Batter-like dough with higher resistant to deformation. Bread volume increased and hardness reduced. The addition of transglutaminase (0.1%) increased the volume of bread. Crumb hardness and chewiness also decreased.	Pongjaruvat et al. (2014)

Bread/dough	Basic ingredient	Additive used	Observations	References
Bread	Maize flour and starch, rice flour and buckwheat flour, potato starch	Analysis of different recipes	CO_2 retention and rheological properties improved.	Pruska-Kędzior et al. (2008)
Bread	Gluten free bread mix (deglutenized wheat starch and others)	Pre-gelatinized oat and barley flour	Pre-gelatinized oat flour reduced amylopectin retrogradation in loaves. Pre-gelatinized flour showed higher hardness as compared to control. Water retention of loaves also increased.	Purhagen et al. (2012)
Bread	Corn, rice and buckwheat flour	Lyophilized buckwheat sourdough	Lyophilized buckwheat sourdoughs increased the bread volume and decreased the hardness of the crumb. Shelf life of bread increased to the extend depending upon the freeze-drying factors.	Rózylo et al. (2015)
Bread	Rice flour and gluten free wheat starch	Green plantain flour	Functional properties and resistant starch content of breads improved. Desirable colour of bread crust and crumb was developed. Water addition, baking time and temperature are critical parameters.	Sarawong et al. (2014)
Bread	Rice flour, corn flour, soy flour	Analysis of different recipes	Final viscosity and setback viscosity of batter decreased. Batter consistency and bread volume improved. Breads made from rice, corn and soy flours showed best properties. Specific volume, crumb appearance and texture improved while staling rate reduced. Soya flour increased softness of bread crumb and reduced bread staling.	Sciarini et al. (2010)
Bread	Rice flour	Carob flour	Dough with improved viscoelasticity was developed that produced high-quality breads.	Tsatsaragkou et al. (2014)
Bread	Corn and potato starches	Buckwheat flour	Loaf volume improved with delayed staling and darker crumb.	Witczak et al. (2016)

(continued)

Table 1 (continued)

Bread/ dough	Basic ingredient	Additive used	Observations	References
Bread	Rice flour and potato starch	Corn starch, xanthan gum and guar gum.	Roundness and weight of bread improved. Water retention increased while reducing hardness.	Mahmoud (2013)
Bread	Sorghum flour	Cassava, corn, potato and rice starch	Improved crumb softness of bread	Onyango et al. (2011)
Bread	Rice flour	Xanthan gum and guar gum	Specific volume of bread improved and hardness decreased.	Demirkesen et al. (2010)
Bread	Potato and corn starches, corn meal	Guar gum, xanthan gum and pectin	Sensory properties improved. Specific volume of bread increased while reducing the hardness.	Gambu and Sikora (2007)
Bread	Fermented cassava	Sweet potato and sorghum flour	Specific volume, aging and textural properties of bread increased.	Charlène et al. (2019)
Bread	Gelatinized cassava and sorghum starches	Microcrystalline cellulose, carboxymethyl cellulose, methyl cellulose, hydroxypropylmethylcellulose and hydroxypropylcellulose and, Glycerol monostarate, sodium stearoyl-2-lactylate and diacetyl tartaric acid esters of mono- and diglycerides.	Cellulose treated dough showed lower rate of deformation than emulsifier treated dough. Emulsifiers decreased firmness and staling of bread.	Onyango et al. (2009)
Bread	Corn, cassava and rice starches	Soya flour	Improved crumb structure	Sanchez et al. (2002)
Bread	Rice starch	Hydroxypropylmethylcellulose, yeast β-glucan, and whey protein	Increased bread hardness while decreasing the specific volume. Hydroxypropylmethylcellulose and whey protein increased cohesiveness and chewiness of breads.	Kittisuban et al. (2014)
Bread	Rice flour and cassava starch (dough was treated with hydroxypropyl methylcellulose)	Soya protein isolate and egg white solids	Addition of egg white proteins in the hydroxypropyl methylcellulose treated dough improved the loaf volume and crumb regularity. Soya proteins decreased the stability of dough.	Crockett et al. (2011a)

Bread/ dough	Basic ingredient	Additive used	Observations	References
Bread	Rice flour	Transglutaminase, albumin and casein	Improved the specific volume of bread while reducing hardness.	Storck et al. (2013)
Bread	Cassava starch	Corn and soya flour	Spongy bread crumb was obtained by increasing specific volume.	Milde et al. (2012)
Bread	Sorghum flour	Pregelatinized cassava starch (Both sorghum flour and pregelatinized cassava starch were modified using microbial transglutaminase)	Firmness and chewiness of bread improved. Decreased cohesiveness, chewiness and resilience of bread with increasing storage time.	Onyango et al. (2010)
Bread	Rice flour, cassava starch and full fat active soya flour	Emulsifiers- diacetyl tartaric acid ester of monoglycerides, sodium stearoyl lactylate, Enzymes- glucose oxidase,α-amylase, Hydrocolloids-xanthan gum, carboxymethyl cellulose, alginate and carrageenan	Addition of emulsifiers increased initial firmness and staling rate of bread. Enzymes improved the specific volume of bread while reducing hardness. Hydrocolloids decreased initial firmness and stalling rate of bread.	Sciarini et al. (2012)
Bread	Corn starch	Brown rice, buck wheat and soya flour.	Decreased cohesiveness, springiness and resilience of bread.	Moore et al. (2004)
Bread	Brown rice flour	Potato and corn starches, soya flour and skim milk powder	Decreased cohesiveness, springiness and resilience of bread.	Moore et al. (2004)
Bread	Rice and cassava starches	Hydroxypropyl methylcellulose and xanthan gum.	Improved water binding capacity of bread dough. Xanthan gum increased elasticity and hardness of bread while reducing extensibility. Hydroxypropyl methylcellulose reduced hardness of bread.	Crockett et al. (2011b)
Bread	Rice and soya flour, corn starch	Xanthan gum and carboxy methyl cellulose	Xanthan gum improved elasticity of bread while decreasing hardness and carboxy methyl cellulose improved crumb porosity.	Mohammadi et al. (2014)
Dough	Corn starch	Amaranth flour, pea isolate and psyllium fibre	Nutritional and rheological properties of gluten free dough improved and bread staling reduced.	Mariotti et al. (2009)

of dextrin, gelatinization properties and retrogradation enthalpy are reduced which in turn reduces the hardness of breads. With the similar functionality, dextrins may play a role in reducing the staling effect and hardness of gluten-free breads. Dextrose equivalent (DE) is the measure of amount of reducing sugar present in a given product. Certain studies show that the addition of maltodextrins with medium dextrose equivalents (DE) further reduce the retrogradation properties of breads. Maltodextrins with medium DE value show good antistaling property and increase the volume of breads. Maltodextrins reduce the peak viscosity of starch and decrease its water absorption capacity. Lower water absorption capacity improves the stability of dough. Maltodextrins impart a plasticizing effect on the dough by improving its extensibility and reducing the stiffness. Maltodextrins with a medium DE increase the water absorption capacity and decrease the extensibility of dough as compared to those with a low DE. On the other hand, addition of maltodextrins with medium DE improve the volume of the breads made from the flour with low amylolytic activity (α-amylase activity). Maltodextrins have an important role in the gelatinization, pasting and structural properties of starch based dough. Addition of low DE maltodextrins decreases the volume and quality of breads. Low DE maltodextrins produces breads with irregular shapes and size of pores. Incorporation of short chain maltodextrins improve the volume and reduces the staling of breads. Maltodextrins with higher DE value prevent amylopectin recrystallisation (Witczak et al., 2010, 2016; Miyazaki et al., 2004) and can be used as an antistaling agent in the gluten-free breads.

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Fruit and Vegetable Based Ingredients in Gluten Free Breads

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1 Introduction

Bread is the most representative foods eaten around the world. It is one of the oldest staple foodstuffs which constitute the basis of main food consumption. Traditionally, breadmaking involves several processes such as the mixing of ingredients of which the flour, yeast and water are the most important followed by molding, proofing and baking. Among cereal flours, wheat flour is the most commonly used flour in the breadmaking as it provides a light, palatable and well risen loaf of bread. It is the gluten protein in the flour which is responsible for the main structure in wheat bread that confers the dough its unique viscoelasticity and baking quality (Bender & Schonlechner, 2019). Gluten free products are next targeted by food processing industry to eliminate the usage of wheat dependent products and alternate diet to celiac people in the world (Sofi et al., 2019, 2020). Eliminating gluten from bread is technologically challenging as it gives rise to baked goods with compromised quality. Currently, breads obtained from the gluten free flours are mainly starch based and nutritionally deficient. They contain inadequate levels of fiber, vitamins and proteins but contain higher levels of carbohydrates than recommended. They are further characterized by compromised quality having dry crumbly structure, reduced loaf volume, rapid staling, cracked crust as well as poor texture and flavor (Masure et al., 2016). Therefore, high quality gluten-free bread will be achieved by incorporating nutrient dense ingredients from fruits and vegetables with good sensory and nutritional quality.

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Fruits and vegetables are the major functional food ingredients which form part of a well-balanced diet and play an important role in human nutrition due to their nutrient composition (vitamins, minerals and fibers) and associated potential health benefits. The benefits relate to their role in kidney functions, prevention of cancer and cardiac disorders through contribution of ascorbic acid, β-carotene and non-starch polysaccharides besides the biochemical constituents like phenols, flavonoids and alkaloids. Compared to cereals, they contain a substantial proportion of micronutrients, phytochemicals and dietary fibers with a better soluble and insoluble dietary fiber ratio (Redende & Franca, 2019). These bioactive compounds have great potential to be used as functional ingredients in the gluten-free products to combat their nutritional deficiencies. Further, addition of fruit and vegetable byproducts such as peel, pomace, and seeds in breads not only resulted in nutritional improvement in terms of dietary fiber and phenolics but also reduced bread staling (Curti et al., 2016). This effect is mainly attributed to the presence of fiber content in the fruit and vegetable by-products which reduces the retrogradation kinetics of amylopectin molecules (Ronda et al., 2014) and increases the water retention capacity of the final bread (Almeida et al., 2013).

2 Fruit Based Ingredients

Fruits are the essential part of healthy lifestyle and crucial part of healthy diet. They are not only the most acceptable for their delightful taste but also for their nutraceutical properties (Buachan et al., 2014). Fruits contain vitamins and minerals in significant amounts (Kazii et al., 2013). Generally, fruits contain moisture (70–80%), proteins (1.5%), carbohydrates (13–15%), dietary fibers (up to 6%), minerals (501 mg), and vitamins (upto 90 mg). However, this composition varies with fruit to fruit and variety to variety. Fruits also contain a significant amount of minerals, a good quantity of folate, carotenoids, large proportions of phytochemicals and substantial amounts of non-digestible carbohydrates. They are either normally used for direct consumption or for the processing of juices, jam, jellies and wines (Chen et al., 2019). Unfortunately, half the fruits produced worldwide end up as wastes, generating environmental pollution, mainly caused by microbial degradation. Most of the wastes are generated by industrial processing, the so-called by-products. This waste or by-products include pips, kernel, skin or peel, pomace and seeds of the fruit (O'Shea, 2014). The skin (peel), pomace and seeds are also characterized for their potential health benefits (Farooq et al., 2020). These by-products contain various nutrients, such as macronutrients (proteins and carbohydrates), enzymes, natural acids, phytosterols and antioxidants with neutraceutical potential (Coman et al., 2020). Fruit and vegetable by-products contain bioactive compounds (Table 1) and can be used to enhance the quality of gluten free bread with better sensory and nutritional quality.

Table 1 Bioactive compounds in fruit and vegetable by-products

By-product	Fruits/ vegetables	Bioactive compounds	References
Peel/skin	Apple	Polyphenols, flavonoids and anthocyanins	Wolfe et al. (2003)
	Avocado	Polyphenols (hydroxybenzoic,hydroxycinnamic acids derivatives), flavonoids, dietaryfiber.	Tremocoldi et al. (2018)
	Mango	Polyphenols,flavonoids,carotenoids,dietary fiber,vitamins	Ajila et al. (2007)
	Carrot	Polyphenols, flavonoids, tannins, saponins, carotenoids, dietary fiber, vitamins	Nguyen and Scarlett (2016), Clementz et al. (2019)
	Onion	Polyphenols, flanoids and dietary fiber	Benitez et al. (2011)
	Tomato	Lycopene, caffeic acid, ferulic acid, chlorogenic acid, quercetin, quercetin-3-β-O-glycoside, Total dietary fiber	Valdez-Morales et al. (2014)
Seeds	Apple	Phenolic compounds (phloridzin, ellagic acid, epicatechin, caffeic acid, catechin, ferulic acid, protocatechuic acid, gallic acid.	Gunes et al. (2019)
	Avocado	Polyphenols (quinic, citric,1-caffeoylquinic, 3-O-pcoumaroylquinic,4-caffeoylquinic acids), Tannins (A-type procyanidindimer, trimer and tetramer andB-type pentamer and hexamer)	Tremocoldi et al. (2018)
	Mango	Polyphenols, tannins, proanthocyanidins	Dorta et al. (2013, 2014)
	Carrot	Essential oils, Terpenes, Carotol, Sabinene, A-pinene, Daucol, Monoterpenes, Sesquiterpenes	Smigielski et al. (2014)
Pomace	Beetroot	Polyphenols, betalain,flavonoids, dietaryfiber, vitamins, betanin, betacyanin, betaxanthin, gallic acid, catechin	Vulic et al. (2012, 2013, 2014)
	Apple	Dietary fiber (pectin, cellulose, hemicelluloses and lignin), tannins, resins, reducing sugar and pigments	Dhillon et al. (2013), Masoodi et al. (2002)
	Grape	Dietary fiber, phenolic acids (ferulic, p-coumaric, caffeic, gallic, vanillic and p-hydroxybenzoic), flavanols (proanthocyanidins),flavonols (kaempferol-3-O-glucoside, quercetin-3-O-glucoside, quercetinand myricetin), and stilbenes (resveratrol, piceid and astringin), and anthocyanins (enocyanins in red grape cultivars)	Machado and Dominguez-Perles (2017), Mattos et al. (2017)

GCAE guaiacylglycerol-β-caffeic acid ether, *GFAE* guaiacylglycerol-β-ferulic acid ether, *TCOA* N-trans-coumaroyloctopamine, *TFOA* N-trans-feruloyloctopamine

2.1 Peel

Peel is the waste obtained from the food industrial processing which is expected to increase with the development and progression of industrial manufacturing process that use fruits either green or ripe (Eshak, 2016). However, the traditional treatment for peel residue includes disposal to landfill, composting and incineration, causing environmental pollution due to emission of harmful gasses (Hu et al., 2020). Fruit peels are functional food ingredients rich in dietary fiber and antioxidant compounds (Ferreira et al., 2015). Peels from citrus fruit have a large number of benefits regarding health issues like scurvy, peptic ulcer, diabeties, skin, hair, cancer, and antimicrobial activity (Ahmed et al., 2018). Orange peels contain many phytonutrients such as flavanones, particularly hesperidin which accounts for 50% of the total phenolic compounds, flavones such as neodiosmin and hydroxycinnamic acids such as ferulic acid (Fernandez-Lopez et al., 2009). Citrus peels are an important source of essential oils, with D-limonene being the major component (32–98% of total oils) (González-Molina et al., 2010) and a valuable source of commercial pectin (Maric et al., 2018). Apple peels contain substantial levels of calcium and magnesium. Quercetin is one of the main flavonoids found in apple, predominantly in apple peel. It has been linked with reduced incidences of breast cancer and leukaemia (O'Shea et al., 2012). Addition of apple peel powder to muffins improved their phenolic and antioxidant content besides enhancing the flavor (Rupasinghe et al., 2008). Mango peel particularly the ripened one is also a good source of antioxidants (vitamin C and vitamin E) (Ajila et al., 2007). It is also a rich source of pectin, with potential to compete with apple pomace as it is less susceptible to enzymatic degradation (Geerkens et al., 2015). Thus, the addition of mango peel to a food product can improve the nutritionalquality of the product by increasing the dietary fibre and phytochemical levels without negating the quality of the product. Banana peels, which accounts about 35% of the total fruit weight, gained attention due to their elevated contents of phenolics (hydroxycinnamic acids), flavonoids, phytosterols, carotenoids (lutein,β-carotene, α-carotene, violaxanthin, auroxanthin, neoxanthin, isolutein, β-cryptoxanthin and α- cryptoxanthin), anthocyanins, biogenic amines, vitamins (B3, B6, B12, C and E), dietary fibers (cellulose, lignin, resistant starch, pectin, hemicelluloses) and many other phytochemicals with antioxidant properties like dopamine and L-dopa (Amini Khoozani et al., 2019). Some phytochemicals were reported to be present in higher amounts in peels than in pulp (e.g., gallocatechins are five times greater in peels). Due to their complex chemical composition, banana peels represent a proper source of nutritional constituents for value-added food products (Segundo et al., 2017). Banana peel flour incorporated in bread showed a significant decrease in glycemic and hydrolysis indexes as compared to wheat flour bread (Juarez-Garcia et al., 2006).

2.2 Seeds

Seeds are the part of the fruit that is usually thrown as waste. Apple seeds representing ~3–4% of apple pomace are the best sources of antioxidants and phenolic compounds which include quercetin, many phenolic acids (ferulic, p-coumaric, caffeic, chlorogenic and gallic) and naringenin derivatives, catechin and epicatechin, phloridzin (Du et al., 2019). Avocado seeds, which represents ~33% of the fruit are potentially rich in the bioactive content including phenolic acids (hydroxybenzoic and hydroxycinnamicacids), condensed tannins (procyanidins) and flavonoids (flavonols) having antioxidant, anti-proliferative and anti-inflammatory effects (Figueroa et al., 2018). Avocado seeds being the richest source of fiber fulfill the recommended daily fiber intake (Reynolds et al., 2019), thereby, act as functional ingredients in foods due to their total dietary fiber composition (lignin, cellulose and hemicellulose) (Barbosa-Martın et al., 2016). Grape seeds represent 2–5% of grape weight and ~38–52% (dry weight) of grape pomace (Brenes et al., 2016). Grape seeds contain polyphenols (mainly gallic and caftaric acid, catechin, epicatechin, epicatechin gallate and procyanidins B1 and B2) besides lipids (e.g., linoleic acid), proteins, carbohydrates and vitamin E (Maier et al., 2009). Mango seed, which constitute 10–25% of fruit weight contain important levels of bioactive compounds even more than the fruit pulp (Kim et al., 2010), such as phenolic compounds, carotenoids, vitamins and dietary fibers, known to have high antioxidant properties (Dorta et al., 2014). Recent literature has shown that mango seed kernel powders can be incorporated as a potential source for functional food ingredients into foods, such as biscuits and macaroni (Ashoush & Gadallah, 2011), improving their sensory scores and possible nutraceutical properties.

2.3 Pomace

Fruit pomace is a by-product from fruit juice and concentrate production, containing substantial quantity of dietary fibers and phenolic compounds. For example, apple pomace, which constitutesup to 30% of the original fruit, is the main processing waste generated after apple juice manufacturing. It mainly contains 95% of peels/flesh, 2–4% of seeds and 1% of stem (Bhushan et al., 2008). It also contains dietary fiber (35–60%), out of which pectin comprises of 5–10%, cellulose 7–40%, hemicelluloses 4–25%, lignins 15–25% (Dhillon et al., 2013). The apple pomace is rich in various substances like tannins, resins, reducing sugars, pigments,together called as 'extractives' due to their solubility in water or in organic solvents (Vendruscolo et al., 2008). Apple pomace in comparison to the cereal brans and legume hulls has certain advantages, as it lacks phytic acid, which renders minerals like zinc unavailable. Recent studies use apple pomace as a dietary fiber source in some baked food products (Figuerola et al., 2005). Grape pomace is the main by-product of wine making industry. Grape pomace, which represents 15–20% of the total grape weight, consists of skins, stems, residual pulp and seeds. An average of 5.6–7.5 metric tonnes per year of grape pomace is generated. Since, ancient times grape pomace was mainly used to produce some distillates, or as animal feed and fertilizer (Bordiga, 2016). Thus, the by-product have been undervalued for a long time, however, recent approach to valorize grape pomace by-product have emerged (Lavelli et al., 2016). It is a rich source of hemicelluloses, cellulose, and pectin (total dietary fiber) (Kammerer et al., 2005), but the ratio of soluble dietary fiber/insoluble dieatary fiber depends largely on variety (González-Molina et al., 2010). The pomace also contains significant quantity of phenolic acids like ferulic, p-coumaric, caffeic, gallic, vanillic and p-hydroxybenzoic, flavanols like proanthocyanidins,flavonols like kaempferol-3-O-glucoside, quercetin-3-O-glucoside, quercetin and myricetin, and stilbenes like resveratrol, piceid and astringin, and anthocyanins (Machado & Dominguez-Perles, 2017; Mattos et al., 2017). Orange pomace is a good source of flavonoids particularly hesperidin and represents 45–60% of the fruit. Similarly, peach, plum and apricot pomace have high content of phytochemicals (Dulf et al., 2017).

Olive pomace is the solid by-product generated through extra-virgin olive oil extraction and is highly rich in polyphenols, where secoiridoid glycoside was the prominent phytochemical (Obied et al., 2005). Apricot pomace has high nutrient and phenolic compound content, and the kernel contains tocopherols protecting against oxidative stress, unsaturatedfatty acids like oleic and linoleic, and also amygdalin with anticancer, and anti-inflammatory effects (Pavlovic et al., 2018). Peach pomace containsphenols, carotenoids and cyanogenic glycosides, with anti-aging, antidiabetic,antioxidative, and anti-obese properties (Nowicka & Wojdyło, 2019).

3 Vegetable Based Ingredients

Vegetables comprise major proportion of the human dietin many parts of the world (Dias, 2012). The fresh vegetables upon consumption gives the consumer a variety of compounds such as phytochemicals, vitamins, minerals and dietary fiber, that have a positive influence on human health. The phytochemicals found in fresh vegetables have antiflammatory, enzyme inhibiting and bioactive potential capable of combating the activities of oxidants such as lycopene found in tomatoes in higher amounts, lowers the incidences of prostrate, lung and digestive tract cancers (Marowa-Wilkerson et al., 2007). A high vegetable diet has been associated with lower risk of cardiovascular diseases. Low vegetable intake, in unbalanced diets, has been estimated to cause about 31% of ischemic heart disease and 11% of stroke worldwide (Dias, 2012). Most often vegetables are either eaten in raw or cooked form. However industrial processing of vegetables produces a large amount of waste in the form of pomace, peels and florets and stalks, and is becoming a serious nutritional, economic and environmental problem. These by-products are source of potentially valuable bioactive compounds such as carotenoids, polyphenols, dietary fibers, vitamins, enzymes and oils among others. These phytochemicals can be utilized in the gluten free breads for the development of functional or enriched bakery product.

3.1 Pomace

Pomace, the byproduct of the vegetable processing industry is rich source of dietary fiber, minerals, carotenoids and phytochemicals. The carrot pomace is a good source of phenolic compounds such as chlorogenic acid, caffeic acid, parahydroxybenzoic acid, ferulicacid and cinnamic acid isomers. Therefore, its addition to food products would be beneficial due to its high antioxidant content. Tomato pomace, isrich source of carotenoids like lycopene and beta-carotene, dietary fiber, protein and phenolic compounds. Altan et al. (2008) explored the utilization of tomato pomace in a barley-based extruded snack with good texture and sensory properties. The beetroot pomace contain high amounts of vitamins, minerals, carbohydrates, betalains, amino-acids, carotenoids, and phenolic compounds (Vulic et al., 2013), with high antioxidant activity. The beetroot pomace also possesses antimicrobial and cytotoxic activity and can be added to relevant functional foods without introducing negative functional issues while retaining the sensory properties (Kushwaha et al., 2018). Beetroot powder incorporated in bakery products resulted in fiber enrichment with positive effects on the farinographic and physical properties, and reduced caloric density (Kohajdova et al., 2018).

3.2 Peel

Peel is the primary residue of industrial processing and causes remarkable environmental problems. However, the beneficial bioactive compounds extracted from peels find application of this bio-waste in functional foods, medicines, pharmaceuticals, and cosmetics (Nguyen & Scarlett, 2016). Carrot peel waste, an important constituent of pectin, also contains a high amount of phenolic compounds (carotenoids and anthocyanin), soluble and insoluble dietary fibers which helps to prevent constipation, control blood sugar level, cancer, and heart diseases (Sharma et al., 2012). Carotenoids (α- and β-carotene) are the most valuable constituents of carrot peel (Clementz et al., 2019). Polyphenols such as found in carrot peel fight against free radicals, reduce the risk of oxidative damage, have antimutagenic, anti-inflammatory, plasma lipid modification and antitumoractions (Sharma et al., 2012). Tomato peels are also rich in phytochemicals, particularly carotenoids and vitamin C and lycopene (Clinton, 1998). Phenolic compounds with important biological activities like caffeic, ferulic, and chlorogenic acids, quercetin and quercetin-3-β-O-glycoside were also identified in tomato peels (Valdez-Morales et al., 2014). Potato peel, a major waste by-product of the potato processing industry, contain highest amount amount of polyphenols (cholorogenic acid, gallic acid, caffeic acid and protocatechuic acid) and flavonoids including flavonols, favanols and antocyanins (Akyon et al., 2016) with antimicrobial and antioxidant properties. The incorporation of potato peel in a bread decreased hardness and gumminess of the bread, good sensory and nutritional quality (Kaack et al., 2006).

4 Fruit and Vegetable Based Ingredients Used in Gluten Free Breadmaking

Since most of the studies have shown that fruit and vegetable by-products are nutrient dense ingredients incorporated in gluten free bread enhance their nutrient composition and sensory quality. The fruit and vegetable based ingredients such as peel, pomace and seeds besides nutrient composition aids in dough structure and functional properties of gluten free breads.

4.1 Fruit Ingredients

Fruit extract and seeds are the rich source of minimal, vitamins and soluble sugars increase the nutritional profile and mineral composition of gluten free bread with good consumer acceptability. Incorporation of raisin juice (Sabanis et al., 2008), extracts from green kiwifruit puree (Sun-Waterhouse et al., 2009), and strawberry and black currant seeds (Korus et al., 2012) into gluten free bread making resulting in acceptable products with improved nutritional compositions. Studies on the addition of fruit ingredients in gluten free breads involved the use of apple pomace by Parra et al. (2014). Gluten free bread was prepared based on cassava starch, rice flour and egg white mixture with apple pomace as fibre source. Response surface methodology was used to study the effect of apple pomace and amount of water on batter rheology and quality of product. Results indicated that incorporation of apple pomace in gluten free bread led to increased crumb hardness and decreased cohesiveness and resilience. However, a well-balanced quantity of apple pomace and water render gluten free bread with improved specific volume and sponginess. In another study, the effect of apple pomace levels on the pasting properties and microstructure of composite starch system while preparing gluten free bread based on cassava starch, rice flour and egg white mixture was evaluated (Parra et al., 2015). Peak viscosity and final viscosity of the cassava starch-water dispersion decreased with increased level of apple pomace. However, microstructure of starch-apple pomace pastes revealed a non-uniform system with predominant starch matrix while fibre particles embedded in the matrix. Water imbibing capacity analysis reported that apple pomace particles, being rich fiber source had ability to capture water to a higher extent than starch. This could lead not only to less water availability in starch suspensions during gelatinization but also to a certain compensation for viscosity loss due to apple pomace particle swelling (Parra et al., 2015). Orange pomace, the by-product of fruit and vegetable juice industry basically consists of albedo and seeds which accounts for about 45–60% of fruit. The remarkable aspect of orange pomace is its high potential as dietary fiber sources with gelling, water and structure binding and fat replacer properties. O'Shea et al. (2013) studied the effect of orange pomace on dough rheology, microstructure and sensory attributes of bread. Incorporation of orange pomace in gluten free bread improved the robustness of gluten free batters and decreased starch gelatinization. Sensory analysis revealed that orange pomace incorporated gluten free bread had good sensory acceptability in terms of flavor, crumb appearance and overall acceptability compared to control. In another study, O'Shea et al. (2014) developed gluten free bread formulation with improved specific volume at longer proofing times and lower pomace levels. Orange pomace incorporation in gluten free bread nutritionallyincreased the total dietary fibre content. Nevertheless, dried fruit pomace can be added in gluten-free bakery products as flour, sugar, or fat replacers, reducing energy load while increasing fiber and antioxidant contents (Salehi & Aghajanzadeh, 2019). The effect of green plantain flouras a functional ingredient on functional properties (loaf volume, crumb firmness and porosity) of gluten free bread and resistant starch content was studied by Sarawong et al. (2014). The addition of green plantain flour significantly improved the quality of gluten free bread with higher loaf volume, softer crumb firmness, regular porosity structure and maximum resistant starch content. In another study, a functional gluten free bread enriched with polyphenols and antioxidants derived from natural aqueous extract from ripe green kiwifruit was prepared (Sun-Waterhouse et al., 2009). Results indicated that the incorporation of high phenolic and vitamin C aqueous extract rendered gluten free bread of high sensory quality with softer and smoother texture compared to plain gluten free bread. Therefore, the natural aqueous extract of kiwifruit can be considered as a functional ingredient for gluten-free bread formulation because of the feasibility of delivering polyphenols and other antioxidants into the finished product. Korus et al. (2012), developed gluten free bread using defatted blackcurrant and strawberry seeds as functional ingredients. Incorporation of defatted seeds from both sources significantly improved the dough viscoelastic characteristics while as lowered dough consistency coefficient and flow indices values. Compared to control, texture profile analysis revealed that defatted seeds supplemented gluten free bread had diminished hardness. On the other hand, addition of the functional ingredient greatly influences the color parameters of crumb, by lowering the L^* and b^* values while as improving a^* values. Inclusion of 5% defatted blackcurrant seeds and 10% defatted strawberry seeds did not affect the overall acceptability of the bread and also proved to be a good source of dietary fibre, protein and polyphenols. In another study, Sabanis et al. (2008) studied the effect of raisin juice on the baking, textural and sensory properties of gluten free bread. Addition of raisin juice resulted in improved loaf volume, color and crumb hardness of gluten free bread as well as increased the shelf life due to its moisture absorption properties. On the other hand, 3% raisin juice rendered gluten free bread with improved sensory quality. Further, Arslan et al. (2017) studied the effect of guava pulp powder on the dietary fiber and antioxidant potential of gluten free bread. Guava pulp powder was added at 5 different levels *viz* 0%, 2.5%, 5%, 7.5% and 10%. Results indicated that guava pulp powder significantly increased the crude fiber and total phenolic content of gluten free bread from 0.92% to 2.45% and 14.46 to 103.77 mg GAE/100 g. Addition of guava pulp powder also resulted in increased loaf volume upto a level of 492.00 cm^3 and decreased hardness from 2.58 to 2.38 N. Further, the L^* value of gluten free bread decreased as the concentration of guava pulp increased. Compared to control, sensory analysis showed that gluten free bread supplemented with 5% of guava pulp powder showed better overall acceptability.

4.2 Vegetable Based Ingredients Used in Gluten Free Breadmaking

By-products of vegetable processing industry differ in composition based on their botanical source. Protein is found to be the main nutrient in vegetable seeds. Besides that vegetable ingredients are rich in bioactive molecules (carotenoids, vitamins, phenolic compounds) and dietary fibers. So they can be used as additional nutrient sources and functional ingredients (O'Shea et al., 2014). Further, findings have revealed that diets rich in brassica vegetables can prevent the cardiovascular diseases as well as cancer. Cruciferous vegetables such as broccoli, cabbage and cauliflower contain large group of sulphur containing glucosides known as glucosinolates. These glucosinolates are hydrolyzed by the endogenous enzymes into biologically active isothiocyanates and indoles especially when plant tissue is crushed or chewed. Various *in vivo* and *in vitro* research studies have shown the chemopreventive activity of these isothiocyanates and indoles (Angelino & Jeffery, 2014). Saccotelli et al. (2018) studied the effect of different vegetable flour mixtures (broccoli, cauliflower, artichoke, fennel, zucchini and mushroom) on sensory quality, antioxidant properties and glycemic responses of gluten free bread. Resulted showed incorporating fennel flour increased the sensory quality of bread. Further the addition of artichoke and zucchini flours increased the total phenol and flavonoids content and improved antioxidant activity. However, incorporation of 15% vegetable flour decreased the glycemic index rendering the gluten free bread with improved nutritional and sensory properties. In another study, the effect of quinoa and amaranth flour on the specific volume, firmness, color, water activity, proximate composition, gross energy and crumb microstructure of the gluten free bread was studied by Machado Alencar et al. (2015). Compared to control, quinoa and amaranth flour enriched gluten free bread showed higher protein, lipid and ash content with larger alveolar area. Further, the addition of amaranth flour as starch replacer render gluten free bread with improved sensory and physicochemical properties. Martínez et al. (2014) study the effect of insoluble fibers (oat and bamboo, fine and coarse, potato and pea) and soluble fibres (Nutriose and polydextrose) on gluten free bread. The bread formulation containing soluble fibres presented decreased dough consistency, improved volume during fermentation and increased specific volume, lower hardness, lower luminosity and greater cell density of produced breads. On the other hand, addition of insoluble fibres rendered gluten free breads with lower specific volume and greater hardness as insoluble fibres remained whole, disrupting the structure created.

5 Conclusion

Baking of bread without gluten is technologically challenging as gluten plays an important role structure development, appearance, texture and shelf life of bread. Several alternative nutrient dense ingredients are used in gluten free bread making to increase the nutritional profile of gluten free breads. Fruit and vegetable based ingredients such as peels, pomace, pulp and seed have been usedto improve the nutritional quality, textural and sensory qualities of gluten free breads. Gluten free bread incorporated with fruits and vegetable based ingredients are new nutraceutical food suitable for gluten intolerant people with health benefits. Despite the fact there is a huge potential for gluten free product marketing, extensive research is required for the development of gluten free products fortified with fruit and vegetable functional ingredients and made available to gluten intolerant patients, which will help them adhere to a strict gluten free diet and reduce diet malnutrition and improve their quality of life.

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Flour Modification for the Development of Gluten Free Bread

Rajan Sharma, Antima Gupta, and Savita Sharma

1 Introduction

The trend of Gluten Free (GF) products has emerged globally in the past few years owing to updated consumer awareness about wheat allergy, gluten sensitivity and intolerance or a widely spread belief that GF products are healthier (Golley et al., 2015). Gluten sensitivity is the major driving force for the scientists to explore opportunities to develop technologies developing high quality gluten free breads. In particular, gluten sensitivity may arise as a result of celiac disease, non-celiac gluten sensitivity and wheat allergy. It has been estimated that about 1% of the world population has encountered with celiac disease and the only treatment available till date is strict exclusion of gluten containing ingredients from diet (Ronda & Roos, 2011). Although GF breads are available in the market, improvement in their quality to enhance acceptability is still challenging. In case of breadmaking, it is essential to mimic the textural properties obtained when prepared with wheat dough. Gluten proteins are critical components of wheat giving bread a unique body and texture due to their visco-elastic properties (elasticity by glutenins and viscosity by gliadins). Formation of gas cells and their stability provide excellent loaf volume and crumb texture to wheat-based breads. Absence of gluten hinders the dough rheology and production process of breadmaking. Gluten free dough is very sticky and difficult to handle due to its low elasticity and cohesiveness, which is a crucial property for breadmaking (Matos & Rosell, 2015).

Major ingredients avoided in production of gluten free products are wheat, rye and barley. Several other nutritional ingredients such as maize, rice, millets, quinoa, soya etc. have been evaluated for their potential as GF bread ingredients. There are number of challenges in development of gluten free products specially bread

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including poor texture, undesirable taste and flavor, reduced expansion and inferior crust and crumb characteristics. Several approaches have experimented to overcome these issues. Functionality of sourdough fermentation in gluten free bread making has been positively correlated to improvement in quality attributes such as sensory properties, nutritional profile and shelf stability (Moroni et al., 2009). Similarly, incorporation of certain additives such as starches, flours, gums, hydrocolloids, emulsifiers, and proteins has also been reported to influence the quality of GF bread (Houben et al., 2012). Role of each additive and ingredient and their impact on GF bread quality is detailed in the coming sections. Presently, there are certain novel technologies being evaluated in manufacturing of high-quality GF bread such as enzymatic treatment, high pressure processing, and extrusion technology. This chapter deals with the recent trends of modification of flour to develop gluten free bread.

2 What Is Gluten?

Gluten is a complex protein composed of gliadin and glutenins, which plays a very important role in the bakery industry by offering a wide range of techno-functional properties *viz.*, water holding capacity, elasticity, cohesivity and viscosity to the dough (Fig. 1). When wheat dough is washed thoroughly with water, starch

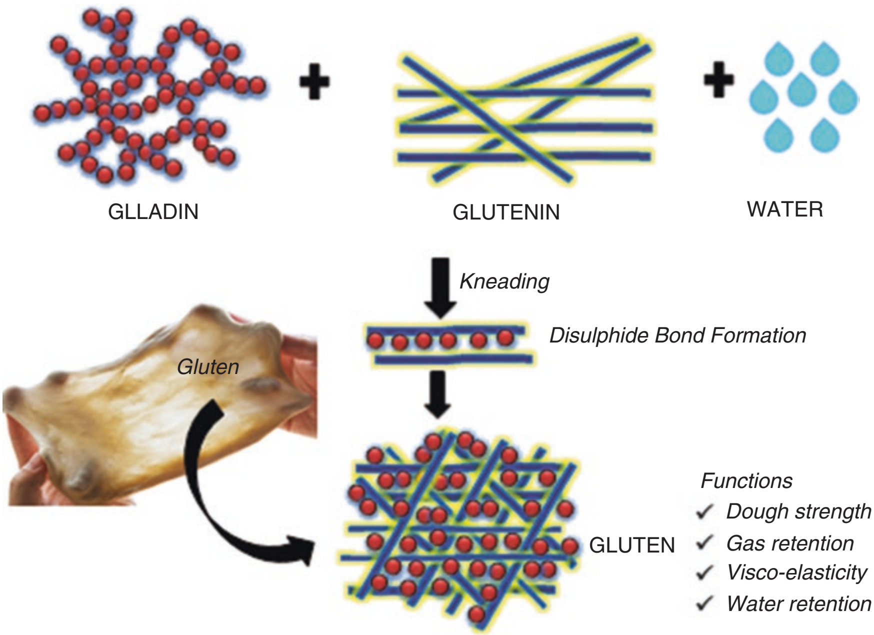

Fig. 1 Gluten formation and its functions

granules and water-soluble constituents are removed leaving behind rubbery mass known as "Gluten". On dry basis, gluten contains protein content ranging from 75% to 85% depending upon the extent of washing and 5–10% lipids, remaining are carbohydrates including starch. Cereals containing gluten are wheat, rye, barley, einkorn, kumut, triticale and spelt. Gluten has a wide range of variability in chemical composition and size because of the difference in genotype, growth pattern and some other technical aspects.

Gluten proteins have been fractionated into two components depending upon their solubility in alcohol-water solution, for instance 60% ethanol; gliadins and glutenins being soluble and insoluble respectively. Gluten proteins have been classified on different basis such as variation in the molecular weight and sulfur content. Another way of classification divides gluten protein on the basis of their primary structures as α, β, γ, and ω gliadins (Shewry & Lookhart, 2003). Most important amino acid affecting the structure and functionality of gluten is cysteine which is present in very little amount (about 2%). Most of the cysteine molecules are oxidized and are responsible for the formation of disulphide bonds within the protein or between separate protein molecules. These disulphide bonds are utmost important sites for redox reactions taking place during milling, dough formation and processing treatments such as baking.

The structure of gluten matrix is maintained by a series of covalent and non-covalent bonds involving ionic bonds, hydrophobic bonds and hydrogen bonds. The gluten matrix corresponds to the quality of dough which further impacts the final product quality such as bread and other bakery products. Gluten is a potential additive used to impart flavor and texture, helps in retention of water due to extending and binding properties. Modification of gluten is also done in accordance to its end product use (Biesiekierski, 2017).

3 Gluten Sensitivity or Gluten Intolerance

"Gluten Sensitivity" or "Gluten Intolerance" is a broader term integrating three major gluten related sensitive conditions *viz*. Celiac Disease (CD), Non-Celiac Gluten Sensitivity (NCGS) and Wheat Allergy (WA) (Fig. 2). Although these three disorders share some common symptoms including upset stomach, diarrhoea and vomiting on consumption of gluten products, they have remarkable variation in terms of causes, laboratory markers and histopathological intestinal conditions. Gluten proteins, a mixture of prolamins resist hydrolysis in the gastrointestinal tract with the action of proteases resulting in the occurrence of pathogenic protein subunits or peptides which are able to cause celiac disease and wheat allergy (Balakireva & Zamyatnin, 2016).

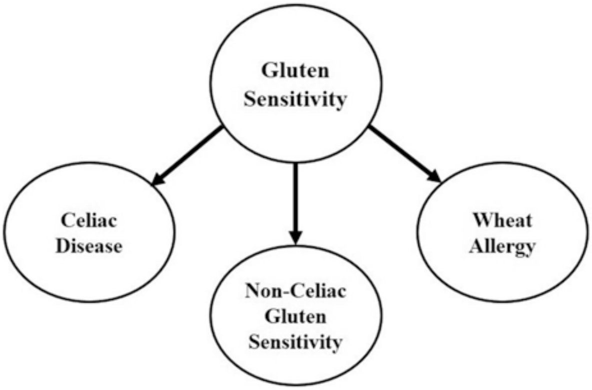

Fig. 2 Different forms of gluten sensitivity

3.1 Celiac Disease (CD)

Celiac Disease is a chronic condition influenced by immunological response triggered by ingestion of gluten and related proteinaceous compounds. CD has been reported in 1% of the total population worldwide with most of the cases undiagnosed. There has been four to five times increase in the prevalence of CD in past 50 years, however, the reason for such upsurge is not clearly known. This disorder is observed in inherently predisposed individuals and better understood as action by certain autoantibodies against transglutaminase-2 (tissue), deaminated peptides (gliadin) and endomysium (Ludvigsson et al., 2013).

CD is a result of incomplete digestion of gluten protein leaving behind peptides containing about 33 amino acid units which in the intestinal tract pass through the epithelial cells barrier to enter lamina propria either via paracellular or transcellular pathway. There occurs an adaptive immune response reaction when CD4 positive cells recognize gliadin peptides through antigen presenting cells leading to the emergence of proinflammatory cytokines, specifically interferon-γ (Sollid, 2002). In addition to adaptive response, innate immune responsive reaction is also activated which is detected by the action of enterocytes as enhanced expression of interleukin-15 causing the stimulation of intraepithelial lymphocytes cells triggering occurrence of receptor NK-G2D recognized as the natural cell killing expression (Mention et al., 2003). These receptor cells impart cytotoxicity by damaging enterocytes by a similar mechanism noted in case of infection due to expression of a cell surface antigen emerged out of stress (Meresse et al., 2004; Green & Cellier, 2007). Although the actual mechanism for such interactions in the lamina and epithelium has not been systematically explained, recent reports suggest non gluten proteins also initiate cell damage in innate epithelial (Junker et al., 2012). Impact of CD on nutrient absorption is illustrated in Fig. 3.

Genetic factors have been seen to play a significant role in occurrence of CD. This condition does not arise unless any individual has HLA gene products encoding for

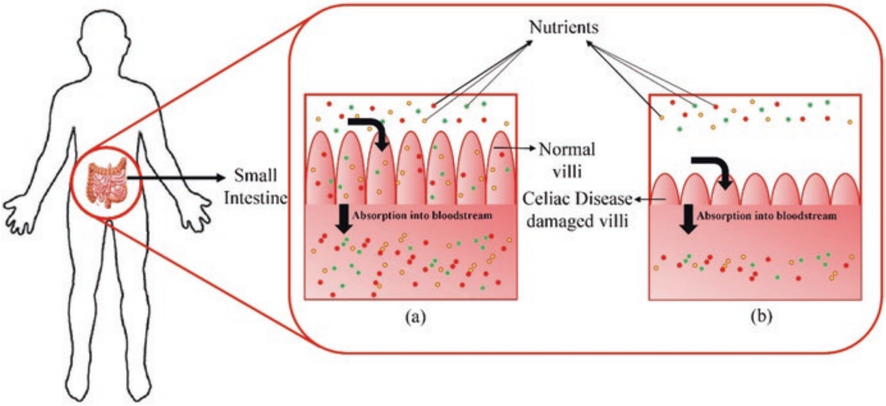

Fig. 3 (**a**): Absorption of nutrients through normal villi and (**b**) Poor absorption of nutrients through CD damaged villi

HLA-DQ8 or HLA-DQ2 kinds of proteins (Sollid & Lie, 2005). These two alleles are considered crucial for the identification of CD in an individual since their presence in general population is about 30–40%. In case of CD positive patients, HLA-DQ2is present in more than 90% cases and remaining patients are found positive for HLA-DQ8. Therefore, absence of these genes confirms the negative results for CD. However, a non-HLA class of genes has also been identified to have a role in CD, but their impact has not been confirmed.

In relation to environmental effects, it has been observed that introduction of gluten to weaning foods and breast feeding are two very critical factors. Infants below the age of 4 months administrated with feed containing gluten are at higher risk to adapt to CD (Norris et al., 2005). Moreover, certain drugs and proton pump inhibitors have also been reported to cause onset of CD, but this impact is inconclusive since there is possibility of undiagnosed CD in such case rather than its origin. Gastrointestinal discomforts including disorders relating to pancreas and liver have also been documented with the development of CD severity ranging from enhanced level of transaminases in serum to deadly liver failure and cancer. However, relation of CD to cardio-vascular diseases is contradictory.

3.2 *Non-Celiac Gluten Sensitivity (NCGS)*

The term NCGS refers to the condition triggered by ingestion of food containing gluten presenting intestinal and extra intestinal symptoms in patients tested negative for CD. This gluten related disorder has been proposed to be more frequent in comparison to CD, however, data for exact frequency is limited due to lack of absolute biomarkers. Such condition disappears on withdrawal of gluten from diet within hours and immediate symptoms are observed again with introduction of gluten in diet (Leonard et al., 2017). The extraintestinal impact of this disorder is characterized in terms of foggy brain, which is expressed by symptoms of muscle pain, weakness, dermatitis, numbness (arm/leg), headache along with neurological impacts such as decline in alertness level, memory interruption and slow thinking. Another impact of gastrointestinal disorder appears in terms of diarrhoea, constipation, stomach or abdominal pain, bowel irregularity and bloating.

The pathophysiology details of Non-Celiac Gluten Sensitivity are imprecise and not conclusive. Adaptive immune response and enhanced interferon γ response are the underlying evidences in few reports studying the gluten challenge. The differentiation of CD from NCGS is known due to lack CD specific antibody biomarkers but in certain cases, antibodies in reference to gliadin proteins are observed which are having lower specificity for CD and such antibodies -disappear with the onset of GF(GF) diet, for example IgG (Caio et al., 2014). Gluten proteins have been reported for certain intrinsic biological properties altering the morphology of the cells leading to motility, and organizational setup of cytoskeleton due to constricted junction proteins (Roncoroni et al., 2013; Casella et al., 2018). Binding of TLR2 receptors cells with gliadin protein subunits has been reported to enhance the production of Interleukin 1 (proinflammatory cytokine) via intermediation of Myd88, which is a known compound for the release of zonulin upon consumption of gluten to increase the permeability of the mucosal cells (Palová-Jelínková et al., 2013).

Certain reports argued that the term "non-celiac wheat sensitivity" better describes the condition as the actual reason for underlying disorder may be the other constituents present in wheat. However, NCGS has been suggested as the most common gluten related disorder with the absence of diagnostic biomarkers. In addition to gluten, believed possible components responsible for such condition are FODMAPs (fermentable sugars such as mono-, di- and polysaccharides), sugar alcohols and anti-nutrients like amylase-trypsin inhibitors (ATIs). Intake of FODMAPs has been correlated with the positive symptoms of NCGS; and the diet limited in FODMAPs for 2 weeks resulted in self-reported NCGS improved symptoms in a recent study. ATIs have also been reported to have significant impact in NCGS, although they represent only 4% of the total protein present in wheat. They are also responsible for activation of innate immune response in small intestine and colon inflammation as reported in both *in vitro* and *in vivo* studies and thereby triggering the expression of myeloid cells of mesenteric lymph node and activate Toll-like receptor-4 due to ATIs being resistant to heat and proteases enzymes (Zevallos et al., 2017; Barbaro et al., 2018).

3.3 Wheat Allergy (WA)

One of the most general reasons of food allergies related to consumption (inhalation or ingestion) is wheat and some of the proteins out of many present in wheat (more than 100) are considered responsible for allergies. Wheat proteins have been classified as albumins, globulins, gliadins and glutenins on the basis of their extraction in different solvent systems. Majorly, the wheat allergy has been reported to be seen with glutenin fractions (low and high molecular weight compounds) along with globulins (α, β, γ, and ω fractions). Certain components of albumins and globulins such as proteinases inhibitors, α-amylase inhibitors, β-amylase, puroindolines, lipid transfer proteins (LTPs) and other surface-active protein moieties. Since wheat belongs to family Poaceae and is a grass, allergens like lipid transfer proteins and α-amylase cross react with other pollen allergens of grass. The most common allergies observed in case of wheat are wheat-dependent exercise-induced anaphylaxis (WDEIA), anaphylaxis and Baker's asthma owing to heat resistant allergens including trypsin and α-amylase inhibitors (Ricci et al., 2019).

Wheat allergen belonging to the class of nsLTP (Tri a 14) is a significant candidate to cause allergy through IgE-mediated foods along with Baker's asthma and WDEIA. Similarly, water insoluble Tri a 19 (ω-5-gliadin) has also been categorized as one of the potential allergens in reference to WEDIA. Presence of antibodies against mechanism of Tri a 37 (highly resistant to digestion and high temperature, a plant defensive protein) in an individual is a great risk to develop allergy upon consumption of wheat (Cianferoni, 2016). WA is generally attributed to outcome of IgE-mediated reactions showing the impact characterized by nausea, bronchial obstruction, urticaria, abdominal pain, angioedema or anaphylaxis within 2 h of ingestion of wheat product.

Pathogenesis of WA has been explained as the clinical indications of WA are attributed to the release of mediators from basophils and mast cells (such as leukotrienes, histamine and platelets activator factor). Contact of any particular allergen with specific IgE antibodies at their receptor point accelerates the cross linking IgE receptor (FcεRI) to trigger the activity of basophils and mast cells. Such release of antibodies due to wheat are a result of Th2-biased immune dysregulation and oral tolerance. Intrinsic profile of wheat allergens also determines the whether specific allergen induces immune response or not. Generally, food allergies are caused due to glycoproteins which are comparatively resistant to acid, temperature and proteases digestion (Lee & Burks, 2006; Lack, 2008; Radauer & Breiteneder, 2007).

Derivative allergies are also important in case of wheat. With the actions of enzymes, proteolysis occurs cutting the protein molecules into simpler units with addition of water molecules at the site and the process is also known as enzymatic hydrolysis yielding polypeptides, peptides and protein hydrolysates. Such hydrolysates are also of allergic nature which was earlier not present in wheat. During the generation of simpler units from proteins such as polypeptides, exposure of buried regions to the surface is observed and these sites are believed to be antigenic. These allergens are not present in wheat as an ingredient in any food, however protein hydrolysates are used as additives in many food commodities (Akiyama et al., 2007; Pasha et al., 2016).

4 Role of Gluten in Bread Making

The proteins belonging to the gluten complex in wheat are of great importance in reference to bread making. Many *in vivo* and *in vitro* investigations have proposed protein structural-functional relationship as critical parameter of functionality in food processing. The major protein of wheat is gluten, which is a mixture of many distinct but related proteins fractions mainly comprising of glutenin and gliadin. Functionality of gluten is known in terms of its heat stability, its potential as binding agent, retention of moisture, and being an additive, it is used to improve the flavor and texture of the bakery products (Biesiekierski, 2017).

The unique rheological behaviour of gluten is the crucial factor for its suitability in many food products. The unusual functional characteristics of gluten are attributed to the ratio of glutenin to gliadin leading to the changes in the interactions. Viscoelastic properties and quality of the bread depend upon the gluten proteins. The strength and elasticity to the dough are imparted by glutenins while dough extensibility and viscosity are contributed by hydrated gliadins molecules. Both the quality and quantity of proteins are vital in breadmaking. The variation in the bread quality from different wheat cultivars is due to the qualitative and compositional differences. For good quality bread, disulphide bonds in linking the subunits of glutenins are of utmost importance.

It is very well known and documented for many years that higher the protein content of wheat, better is the bread quality. The functional properties of gluten including elasticity, viscosity and extensibility leads to the entrapment of carbon dioxide in the dough, released by yeast during fermentation. The gluten network is altered with porous structure which becomes permanent during high temperature treatment (baking). As mentioned earlier, the balance between glutenin and gliadin is critical because high glutenin level will increase the elasticity limiting the expansion of the loaf while retention of carbon dioxide is affected poor elasticity due to lower proportion of glutenin (Shewry et al., 1995).

The first step of gluten development in bread making is the hydration of wheat flour followed by kneading which gives mechanical energy to the system and dough with elastic nature is formed. Dough formation in breadmaking is considered poor or good depending upon the quality and volume of the loaf. A good loaf volume and silky crumb is the desirable feature of the bread. The quality of the final product is determined in terms of:

(a) Retention of carbon dioxide and to develop porous small gas cells,
(b) Balance between elasticity and viscosity for adequate expansion and retention of shape.

The contact of wheat proteins with oxygen is also equally important for development of gluten network in dough. There occurs the formation of intermolecular disulphide bonds with interactions between the protein fractions of the wheat leading to the resultant three-dimensional matrix of the dough. Blending is also critical to allow complete hydration and to supply mechanical energy required for rearrangement of gluten proteins. Mixing is a complex process favouring mechanical as well as chemical alterations during the formation of dough. Oxidation of sulfhydryl groups of proteins leads to the formation of disulphide bonds. Although these bonds are low in number, they provide significant difference in the quality of the resultant dough (Sluková et al., 2017). Gluten proteins are hydrated and developed with the aim of inclusion of air into the dough control on the number and size of gas cells during mixing.

5 Challenges in Gluten Free (GF) Bread Making

Since strict GF diet is the only way to combat CD, NCGS and WA, it is important to find alternatives for baking industry. Wheat, rye and barley are the major cereals to be avoided for GF food formulations. Gluten is a complex protein matrix giving wheat unique functional properties which are difficult to replicate with other cereal crops. Commercially available GF food products are incomparable with those made from wheat due to lack of excellent functionality in terms of texture, organoleptic profile and visual appeal. GF breads give poor texture and volume to crumb and crust along with undesirable mouth feel and taste due to added additives. Starch is the primary structural ingredient in GF bread and thereby such products are low at nutritional value and undergo staling readily by retrogradation. Also, expansion and gas retention in GF breads are poor since gluten is the only matrix to hold these unique properties. Consistency of dough from non-gluten ingredients is more likely to that of batter resulting in loss of baking quality by producing crumbly texture of the bread. Yazynina et al. (2008) reported that elimination of gluten from bread is associated with loss of iron, folate and vitamin B complex along with reduced level of minerals and fibre. Fat content has been reported as double as that of gluten containing bread (Pellegrini & Agostoni, 2015). Lysine content of commercially available GF breads is lower while fat and carbohydrate content is high (Naqash et al., 2017). Although many ingredients such as teff, sorghum, oats, buckwheat, rice and maize have been used with the incorporation of additives like starches, proteins, hydrocolloids, emulsifiers and in certain cases gluten has been removed from traditional recipe, the texture and aroma due to production of unique volatile compounds have not been replicated to date (Pacyński et al., 2015).

Water binding capacity of gluten is attributed to high content of glutamine and hydroxyl amino acids which almost represent 10% of the gluten and hydrogen bonding between these fractions give rise to cohesion and adhesion characteristics. Cysteine fractions, contributing to 2–3% of total amino acids help in the formation of dough due to interchange reactions between sulfhydryl-disulfide giving gluten matrix an extensive polymerization. Gliadin fraction of gluten imparts desirable viscosity and extensibility to the wheat dough. Therefore, due to unique structural orientation and excellent functional properties of gluten protein complex, it is technologically an extreme challenge to mimic the rheological and sensory profile of wheat bread using gluten-free ingredients and additives to replicate desired properties (Arendt et al., 2008).

6 Gluten Free Bread Formulations

Owing to the challenges like CD, NCGS and WA, cereal technologists are working in the direction to cater the need of GF food products. Considering the knowledge available at present scenario, strict GF diet is the only solution to this sensitivity as the symptoms immediately disappear upon withdrawal of gluten. Scientists and technologists worldwide have tried their best to overcome this challenge by incorporating alternatives to wheat in bread either with addition of nutritional ingredients and additives or by technological modification of GF flour. An overview of challenges and opportunities of GF (GF) bread formulations is demonstrated in Fig. 4. Addition of hydrocolloids have resulted in the viscoelastic properties of the dough and at the same time, bio-functional ingredients such as buckwheat, nutri-cereals, teff and brown rice produced highly nutritious product (Moroni et al., 2009). Recipe of GF breads is heterogeneous in nature being a combination of different cereals such as maize, rice, nutri-cereals, super grains along with additives to impart techno-functional properties including starches, non-gluten proteins, fats, enzymes and hydrocolloids.

In the absence of gluten, it becomes very important to add such additive which can mimic the role of gluten in forming dough and imparting good texture and volume to the crumb without affecting the organoleptic and functional properties of the bread. Milk proteins are potential ingredients in the ability to form gluten like matrix in the bread giving improved crumb texture and prevent staling for a significant timing. Use of proteases enzymes from microbiological origin such as *Aspergillus oryzae* and *Bacillus stearothermophilus* and transglutaminase have been reported to improve the rheological properties of the bread by promoting network formation (Mohammadi et al., 2015).

7 Additives in Gluten Free Breads

Since no ingredient other than gluten can yield final product with excellent textural properties as obtained with use of wheat, it becomes essential to incorporate certain additives in dough making to achieve similarity to wheat-based bread as maximum as possible. In past few years, it has been found that addition of additives like starches, hydrocolloids, emulsifiers and proteins at specific levels to non-gluten ingredients yield breads mimicking the structure and visco-elastic profile of conventional bread dough.

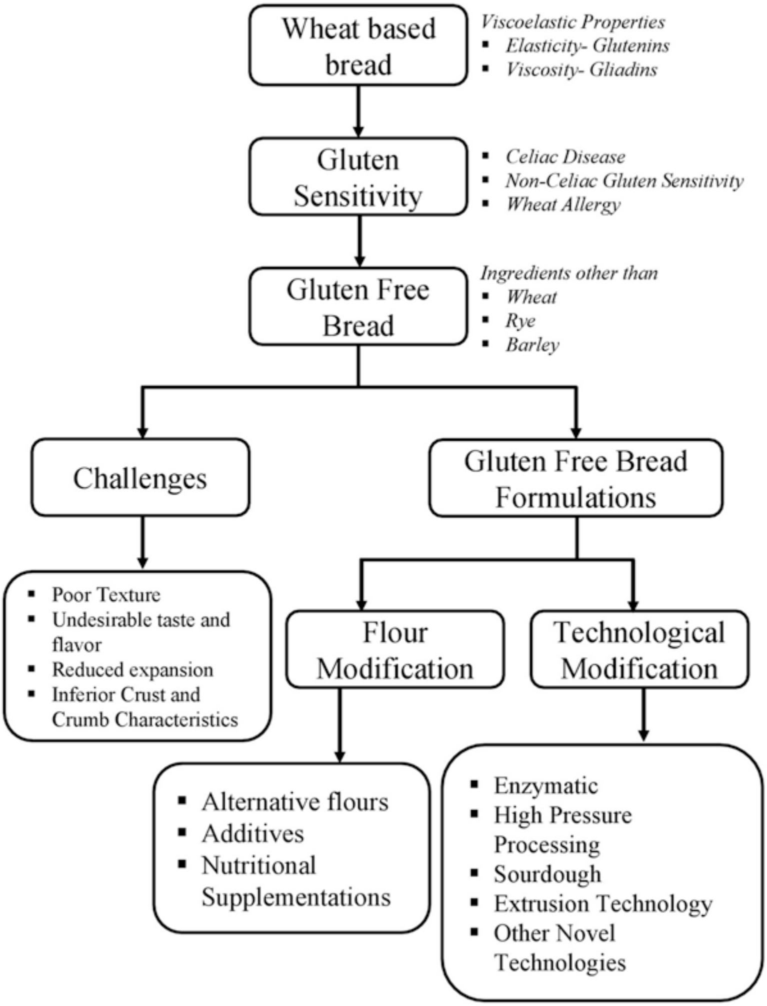

Fig. 4 An overview of challenges and opportunities of Gluten Free (GF) bread formulations

7.1 Starch as Additive

In GF bread formulations, starch is responsible for primary texture and structure of the bread; added in both native and modified forms such as resistant starches, chemically modified and maltodextrins. Maltodextrins having varying degree of dextrose equivalents (DE) has been examined for quality and stability of GF bread. Chemical modification of starches has been studied for improving the volume of the loaf and elasticity of the crumb structure. Chemically modified starches like acetylated di-starch adipate and hydroxypropyl di-starch phosphate when used for preparation of

GF bread, resulted in elastic crumb and decreased value for hardness and chewiness (Ziobro et al., 2012).

A physically modified starch such as pre-gelatinized tapioca starch has also improved the crumb volume and softness. Resistant starches not only improved the nutritional quality of the bread, but also the rheological profile of the bread in terms of improvement in the elasticity and reduction in the hardness of the crumb. The property of resistant starch as elastifying agent in rice-based products has also been reported (Naqash et al., 2017). Starch functionality of gelatinization and retrogradation plays important role in the formation of dough as it absorbs about 45% water and act as continuous filler in dough matrix. On heating in the presence of moisture during baking process, starch molecules gelatinize but still maintain their granular behaviour.

7.2 Proteins as Additive

To mimic the role of gluten in GF bread, proteins from animal origin such as casein and egg proteins and of plant origin involving soya and other legumes can also be added. Milk proteins have similar chemical structure to one of the gluten proteins, giving GF bread desired shape and texture. Functionality of milk proteins is dependent on the constituent protein fractions. For instance, caseinate fraction acts as good emulsifier and provide stability to the batter; good water binding capacity is attributed by skimmed milk powder and whey proteins are excellent in forming gels (Houben et al., 2012).

Performance of egg in GF bread is mainly due to albumin providing stabilization to foam to retain gas and to give better structure to loaf in absence of gluten (Deora et al., 2015). Plant proteins such as isolated pea and soya proteins also have remarkable water holding properties improving mechanical properties of the dough. Plant proteins have illustrated enhancement in the specific volume, sensory profile along with decline in the retrogradation. They also make the final product more softer and elastic by improving the viscoelastic properties of the dough (Matos et al., 2014; Ziobro et al., 2016).

7.3 Hydrocolloids as Additives

Hydrocolloids are class of water-soluble polysaccharides, commonly known as gums having the property to control texture and rheology of food systems and also capable of stabilizing the emulsions, gels, foams and suspensions. They provide variety of functional properties making them potential additives in food processing (Naqash et al., 2017).

These are added to mimic the formation of gluten network in absence of wheat proteins. Xanthan gums and carboxymethyl cellulose (CMC) are two important hydrocolloids to improve the size of gas cells resulting in improvement of the crumb porosity. Korus et al. (2015) examined the linseed mucilage as alternative to the hydrocolloids to form structure of the dough and found improvement in the sensory and textural properties of the bread along with rheological profile of the dough.

Hydrocolloids such as xanthan gum, locust bean gum, guar gum and tragant have been evaluated for activity as binding agents and found favourable results for bread volume and firmness. According to FAO/WHO Expert Committee on Food Additives, the daily intake of certain hydrocolloids such as pectin, guar gum, carrageenan, xanthan, CMC and locust bean gum is 'not specified' which means that these ingredients do not possess any hazard to health at levels necessary to impart desirable impact on the final product (Anton & Artfield, 2008).

Among several hydrocolloids studied for their application in GF bread formulations, xanthan gum and hydroxypropylmethylcellulose (HPMC) are best suited for mimicking the functionality of gluten. Xanthan gum, an exocellular carbohydrate obtained from microorganisms, improves the rheological profile of GF dough. The possible mechanism has been described on the basis of chain conformation and molecular structure of the gum resulting in the intermolecular interactions of the chain. At lower shear rates, xanthan gum exhibited better elasticity among other hydrocolloids, reason may be its weak gelation and higher viscosity (Lazaridou et al., 2007).

7.4 Emulsifier as Additive

Emulsifiers, also known as surface active agents and surfactants are often considered as dough improvers. They are functional additives in bakery products enhancing strength of the dough and crumb softness which is mainly due to amphiphilic nature. They are active at interfacial sites of two phases and forms dispersion (Stampfli & Nersten, 1995). Emulsifiers are important ingredients of bakery industry owing to their ability to have interaction with various dough ingredients and flour components giving rise to desirable texture of the final product (Demirkesen et al., 2010).

Emulsifiers, when added to GF bread, have contributed in improving the stability of breadmaking thermodynamically lesser stable system (Gómez et al., 2004). They have been reported to positively impact the dough structure with decreased crumb firmness (Onyango et al., 2009). Properties like antifarming and dough improvement by emulsifiers are generally attributed to their ability to reduce repulsive charges between protein fractions, thereby leading to their aggregation. They have also been reported to delay or retard retrogradation by limiting the water movement within starch molecules (Stauffer, 2000). Another important property of emulsifiers is generation of liquid films around gas cells providing them protection and stabilization. Most common emulsifiers used to functional additives in GF breadmaking are diacetyl tartaric acid esters of monodiglycerides, Sodium stearoyl-2-lactylate, polyglycerol esters of fatty acids, distilled monoglyceride and lecithin (Eduardo et al., 2014).

8 Nutritional Enrichment of Gluten Free Bread

Number of cereals, legumes and pseudocereals flour has been used in the development of GF breads. These flours are added to breads to improve the nutritional quality since they possess bio-functional properties. GF bread formulation constituting quinoa, amaranth and alternative sweeteners yielded product of similar quality to that of control wheat bread. In a similar study with quinoa and buckwheat, increased crumb volume was observed along with enhanced cohesiveness and springiness. Pseudocereals have also been reported to better withstand high temperature and shear in comparison to rice flour.

Dietary fibre is an important food component owing to its unique functionality and excellent nutritional characteristics. Consumers have shown inclination towards fibre enriched bakery products even with reduced functionality, for instance, reduction of loaf volume and hardness of the crumb with particular flavour. Addition of both soluble and insoluble fibres has been evaluated in GF breads. Dietary fibre from cereal sample (oat and maize) when added in GF bread gave rise to improved loaf volume and texture in comparison to control GF bread. Oats are rich source of β-glucan (soluble dietary fibre) which is associated with several health promoting properties like reduction in low density lipoproteins, attenuation of insulin level and post prandial blood glucose.

Proteins are added to GF breads with double objective of enhancing the nutritional and functional profile since they improve flavor, texture and amino acid content. Proteins from different sources such as dairy, legumes, eggs and cereals have been added to GF bread formulations.

Houben et al. (2012) suggested that eggs create improved crumb structure due to their emulsifying and foaming properties. These properties promote the retention of gas during baking and build desirable structure. For nutritional enhancement of GF breads, fruits and vegetables in dried and native forms have been added such as green kiwifruit puree, strawberry seeds, raisin juice, orange pomace and banana flour (Capriles & Arêas, 2014).

9 Technological Modification for the Development of Gluten Free Bread

Gluten Free bread is gaining popularity these days due to the upsurge cases of gluten intolerance. But, development of GF bread is itself a challenge due to the absence of gluten, which is well known as a heart of bread. Additives can be added to overcome these challenges but again, due to their chemical origin, their use is limited. To overcome such issue and to maintain the acceptability of GF bread among consumers technological modifications are carried out in the food processing industry by various techniques/technology like enzymatic modification, high pressure (HP) processing, sourdough fermentation, extrusion technology, germination, heat treatment, hydrothermal treatment etc. which are discussed below (Table 1):

10 Enzyme Modification

Enzyme modification is a natural way of modifying GF flour because they are substrate specific therefore can modify specific properties according to its applicability. Enzymes are generally recognized as safe (GRAS), so they do not have any negative health implication as that of chemicals (Rosell, 2009). Recent past studies, have been done to improve the rheological properties of GF dough by oxidation, hydrolysis or protein cross-linking. Enzymes like transglutaminase (TGase), glucose oxidase (GO), amylases, cyclodextrin glycosyltransferases, protein cross-linking enzymes and many more are used in GF formulation to improve its rheological and sensory properties (Fig. 5).

Enzymes are added to improve the functionality of GF bread to produce desirable loaf texture and transglutaminase (TGase) is one of the best candidates for improving cross-linking to impart characteristic texture. Mechanism of TGase has been reported in three terms as deamination, crosslinking and amine incorporation. Intermolecular and intramolecular iso-peptide bond interactions are induced due to cross linking when ε-amino group in lysine work as acyl receptor. TGase also plays important role to link different proteins such as caseins, soya proteins, wheat proteins and albumins. Modification of proteins by amine incorporation occurs when primary amines are absent in the bread formulation and thus water acts as acyl acceptor for the deamination of glutamine units (Motoki & Kumazawa, 2000).

Studies reported that TGase increased the protein cross-linking in oat and rice dough, respectively, causes improvement in viscoelastic and gas retention properties of dough. TGase can also catalyse deamination and acyl transfer reactions. TGase reported to improve the dough handling properties of brown rice batter and quality of GF bread prepared from it. These changes in properties are mainly attributed to the formation of highly polymerised structure from large protein complex and stronger hydrophobic interactions among proteins in presence of TGase (Renzetti et al., 2012; Deora et al., 2014). Hatta et al. (2015) examined improvement in bread properties like gas retention and textural parameters with rice protein due to action of proteases by degrading α- and β-glutelin in rice. TGase in addition with hydrocolloids such as guar gum has been reported to improve the bread quality; however, at higher concentration of TGase, increase in the hardness of the crumb was observed (Mohammadi et al., 2015). Use of pre-gelatinized starch along with TGase can potentially be used to produce good quality GF bread. In sorghum batter,

Table 1 Technological modification in the flour in the development of Gluten free Bread

S.No.	Flour treatment	Treatment	Improvement	Reference
1	Enzymatic modification	Pre-gelatinized tapioca starch (30%) and transglutaminase in the preparation of gluten free jasmine rice bread	Decreased dough elasticity Increased resistance to deformation Better expansion of gas cells Specific volume increased to 2.4 $cm^{3/g}$	Pongjaruvat et al. (2014)
		Tyrosinase and laccase in the oat bread development	Firmness of bread treated with tyrosinase increased in comparison to laccase due to cross-linking of oat globulins. Specific volume increased Combination of both enzyme increased the softness of bread	Flander et al. (2011)
		Tranglutaminase and quinoa flour	Overall acceptability of the bread improved with increased softness of bread	Romano et al. (2018)
2.	High Pressure Processing (HPP)	Sorghum dough is treated at 200 and 600 MPa and added to untreated dough @ 2% and 10%	Delayed staling of bread containing 2%, 600 MPa treated sorghum flour. No difference in specific volume.	Vallons et al. (2010)
		Composite dough (oat, millet, sorghum bread) treated at 200, 350, 500 MPa for 10 min.	Excellent nutritional and anti-radical properties. No significant change in specific volume is observed. Little change in staling	Angioloni and Collar (2012)
		Corn starch and rice flour are treated at 600 MPa for 5 min at 40 °C	No significant different was observed in specific volume of bread before and after treatment. Delayed staling was observed.	Cappa et al. (2016)
3.	Sourdough Fermentation	Lactobacillus plantarum AL30 (Amaranth dough)	Visco-elastic properties of dough were similar as that of wheat dough	Houben et al. (2010)
		Lactobacillus amylovorus DSM19280 as starter in quinoa bread preparation	Increase in the firmness of bread Delayed staling Higher specific volume	Axel et al. (2015)
		Lactic acid bacteria and yeast in the preparation of chest flour bread	Higher Specific volume than control Gas retention improved Crumb softness increases	Aguilar et al. (2016)

(continued)

Table 1 (continued)

S.No.	Flour treatment	Treatment	Improvement	Reference
4.	Extrusion Technology	Acidic extruded rice flour bread	Improvement in color and texture of bread	Pedrosa Silva Clerici et al. (2009)
		Composite flour (Buckwheat, rice, maize and extruded maize) bread	Develops regular porosity in the bread crumb No significant change in specific volume Softer crumb than the bread containing without extruded maize flour	Ozola et al. (2011)
		Extrusion effect on rice bread	Improved dough consistency Increase in specific volume Delayed staling	Mario et al. (2014)
5.	Germination	Germinated quinoa and oat bread preparation	Improvement of specific volume Improvement of crumb texture Germinated quinoa only adds to the flavour and nutritional properties of the bread.	Makinen et al. (2013)
		Amaranth, Millet, Corn, Lentil, Lupin, Pea and quinoa were sprouted and added @5%	Good specific volume and reduced hardness in comparison with control. Amaranth based bread was found to have the highest specific volume.	Horstmann et al. (2019)
6.	Heat treatment	Heat treated flour	Increased elasticity of dough Increased specific volume	Gêlinas et al. (2001)
		Heat treated (125 °C for 30 min) sorghum based bread	Improved dough handling properties Increased specific volume	Marston et al. (2016)

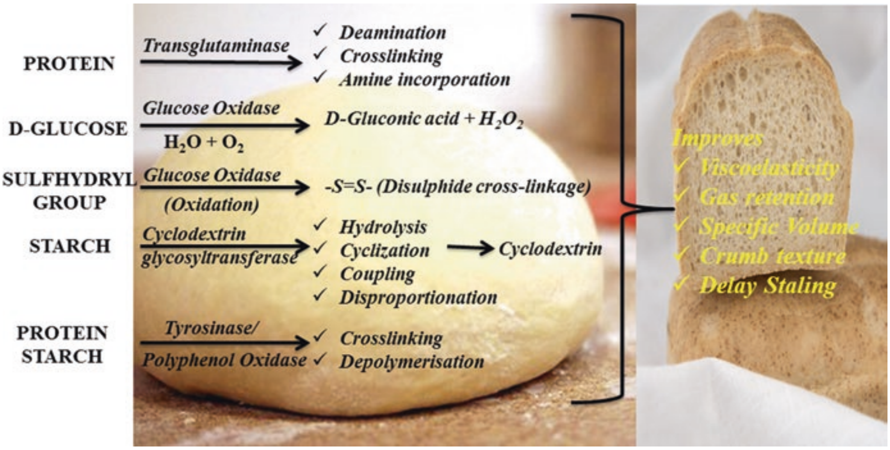

Fig. 5 Action of enzymes on macromolecules during the development of GF bread

dough handling properties were found to be significantly increased by incorporation of pre-gelatinized cassava starch followed by modification using microbial transglutaminase (MTGase). MTGase decreased the resistance to compliances and deformation while increased the zero shear viscosity and elastic recovery in sorghum based batter (Onyango et al., 2010).

Glucose oxidase (GO) is the charm of bakery industry, it carries out the oxidation of β-D glucose into D-gluconic acid and a molecule of hydrogen peroxide. GO also promotes the oxidation of free sulfhydryl into disulphide cross linking, thereby results in moderating the rheological properties of GF dough. Moreover, hydrogen dioxide produced during oxidation also plays a role in modulating textural characteristics. Gujral and Rosell (2004), reported improved elasticity of rice flour dough due to the disulphide cross-bridge formation in the presence of GO. Another study confirms the enhancement of elastic behaviour of sorghum and corn flour dough, author correlates the improved dough handling properties with aggregation of protein structure and polymerization of sulfhydryl groups into disulphide cross-bridges (Renzetti & Arendt, 2009).

$$\beta - \mathrm{D} - \mathrm{Glucose} + \mathrm{H_2O} + \mathrm{O_2} \xrightarrow{\text{Glucose oxidase}} \mathrm{D} - \mathrm{Gluconic\ acid} + \mathrm{H_2O_2}$$

$$-\mathrm{SH} + -\mathrm{SH} \xrightarrow{\text{Glucose oxidase}} -\mathrm{S} = \mathrm{S} - \left(\mathrm{Disulphide\ cross - linking}\right)$$

Cyclodextringlycosyltransferase (CGTase) is another enzyme used widely in bakery industry with the capacity to hydrolyze α-1,4 glycosidic linkages in starch molecule and linking reducing and non-reducing end to produce cyclic molecule. CGTase improve the pasting properties of GF flour by cyclization of starch and glucose into cyclodextrins. Cyclodextrins are the amphiphillic molecule having hydrophilic outer part and hydrophobic internal cavity. Rice proteins are hydrophobic in nature, so traditionally addition of conditioner and improver into flour was of no use in improving textural properties of rice bread, but incorporation of CGTase cause improvement in loaf volume, crumb texture and delaying the bread staling (Gujral et al., 2003). This Antistaling property of CGTase is due to the formation of complexes with protein and lipid.

Tyrosinase and polyphenol oxidase are oxidative enzymes with the capacity to catalyze crosslinking of macromolecules by their phenol moiety, which causes improvement in viscoelastic properties of gluten-free dough. It has been reported that tyrosinase and polyphenol oxidase strengthen the oat dough by promoting intermolecular covalent bonding or cross-linking of oat protein (Buchert et al., 2010; Mattinen et al., 2005). Renzetti et al. (2010) reported improvement in the specific volume of oat bread due to the depolymerisation of β-glucan and polymerisation of protein.

Thermoase, a protease enzyme obtained from *Bacillus stearothermophilus* when evaluated for development of GF rice breads resulted in improvement of visual properties, loaf volume and texture. Positive impact of thermoase in relation to staling of the bread was also noted. (Kawamura-Konishi et al., 2013). With the application of enzymes, it has also been observed that gelatinization temperature is decreased. Enzymes are also useful in mimicking the perforated structural organization of wheat-based breads in GF breads with smooth surface appearance as revealed in microstructural evaluation (Naqash et al., 2017). Therefore, application of enzymes in GF bread formulations is a promising approach to improve the quality.

11 High Pressure Processing

High Pressure (HP) processing is a “non-thermal technology” in which food is subjected to elevated pressures, to achieve the microbial inactivation while retaining sensory characteristics and nutritional value of the food products. At the present time, HP is gaining considerable attention in flour modifications as well. HP results in remarkable change in the macromolecular structure which enhances the functionality of the flour leading to the development of newer product with desirable sensory characteristics. HP treatment majorly affects the starch and protein structure through starch gelatinization and disruption in protein structure (Ahmed et al., 2007).

HP can be used as one of the promising technique with the potential area in food texture engineering (Deora et al., 2014). Pressure used for modification varies from 100–1000 MPa. The application of HP causes swelling and gelatinization of starch without disturbing the granule integrity. Extent of swelling and gelatinization depends upon the applied pressure, duration, temperature, type and concentration of starch (Stolt et al., 2000; Vallons & Arendt, 2009). Starch is one of the key ingredient in the development of the GF products. It is demonstrated that application of HP lowers the gelatinization temperature of starch and form paste with creamy texture. These altered properties can be directed to improve the consistency of GF batter, used to develop products with properties similar as that of wheat-based product (Stolt et al., 2000; Liu et al., 2008; Deora et al., 2014).

Studies have been conducted to investigate the effect of HP of 200, 400 and 600 MPa for 10 min on the viscoelastic properties of GF flour. The result of the investigation confirmed that HP treatment improves the functionality of GF flours by prompting the protein cross-linking and starch gelatinization, which indirectly reflects the improvement in viscoelastic properties of teff, white rice and buckwheat (Vallons et al., 2011). Huttner et al. (2009), applied the pressure of 200, 300, 350, 400 and 500 MPa for 10 min on oat batter, observed increased viscosity of batter at 300 MPa but at 350 MPa, the elastic component was predominant. These changes attributed to the starch gelatinization and formation of disulphide bonds or urea-insoluble complexes in the oat batter. Vallons and Arendt (2009) also reported elasticity in the sorghum batter above 300 MPa. Huttner et al. (2010) treated oat dough at 200, 350 and 500 MPa for 10 min, and replaced untreated oat dough with treated oat flour @ 10%, 20% and 40%. They reported 10% oat dough treated at 200 MPa was best in improving the crumb volume, appearance and reduced the bread staling rate. Vallons et al. (2010) treated sorghum dough at 200 and 600 MPa, and added HP treated sorghum to untreated sorghum @ 2% and 10%. They reported delayed staling of bread containing 2% of sorghum treated at 600 MPa due to the inactivation of enzymes responsible for bread staling while 10% resulted in poor bread quality because of low specific volume. They found no difference in bread containing sorghum treated at 200 MPa with that of control bread. Therefore, with above studies, it can be concluded that when GF flours are subjected to HP, there is an improvement in the viscoelastic properties which is a major challenge of GF bread and these flours with altered functionalities can be directed to make GF product. To realise the potential of HP processing in GF flour modification, more research is required to improve the specific properties like dough expansion, structure and gas retention of the dough as well as shelf life and cost for the development of GF bread.

12 Sourdough Fermentation

Sourdough fermentation is one of the oldest biotechnological process to leaven baked good, and it is known to improve texture, appearance, volume, aroma and shelf life of the bakery products. Sourdough is a blend of flour, water and other ingredient which is spontaneously fermented by lactic acid bacteria (LAB) or yeast under controlled condition. Fermentation enhances the dough handling properties as well as improves the textural, sensorial and nutritional properties of the GF bread. During fermentation due to various metabolic processes different exopolysaacharides (EPS), organic acids, antimicrobials and antifungal agents are produced (Moroni et al., 2009).

Fermentation process triggers the naturally occurring enzyme in the grain. These enzymes increased the bioavailability of the nutrients. Starch gets hydrolysed into simple sugars and disaccharide due to the increased amylolytic activity resulting in maltodextrin, maltose and glucose. Specific sourdough bacteria breaks sucrose and produce exopolysaccharide which leads to improvement in the techno-functional properties of GF breads by increasing the water binding capacity of flour. Increase in fiber content lowers the glycemic index of GF bread and does not cause rapid rise in blood sugar. Production of organic acid like lactate, formate, succinate, acetate and citrate improves the sensory property of bread. Antimicrobial and antifungal agents produced extend the shelf life of dough during storage. Carbon dioxide produced during heterofermentation by LAB and yeast affects the leavening process of final dough and indirectly improves the bread softening. Moreover, Sourdough bacteria breaks the anti-nutritional compounds during proofing process which inturn improves the bioavailability of the nutrients especially minerals like Ca, Mg, Fe, Zn, K and Mn. Incorporating sourdough in GF bread can potentially replace the chemical preservative (Carbo et al., 2020).

Fonio based bread was found to be with improved dough strength and gas holding capacity due to increased water absorption capacity (Edema et al., 2013). Sorghum based GF bread was prepared, sourdough fermentation, they reported EPS formed masked the organic acid effect and led to softer crumb of bread. In addition, modification in the macromolecules resulting from metabolic processes (Galle et al., 2012). Bread prepared from GF flour including quinoa, buckwheat, sorghum and teff, employed with sourdough fermentation by *Weissellacibaria* MG1, leads to acidification causing increased crumb porosity which inturn decreased hardness. Furthermore, staling of bread was significantly reduced (Wolter et al., 2014). Bender et al. (2017) studied the effect of selected *lactobacilli* on the functional properties of and stability of GF sourdough bread. They suggested *Lb. sanfranciscencis* strain was able to enhance the all the functional properties of millet and buckwheat based GF bread. Sourdough fermentation leads to the improvement of the elasticity and delayed the process of staling, this may be attributed to the breakdown of starch and non-gluten proteins by LAB. Therefore, sourdough fermentation of GF bread is a promising approach to improve the quality, but still lot of research is required in microorganism optimization.

13 Extrusion Cooking

Extrusion cooking is one of the processing techniques to modify the functional properties of the GF flour. It involves mixing of different ingredient that are forced through a small opening of specific shape and are cut into specific size by blade. Extrusion bring gelatinization of starch, denaturation of proteins, structural changes in lipid and decrease in anti-nutritional factors content leading to the overall change in the GF flour. In GF product, the main textural properties depend upon starch. During extrusion gelatinisation of starch occurs that enhances the water binding capacity of flour and extruded flour make abundant hydrogen bonds with water that ultimately ease the dough development.

Pedrosa Silva Clerici et al. (2009) develops the GF bread by addition of acidic extruded rice flour with improved color and texture of bread. Alongside, extruded maize flour is also used to make GF bread and reported to have excellent physicochemical properties (Ozola et al., 2011). Defloor and Delcour (1999) noticed addition of extruded starches improved the specific volume of bread made with tapioca and soya flour, this was due to the partial gelatinization of starch and increased consistency of dough which improves the gas retention properties of dough. Due to the complex formation between amylose and lipids there is a delay in staling of bread. Mario et al. (2014) reported extruded rice flour improved dough consistency and effect was more noticeable when percentage of extruded flour was higher. Bread obtained is of higher specific volume and water requirement to make same consistency of dough was higher than the control and staling of bread was less noticeable till 72 h with a large particle size of extruded rice flour.

Enzyme liquefaction can be accompanied with extrusion technology to speed up the process. This method can be used to concentrate protein which can be utilised as gluten substitute in GF formulation. In this processing, flour is first extruded through which starch is gelatinized which more prone to enzymatic activity, thereby liquefying enzyme break all the starch and concentrate protein in the remaining mixture. Sorghum proteins are capable of contributing to the viscoelastic properties to the GF dough, so sorghum protein can be concentrated by extrusion-enzyme liquefaction technology and can be further added to the GF formulation in the development improved quality bread with good digestibility (De-Mesa et al., 2009; De Mesa Stonestreet et al., 2012). Mario et al. (2014) reported addition of lipase in extruded rice flour positively affected the bread volume, this was may be due to the fact that lipases hydrolysed the lipids in dough that acts as emulsifier which results in increase the volume of GF bread. Enzyme treated dough have higher capacity to incorporate air and prevent coalescence phenomenon (Sahi & Alava, 2003).

14 Others Technologies

The Inclusion of germinated flour in the cereal products has been one of the upcoming trends in the market. Germination is natural and inexpensive way of modifying GF flour. During germination enzymes gets triggered cause hydrolysis of the macromolecules and results in enhancement of nutritional bioavailability, digestibility, antioxidant and functional properties of the flour. Therefore, germinated grain with full of enzymatic activity can be used as functional food ingredient in the prepared of GF product. Makinen et al. (2013) studied the potential of germinated oat and quinoa in bread preparation. They reported germinated oat at a concentration of <1% results in improvement of specific volume and crumb texture whereas germinated quinoa only adds to the flavour and nutritional properties of the bread. In oat bread improved sensory properties was due to increased water absorption capacity of germinated oat flour. GF bread prepared from germinated soy was found to have good specific volume then heat treated soy flour (Shin et al., 2013). Horstmann et al. (2019) conducted comparative study of GF sprouts (amaranth, millet, corn, lentil, lupin, pea, quinoa) at 5% w/w concentration in the development of GF bread. They reported all the breads with germinated flour have good specific volume and reduced hardness in comparison with control and among all amaranth bread were having highest specific volume and this is assumed because of the increased α-amylases activity that causes decrease in viscosity which allows greater gas cell expansion.

Heat treatment is one of the methods used to improve the bread quality in weak, poor or GF flour. Heat causes unfolding of protein, partial gelatinization of starch and inactivation of enzymes in the flour while improving volume expansion. In recent past study, it has been reported that bread prepared from heat treated flour showed increased elasticity of dough with positive effect on specific volume (Gêlinas et al., 2001). Marston et al. (2016) reported increase in specific volume of heat treated sorghum based bread. Heat treatment of 125 °C for 30 min was found to be optimum, and this increase in specific volume was because of the oxidation of the free sulfhydryl group to disulphide cross-link Bridge as a result stronger dough will form with resistance to mechanical stress. Hydrothermal pre-treatment to GF flour leads to the partial gelatinization of starch which results in excellent thickening properties and high water absorption capacity and can potentially replace hydrocolloids as additive (Hormdok & Noomhorm, 2007).

15 Conclusion

Gluten is a complex protein composed of gliadin and glutenin. Gliadin is responsible for strength and elasticity of dough while glutenin relates with the extensibility and viscosity of dough. Gluten plays a key role in the rheological properties. However, people with gluten intolerance are not able to digest gluten based products. Thereby, GF flour comes into play with a major challenge to mimic the viscoelastic properties of gluten. GF flours can either be supplemented with additives or can be employed with different technological modification. Although, it appears from the above discussion that technological modifications are showing positive impact on the textural properties of bread. But, still the commercial applicability to improve the GF flour is at its infancy stage. Extensive research is required in this area to increase the usability of these techniques in flour modification that can be aimed to make GF bread or other related products.

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Novel Approaches to Gluten Degradation

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1 Introduction

Gluten being the most important storage protein naturally found in several grains like wheat, barley, rye, and spelt, comprises around 80% of whole grain proteins. It arises entirely in the endosperm of grains and comprises of numerous diverse proteins, mostly gliadins and glutenins in wheat, secalin in rye, hordein in barley, and avenins present in oats, are all referred to as "gluten." Wheat gluten attains a top position amongst all the gluten proteins of various cereals due to the visco-elastic polymeric network capable of exclusive baking performance of wheat flour (Wieser, 2007) as the gluten protein is responsible for the bread making as well as wheat flour properties. In addition, it acts as a binder that holds the food together and thus adding a "stretchy" quality. The gluten proteins are further classified into subgroups as alpha, beta, gamma and omega gliadins depending on the primary structures (Shewry & Lookhart, 2003). The exclusive parameters of gluten are attained from the structure and interaction of gluten proteins bound through covalent and non-covalent forces. The composition of these gluten proteins varies with different wheat varieties (Wieser, 2007).

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2 Gluten Function for Bread Development

The visco-elastic characteristics of dough made with wheat flour makes it suitable for bread making. The dough prepared may be either weak or strong depending mostly on the quantity and quality of the wheat proteins. The protein content of wheat grain varies widely but for bread making it should be at least 11% (Wilderjans et al., 2008; Mariotti et al., 2013). The gluten must be strong enough for bread making so as to hold the gas (carbon dioxide) produced during the fermentation process which allows the bread to rise. The rheological properties of dough are improved by kneading process that leads to development of the gluten structure which further improves the expanding ability of dough owing to the production of carbon dioxide gas through fermentation process (Edwards et al., 2003). This stage of gluten network formation may be referred to as 'ripening' or 'maturing' of the dough and the changes associated with gluten formation requires both the protein hydration of the flour as well as energy application by the kneading process. Moreover, the final bread quality is attributed by the quality of wheat flour from the mill as it contributes the proteins compulsory for gluten formation and beneficial for bread production process. In addition, the ability of gluten formation is unique to wheat and the quality and intensity of gluten proteins depends primarily upon variety of wheat, environmental effects as well as agricultural procedures (Lee et al., 2001).

Furthermore, the bread production depends upon the chemical parameters of gluten proteins that lead to the formation of leavened bread. The glutenin proteins that give bonding and elasticity properties are rich in charged and non-polar amino acids, that allow hydrogen bonding, hydrophobic and electrostatic interactions. In addition to these interactions, the main important bonding of gluten proteins occurs via disulphide bond formation and these bonds also lead to the development of extended protein networks due to the binding of protein subunits. The elastic behavior of gluten dough is achieved from the glutenin subunits which are categorized as high molecular weight (HMW) and low molecular weight (LMW) subunits. The dough elastic behavior can further be improved through several techniques wherein glutenin concentration is improved in the wheat variety like in case of "Bobwhite" wheat variety (Blechl & Anderson, 1996; Altpeter et al., 1996). The deviation in the concentration leads to increased elasticity of dough (Barro et al., 1997).

3 Methods and Techniques for Gluten Degradation

There have been a lot of studies emphasizing gluten degradation and ways to develop gluten-free food formulations. Various techniques used to develop gluten-free foods for celiac patients are discussed below:

3.1 Enzymatic Methods

Enzymes, due to their potency to catalyze various biochemical reactions and due to absence of any negative impact on health, are considered to be novel solutions and safe alternative against the usage of chemical ingredients for inhibiting the immuno-reactivity of gluten present in food systems (Rosell, 2009). In the past few years, enzymes have been utilised as a means for degradation of gluten present in various foods. Various enzymes have been found to break the glutamine and proline-rich gluten effectively inside the gastrointestinal tract. Clinical trials have shown that enzymes such as prolyl endopeptidase (isolated from *Sphingomonas capsulate*), cysteine endoprotease (isolated from barley), and prolyl endoprotease (isolated from *Aspergillus niger*) can degrade gluten effectively in the gastrointestinal tract (Bethune et al., 2006; Ehren et al., 2008; Mitea et al., 2008). Prolyl endoprotease (isolated from *Aspergillus niger*) is available as a dietary supplement in United States market as GlutnGo™ (Bricker Labs, Chandler, AZ, USA) and SpectraZyme® (Metagenics, Aliso Viejo, CA. USA), but is marketed as Tolerase®G by DSM Nutritional Products (Heerlen, The Netherlands). Tolerase®G is aimed to degrade unintentional gluten present in the gluten-free foods and should not be confused with a means for prevention of celiac diseases or as a replacement of gluten-free foods. In an experimental study, Sestak et al. (2016) determined the effect of oral supplementation of Tolerase®G (at recommended dosage) along with reduced gluten barley diet on gluten sensitive rhesus macaques model. The intake of 32–64 mg gluten per day from reduced gluten barley diet was found to get degraded by prolyl endoprotease present in Tolerase®G and the overall effect was found to be similar to that of gluten-free diet.

Another approach to hinder the reactivity of gluten peptides is to reduce their binding ability with HLA-DQ2/8 by introducing a bulky molecule into the system. Transamidation of wheat flour using an enzyme and a suitable amine donor can be used to block the gliadin immunotoxicity by hindering the secretion of interferon-γ by intestinal T cells of celiac diseased patients (Gianfrani et al., 2007; Mazzarella et al., 2012).

Enzymes are widely used for shelf-life enhancement and for modification of rheological properties of dough of various gluten-free products. They are known to improve the rheological characteristics and product quality by forming protein cross-linkages, and promoting hydrolysis and oxidation in the gluten-free batters (Renzetti et al., 2008a, 2008b; Renzetti & Arendt, 2009; Rosell, 2009; Segura & Rosell, 2011). Various enzymes such as transglutaminase, glucose oxidase, tyrosinase, laccase, cyclodextrin glycosyltransferase have been found to modify the dough characteristics resulting in formulation of gluten-free products with desirable texture characteristics and functionality.

3.1.1 Transglutaminase

Transglutaminase can be used to catalyze the formation of intermolecular cross-linkages between protein molecules that may be from a single or multiple sources. Major classes of these proteins include dairy proteins, pea legume proteins, myosin, wheat proteins, oat globulins, lactalbumin, conalbumin, and soy proteins (Babiker et al., 1996; Ikura et al., 1980; Larre et al., 1993; Marco et al., 2007; Rosell et al., 2003; Siu et al., 2002). Transglutaminase has been widely applied to gluten-free bread formulations to modify the viscoelastic behaviour of the batters, and help in formation of protein networks, thus enhancing the quality of final product. For instance, transglutaminase was found to ameliorate the consistency and rheological characteristics of rice dough (Gujral & Rosell, 2004a; Pongjaruvat et al., 2014; Shin et al., 2010). It was found to promote cross-linkage formation between protein fragments present in rice and this was supported by the evidence that with increase in concentration of the enzyme, the concentration of free amine groups was found to decrease (Gujral & Rosell, 2004a; Pongjaruvat et al., 2014). With increasing enzyme concentration, the water binding capacity of dough was also found to enhance resulting in the structural modification of rice proteins. These structural changes and cross-linking of rice proteins improve the visco-elastic properties and handling behavior of dough, thus, making it suitable for formulating gluten-free breads and other similar products (Gujral & Rosell, 2004a; Pongjaruvat et al., 2014; Shin et al., 2010).

Rheological and processing attributes of gluten-free oat dough was found to be enhanced significantly by addition of exogenous proteins and 1% transglutaminase. The thermo-mechanical properties, cooking quality, elasticity, and hardness of noodles made from this dough were found to be enhanced and the cooking losses were lowered. Cross-linking between protein fractions was found to be catalyzed by the transglutaminase resulting in formation of new covalent bonds (Wang et al., 2011). Bread produced from brown rice flour, after treating it with transglutaminase was found to have improved textural properties, better elasticity and enhanced consistency due to polymerization of brown rice protein fractions to form bigger, insoluble complexes. α and β-glutelin subunits were found to be the primary substrates for this polymerization, while the globulin and albumin fragments were barely affected by transglutaminase.

Microbial transglutaminase was found to revamp the dough-handling characteristics of pre-gelatinised cassava starch and sorghum-based batter. Increasing the concentration of enzyme was found to intensify the elastic recovery and zero-shear viscosity of the resulting batter while reducing its resistance to deformation and compliance. Chewiness and firmness of the resulting bread crumb was also found to increase with increase in the concentration of enzyme while unaffecting its cohesiveness and springiness (Onyango et al., 2010). Rheological, biochemical and textural attributes of dough and batters prepared from damaged sorghum or wheat flour were also found to be boosted by incorporation of microbial transglutaminase (Onyango et al., 2010; Renzetti et al., 2008a, 2008b; Rosell, 2009). The enzyme was also proven to promote formation of covalent bonding between lysine and glutamine residues in the gluten-free batter comprising of rice flour, cornflour, xanthan gum and potato starch which resulted in better protein linkage in the resulting batter (Moore et al., 2006).

Although, transglutaminase was found to have enhanced the rheological and textural properties of various gluten-free food formulations, its incorporation into food systems should always be cautious. Studies have proven that the transglutaminase, upon exposure to wheat proteins in the gut, can induce formation of autoantigen of celiac disease (Dieterich et al., 1997; Marsh, 1997).

3.1.2 Glucose Oxidase (EC 1.1.3.4)

Glucose oxidase is a widely used oxidising enzyme in the food industries and is steadily gaining importance in the bakery sector. It produces D-gluconic acid and hydrogen peroxide upon oxidising the β-D-glucose under aerobic conditions. Its ability to oxidise free sulphydryl units present in gluten proteins can be an advantage to tailor the rheological behaviour of wheat dough (Primo-Martin et al., 2003). Glucose oxidase can be used to enhance the rheological behaviour of gluten-free dough formulations by inducing cross-linkage of water-soluble wheat protein fractions, including both disulphide and non-disulphide linkages (Rasiah et al., 2005). Studies have shown that addition of glucose oxidase at a level of 1 unit/g of flour could promote disulphide bridge formation and therefore, reduce the amount of sulphydryl groups by 41.3% (Gujral & Rosell, 2004a).

Glucose oxidase was found to enhance the elasticity of rice flour proteins and modify their structural arrangement by oxidising the free sulphydryl units and forming the disulphide linkages. This functionality was found to rise with increase in level of the enzyme and thus results in formation of stronger dough. Rice flour, with the enhanced elastic behaviour can be further utilised in formulation of gluten-free foods (Gujral & Rosell, 2004b). In a study, Renzetti and Arendt (2009) examined the effect of addition of glucose oxidase in various gluten-free dough formulations. Elastic-like behaviour of sorghum and cornflour were found to increase by enzymatic treatment which was mainly due to polymerization of protein structures. The aggregates formation, as a result of protein polymerization, was found to be favoured by the surplus availability of free sulphydryl groups in these flours (Renzetti & Arendt, 2009).

3.1.3 Cyclodextrin Glycosyltransferase

Cyclodextrin glycosyltransferase is a special enzyme that promotes breakage of 1,4-glycosidic bonds in starch molecules and simultaneously, forming the bond between reducing and non-reducing ends to form cyclic compounds (Ohnishi et al., 1997). It can alter the adhesive properties of various starches by formation of cyclodextrins from their related sugars (Li et al., 2000; Liang et al., 2002). The cyclodextrin molecules, thus formed, can act as molecular container and can entrap hydrophobic fragments within them. Since the rice proteins are hydrophobic in nature, they cannot be used as bread improvers and conditioners; therefore, this enzyme can improve their rheological properties. Due to multiple catalysing nature, cyclodextrin glycosyltransferase could act as a suitable means of modifying the structure of rice proteins making them suitable for formulating gluten-free rice-based bread and other products (Gujral et al., 2003).

Cyclodextrin glycosyltransferase was also found to enhance the baking characteristics and lower the rate of staling of bread (Gujral et al., 2003; Lee et al., 2002). The delayed staling could be attributed to the formation of complex networks between cyclodextrins and proteins and lipids, which decreases the amount of interfacial tensile forces acting due to presence of emulsifiers (Liang et al., 2002; Shimada et al., 1992).

3.1.4 Endopeptidases

The endopeptidases found largely in microorganisms and are used for the enzymatic degradation of gluten peptides to small and lesser immunogenic fragments. The process can also be performed by fungal and bacterial enzymes e.g., Latiglutenase may degrade gluten within the intestinal lumen resulting in non-antigenic peptides (Stepniak et al., 2006; Piper et al., 2004).

3.2 High Pressure Treatment

For the past few years, numerous studies have been conducted to modify the physical structure and the application of high pressure to cereal flours to improve the functional attributes have been done (Bárcenas et al., 2010; Hüttner et al., 2009; Kieffer et al., 2007; Michel & Autio, 2001; Schurer et al., 2007). Most importantly, it tailors the structure of carbohydrates and protein fragments in the food system to induce desirable functionalities (Rastogi et al., 2007). Application of high pressure has been proven to reform the structural and viscoelastic properties of various cereal batters, including, rice, buckwheat, sorghum, teff and oats by changing the structural orientation of protein fractions and gelatinizing the starch present in them (Hüttner et al., 2009; Vallons et al., 2011). It is evident to be a suitable technique to improve the structure of gluten-free batters and doughs (Angioloni & Collar, 2012a, 2012b; Vallons et al., 2011). Various studies have demonstrated utilization of high pressure varying from 100 to 1000 MPa to enhance functionality of proteins and starches in gluten-free foods where it enables the gelatinisation of starch and allows it to swell up without disturbing the integrity of its granular structure (Gomes et al., 1998; Vallons & Arendt, 2009).

High pressure alters the structure of proteins in the same manner as thermal or chemical induced denaturation, although the mechanism of structural alteration varies greatly in this technique. Application of high pressure promotes occurrence of hydrophobic interactions, Van der Walls interactions and hydrogen bonding in the biomolecules which accounts for a greater packing density of the molecules (Knorr et al., 2006). Depending on the amount of pressure applied, this technique can enhance the reactivity of sulphydryl groups and can easily distort the tertiary and quarternary protein structures, however, primary and secondary structure of proteins (α-helices and β-sheet structures) remain unaltered due to their incompressibility (Boonyaratanakornkit et al., 2002; Rivalain et al., 2010). High pressure also promotes polymerisation of protein fragments and can enhance the rheology of batter by improving its elasticity (Renzetti & Arendt, 2009). It acts as a promising technique for development of gluten-free dough using nutritionally rich cereals like sorghum, oats, and millets by improving their machinability and textural attributes (Angioloni & Collar, 2012a, 2012b).

In order to improve consistency of gluten-free batters, formation of a rigid gel is highly favorable. Gelatinization temperature reduces significantly upon application of high pressure to the batters which modifies its rheological attributes (Bauer & Knorr, 2005; Liu et al., 2008; Muhr et al., 1982). High-pressure processing induces formation of creamy texture in barley starch, similar to that of corn, wheat, and tapioca starches, which enhances its rheological properties and help in formulation of gluten-free products using barley flour but having texture similar to that of wheat-based products (Stolt et al., 2000).

High-pressure processing technology have also been found to affect the gluten and gliadin fractions in wheat flour by rearranging the disulphide bonds, thus altering the rheology of dough and batter produced from it (Kieffer et al., 2007). However, it should be noted that these rheological modifications vary greatly with the amount of pressure applied. A low-pressure treatment of gluten at 200 MPa and 30 °C was found to lower the strength of gluten network. An increase in pressure and temperature leads to increase in concentration of insoluble protein fragments which in turn, increases the strength of gluten and its resistance to extend further. However, gluten was found to completely lose its cohesive character upon application of extremely high amount of pressure of 800 MPa at 60 °C (Apichartsrangkoon et al., 1998; Kieffer et al., 2007). Hence, it is very important that high pressure treatment should be applied to enhance the properties of doughs and batters only after proper optimization of processing parameters.

3.3 Sourdough Fermentation Technique

Sourdough is the dough prepared by incorporation of starter culture of lactic acid bacteria and yeasts into flour and water. These starter cultures can either be present in flour as contaminants or may be added intentionally (De Vuyst & Degeest, 1999). This fermentation process is well-known to enhance the flavour, texture, volume, and nutritional attributes of the bread and hinders its spoilage by bacterial or mould infestation (Tafti et al., 2013). In the past few years, this technique has been widely applied for enhancement of the dough-handling attributes of gluten-free batters (De

Vuyst & Degeest, 1999; Houben et al., 2010; Moroni et al., 2010; Schober et al., 2007). A detailed knowledge of microbial interactions happening during the fermentation process is highly required to control their growth and to maintain the uniformity in quality attributes of the gluten-free dough. In this type of fermentation method, the carbohydrate profile of the gluten-free flour is of key importance. For instance, Galle et al. (2010) found that deficiency of maltose in sorghum sourdough, during initial stage of fermentation, was found to hinder the growth of starter strain (*Lactobacillus sanfranciscensis*), while presence of glucose in excessive amount favoured growth of *Weissella* spp.

Sourdough fermentation technique also involves degradation of proteins which alters its viscoelastic characteristics, improves its overall quality and promotes formation of precursors for the flavouring compounds. Various functionalities of sourdough fermentation method are described below:

3.3.1 Gluten Detoxification

An important application of sourdough fermentation method is the exclusion of gluten present in flours by hydrolyzing the toxic metabolic substances. Rizzello et al. (2007) found that gluten (*Triticum aestivum*) concentration could be reduced to less than 10 ppm by using suitable *Lactobacilii* and fungal proteases together. This technique was found appropriate for formulation of pasta for celiac patients using a mixture of pre-fermented durum wheat semolina (*Triticum turgidum L.var. durum*) and buckwheat flour (*Fagopyrum esculentum*). In another study, wheat semi-liquid dough was initially allowed to ferment with selected *Lactobacilii* for a period of 24 h at 37 °C, and was then blended with miller, oat, buckwheat flour and baker's yeast. The resulting dough was then fermented at 37 °C for 2 h and was then baked at 220 °C for 20 min which produced bread tolerated to coeliac patients (Di Cagno et al., 2004).

3.3.2 Formation of Extracellular Exopolysaccharides

Lactic acid bacteria, due to their ability to release extracellular exopolysaccharides, have drawn ample interest of researchers working on development of gluten-free food formulations. These compounds were found to have potential applications as bio-thickeners which can stabilize, emulsify, viscosity and induce gelation of numerous gluten-free foods (De Vuyst & Degeest, 1999; Waldherr & Vogel, 2009). The rheological properties of resulting gluten-free dough were enhanced due to the potency of these polysaccharides to act as replacement for hydrocolloids (Galle et al., 2011, 2012; Katina et al., 2009). Moreover, these extracellular exopolysaccharides were found to promote growth of *Bifidobacteria* in the gut.

3.3.3 Development of Dried Sourdough

Dried sourdough has been used as an appropriate bakery ingredient for over four decades due to consistent quality, lower transportation cost and lesser end-product quality variations in comparison with fresh sourdough (Brandt, 2006). Tafti et al. (2013) produced spray-dried sourdough and determined its physico-chemical and functional attributes. Drying of sourdough was found to drastically decrease the population of lactic acid bacteria. Incorporation of spray-dried sourdough was found to delay the staling process of bread and improve its overall flavour. Although, studies have been performed to formulate and use stable dried sourdough as a crucial ingredient for wheat-based bakery products (Kulp & Lorenz, 2003) its usage for modification of gluten-free formulations still needs an in-depth research in future (Deora et al., 2014).

3.4 Extrusion Technology

Extrusion technology has been proven to improve the functional characteristics like water solubility, rheological attributes, water absorption index, and breaking strength of starch-based food formulations (Choi et al., 2008). Extrusion of rice flour at a moisture level of 20 mL/100 g and barrel temperature of 180 °C was found to modify its functional attributes by gelatinising the starch granules. The resulting extruded rice flour can act as a substitute for gluten in formulating the gluten-free foods. This modified functionality is mainly acknowledged to the formation of ample hydrogen bonds with water which is a result of pre-gelatinisation of starch (Jeong et al., 2011). Clerici et al. (2009) developed gluten-free bread using a mixture of raw rice flour and acidic extruded rice flour which was found to have better textural attributes with improved crust colour. The results advocated for the suitability of acidic extruded rice flour as a novel alternative to gluten for formulating bread for coeliac patients.

The effect of incorporating extruded maize flour in the gluten-free bread formulations with buckwheat flour, rice flour, maize flour and extruded maize flour have been studied (Ozola et al., 2011) and the addition of extruded maize flour was found to impart uniform porosity, higher softness and moisture content to the gluten-free bread. Although, extrusion technology can be greatly used as a cost-efficient and novel method of developing gluten-free bakery products, its application on utilization of other cereals is still a major area of research in near future.

Extrusion technology can also be used to fasten the process of liquefaction. Extrusion-enzyme liquefaction method is based on the principle that extrusion degrades and gelatinises the starch in a thermo-mechanical manner which makes it more prone to be affected by the enzymatic attack. This technique can also be applied to concentrate protein fragments (de Mesa-Stonestreet et al., 2012) which can be further used as replacement for gluten in gluten-free food formulations due to the deficiency of proteins. Proteins from sorghum flour have been successfully concentrated by this method which were further utilized to enhance viscoelastic nature of the gluten-free dough (de Mesa et al., 2009). Liquefaction of starch becomes easy and fast due to extrusion and the sorghum protein concentrate, thus obtained, has better digestibility and desirable functional attributes for its potential usage in food formulations and beverage industries (de Mesa-Stonestreet et al., 2012).

3.5 Genetic Modification

One of the methods to reduce the toxicity of gluten is genetic modification. Wheat has a hexaploid genome AABBDD, wherein chromosomes 1 and 6 predominantly harbor the genes known to code for immunotoxic components of gluten (Marino et al., 1996). Genetic modification of wheat has been done to attenuate immunotoxicity effect which however, may also alter the gastronomic properties of wheat, the yield etc., if these properties are governed by the same or neighboring genetic loci. A study explored a variant formed by the removal of genes on chromosome 1 that code for β, γ, and ω gliadin fractions. While the toxicity was attenuated, the mechanical properties of wheat were not altered. However, when α fraction was attenuated instead, the mechanical properties were compromised while also significantly reducing the dose of immunogenic T cell epitopes (van den Broeck et al., 2009). The International Wheat Genome Sequencing Consortium delivered a high-quality annotated reference genome sequences of the Chinese spring wheat. This has the potential to fast track development of genetically engineered wheat with attenuated immunotoxicity while preserving its gastronomic or agronomic properties.

3.6 Microwave Treatment

Microwave treatment has been used to detoxify wheat gluten proteins (Bevilacqua et al., 2016). The microwave energy is applied prior to milling, for a few seconds to cleaned, hydrated wheat kernels at 15–18% humidity, to reach a high temperature within a short period of time. The process is repeated over several cycles until a temperature of 80–90 °C and moisture of 13–13.5% in the grains is reached. After this, grains are dried over 24 h at room temperature and milled. This process had been proposed to attenuate the immunotoxicity of gluten by 99%, as detected by the R5 monoclonal antibody method, which is a method of detection of gluten immunogenic peptides (Lamacchia et al., 2016). The bread from this flour was called "gluten friendly or GLUFR." However, a later study found the immunotoxicity of this flour to be unchanged, when checked by the G12 method (another antibody-based gluten immunogenic detection test), mass spectrometry-based proteomics and in vitro assay with T cells of celiac subjects (Gianfrani et al., 2017). The microwave treatment causes reconfiguration of the gluten structure that interferes with detection of gluten immunogenic peptides by R5 ELISA method.

3.7 Immune Modulation

The immune modulation may restore gluten tolerance and for this a vaccine may induce immune tolerance to some of the gluten immunogenic peptides. The celiac disease can also be treated by the use of nanoparticle-based therapeutic agents that reverse gluten sensitivity and stimulate immune tolerance by delivering encapsulated gliadin to tolerogenic immune cells (Akbari et al., 2006). Tolerogenic therapies using vaccines have been developed to hypo-sensitize the adaptive immunity and is a potential therapeutic approach to allergic and autoimmune diseases. In a departure from their traditional use of immunization, vaccines are now being tested for desensitization. Examples in the case of Ced, the peptide-based vaccine called NexVax2. It was developed by a US based company, ImmunoSanT, Inc. NexVax2 is composed of three proprietary, immunodominant gliadin peptides named NPL001, NPL002, and NPL003 each of which is 15–16 amino acid long. The vaccine target is the HLADQ2.5-epitope-TCR complex linking the antigen presenting cell to the gluten-reactive CD4+ T cells. It engages specific immune cells and a signature pathway has been discovered based on that. In animal studies in HLADQ2.5 transgenic mice having gluten-sensitive T cells, it was found to be efficacious (Anderson & Jabri, 2013).

The use of oral agents that acts locally in the gut is another way of inducing immune tolerogenesis. The Lactobacillus lactis, genetically engineered to release modified, non-toxic gliadin was administered orally to secrete a deamidated DQ8 gliadin epitope in the intestinal lumen of transgenic NOD-2 mice with ABoDQ8 haplotype. This induced suppression of the lamina propria and systemic DQ8-restricted T-cell responses, downregulation of IL-12 secretion, systemic production of IL-10 and TGF-β and induction of Foxp3+ Tregs in the lamina propria. These findings suggest development of mucosal tolerance to the gliadin (Huibregtse et al., 2009). Similarly a study used Bacillus subtilis spores as a long-lived, protease-resistant adjuvant system for administering gliadin peptides to HLA-DQ8-transgenic mice. The spore-adsorbed gliadin activated the dendritic cells and elicited a T-cell response in the gut. This mechanism (Bonavita et al., 2015) can be utilized for developing immune tolerance.

3.8 Probiotics

Probiotics play a significant part in the intestinal microbial imbalances of individuals with celiac disease due to increased *Bacteroides* spp. and decreased *Bifidobacterium* spp. irrespective of gluten-free diet. The patients suffer from persistent gastrointestinal symptoms due to gut microbiota composition but certain strains of probiotics may act on gluten immunogenicity, assist with intestinal healing, and improve patients' symptoms (Fasano, 2009). Studies have found a reduction in the relative proportion of *Firmicutes, Proteobacteria, Bifidobacterium* and a relative increase in *Bacteroides* and *E. coli* in celiac disease patients compared to controls. Oligofructose-enriched inulin a prebiotic, increased the *Bifidobacterium* count in the gut significantly, with no side effects (Drabinska et al., 2018). These findings point to a possible causative role of gut dysbiosis in celiac disease, although the exact mechanism remains obscure. Many studies have suggested low use of short chain polysaccharides like fructans, lactose, mannitol, sorbitol etc. which are hard to digest, resulting in fermentation in the bowel and flatulence, and are implicated in causing some of the symptoms of Irritable Bowel Syndrome (IBS) (Magge & Lembo, 2012).

4 Conclusions

The gluten replacement with different ingredients and processing techniques in various food products has been done but the feature of these foods is not equal to gluten foods. In addition, the low accessibility, high cost and frequently critical sensory and textural parameters of food products from foods free from gluten add as a burden to celiac individuals. The gluten is removed from the food products by different mechanisms either by using enzymes like peptidases or microbial transglutaminase, microwave treatments or genetic engineering. The superior quality of foods free from gluten can thus be developed with these products (wheat, barley and rye) after removal or degradation of gluten.

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Quality Tests for Evaluating Gluten-Free Dough and Bread

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1 Gluten-Free Products and Quality Parameters

Gluten is a protein fraction obtained from wheat, oats, barley or their cross-breed varieties. Its consumption leads to allergy and coeliac diseases in the people suffering from gluten intolerance. Coeliac disorder is an autoimmune disorder which arises due to the ingestion of gluten from wheat, barley and oats. Gluten is the structure building complex in the wheat based dough. Glutenin and gliadin are the two main proteins that help in the formation of gluten complex which provides the visco-elastic nature to the dough. Unfortunately gluten consumption leads to certain allergic responses (coeliac disorder) among the people with gluten sensitivity. Hence, gluten-free products such as breads, biscuits, cookies etc are gaining global attention in the present day. Since gluten is the chief structure building component of wheat flour, it imparts a desirable texture and organoleptic properties to the bakery products. Development of gluten-free breads with standard attributes is, therefore, a real challenge for the food processors. The quality of gluten-free products can be determined using various tests that hold a considerable significance in determining their overall market acceptability. These include chemical composition, colour, texture, rheology, pasting, gelatinization and other physico-chemical properties.

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2 Quality Tests for Gluten-Free Dough and Breads

2.1 Chemical Composition

2.1.1 Moisture Content

To determine moisture content, 2–3 g of sample (bread/dough) are accurately weighed and transferred to the pre-weighed moisture dish. The sample is kept in a hot air oven at 130 °C for 1 h. It is then cooled at room temperature and weight is noted and the procedure may be repeated at least three times to overcome the chances of error (AOAC, 1990). The moisture content is calculated using the equation given below:

$$\text{Moisture content on dry weight basis}\left(\%\right) = \frac{A - B}{A} \times 100$$

A: Weight of sample before drying (dry weight basis)
B: Weight of sample after drying (dry weight basis)

Significance Moisture content has an important role in the storage life of grain/flour. It also influences the microbial growth in the food products. The average moisture content of bread crumb is 35% to 45%. Moisture content of dough and bread affect its textural and rheological properties. Lower moisture content of the gluten-free bread increases the crumb firmness and firming rate (Giannone et al., 2016). The reduction in the moisture content of gluten-free bread during the storage period increases its staling. Hence, moisture content determines the overall freshness of the gluten-free bread. The weight loss of gluten-free bread during the storage period can be accredited to loss of moisture content. The moisture rapidly transfers from crumb to crust in the gluten-free bread as compared with gluten containing bread. Higher moisture transfer in the gluten-free bread increases its staling rate as compared with gluten containing bread.

2.1.2 Water Activity (a_w)

Water activity is the ratio of vapour pressure of the food to the vapour pressure of pure water under similar and undisturbed conditions. It increases with the increase in temperature. Water activity is determined by equilibrating the water present in the material and vapour phase of water in the head space, it measures the relative humidity of the head space. Water activity meter consists of a fan, dew point sensor, temperature sensor and an infrared thermometer. Dew point sensor measures the dew point of air in the chamber and infrared thermometer measures the temperature of the sample. Then, the relative humidity of head space is measured as the ratio of saturated vapour pressure at dew point temperature to saturated vapour pressure at the sample temperature. The humidity of head space provides the water activity of the sample when the water activity of the sample and relative humidity of air are reached in equilibrium stage (Devices, 2014). Water activity of the dough and bread samples can be analyzed after calibrating the water activity meter using NaCl, LiCl, KCl or distilled water. When the calibration is over, the sample drawer knob of the water activity meter is turned to open or load position and pulled the drawer to open. The sample is kept in the drawer and carefully slided into the meter, turned the sample knob to read position to seal the sample cup with the chamber and screen will show the measurement has started. The length of measurement depends on the temperature difference between the sample and chamber.

Significance Water activity influences the shelf life of food products. Higher water activity of the product favours mold, yeast and bacterial growth, resulting in the lower shelf life. Water activity of dough influences the functional properties of gluten-free breads. Optimum water activity facilitate the proper mixing of ingredients and improves the uniformity of dough (Lassen & Skinhoj, 2004).

2.1.3 Carbohydrate Content

Anthrone Test

Anthrone test is a rapid and convenient method for the estimation of hexoses and aldopentoses as free form or present in the polysaccharides. Carbohydrates are hydrolysed to simple sugars under the acidic condition (HCl) and glucose units are further dehydrated to hydroxyl methyl furfural, resulting in the formation of blue-green solution which have maximum absorbance at 630 nm.

In order to determine the carbohydrate content, 100 mg of sample was transferred into a conical flask. To it was added 5 ml of 2.5 N HCl and the conical flask was placed in a water bath at 100 °C for 3 h. The sample was cooled to room temperature and neutralised with Na_2CO_3. This was followed by the addition of distilled water to the contents to make a final volume of 100 ml. The contents were centrifuged at 5000 g for 15 min and supernatant was collected. Approximately 0.1 ml of the collected supernatant was used for analysis. The working standard solution was prepared using glucose solution (0.1 mg/ml concentration) and added to each test tubes at different concentrations such as 0 (blank solution), 0.2, 0.4, 0.6, 0.8 and 1 ml. The distilled water was added to each test tube including sample supernatant and made up to 1 ml, subsequently added 4 ml of anthrone reagent and made up to 5 ml. All test tubes were kept in a boiling water bath for 8 min and cooled rapidly. Then, took the absorbance at 630 nm. The absorbance of standard solution was used to calculate concentration value of 1 O.D (optical density) and the amount of carbohydrate present in the sample was calculated by multiplying the absorbance of sample with concentration value of 1 O.D (Agrawal et al., 2011).

Phenol-sulphuric Acid Method

It is one of the most commonly used methods for the estimation of carbohydrate. In this method, hydrolysed carbohydrates are dehydrated to furfural complex in the presence of sulphuric acid (H_2SO_4). It reacts with phenol and forms coloured complex that shows maximum absorbance at 490 nm (Albalasmeh et al., 2013). A 2 ml aliquot of carbohydrate solution was transferred into test tubes containing 1 ml of 5% aqueous solution of phenol. Then rapidly added 5% concentrated sulphuric acid into the mixture and allowed to stand for 10 min. Subsequently, the test tube was vortexed for 30s and kept in a water bath at room temperature for 20 min for colour development. Then, the absorbance was taken using spectrophotometer at 490 nm. Perform the blank test with double distilled water in place of 2 ml aliquot of carbohydrate solution (Albalasmeh et al., 2013).

Sulphuric Acid- UV Method

Aqueous solution of furfural complex shows maximum UV light absorption at 277 nm. Glucose and cellulose absorbs UV light at 323 nm after sulphuric acid hydrolysis. The sulphuric acid present in the solution leads to bathochromic shift from 277 to 323 nm. Moreover, it increases with increase in the concentration of sulphuric acid. The advantages of this method as compared with above methods are more accuracy, less time consumption and direct correlation between light absorbance and total carbon content of the aqueous solution (Albalasmeh et al., 2013).

A 1 ml aliquot of carbohydrate solution was transferred into test tubes containing 3 ml of concentrated sulphuric acid and vortexed for 30s. The addition of sulphuric acid rapidly increased the temperature of the mixture with in 10–15 s. Subsequently cool the mixture by keeping in ice bath for 2 min and bring to room temperature. Then, the absorbance was taken using UV- spectrophotometer at 315 nm. Perform the blank test with double distilled water in place of 2 ml aliquot of carbohydrate solution (Albalasmeh et al., 2013).

Resonance Light Scattering Method

When the wavelength of Rayleigh scattering is equal or nearer to molecular absorption band it produce resonance light scattering, which is a special elastic scattering. Here, frequency of both scattered and absorbed light by the electrons is equal. The absorbed electrons shows the rescattering of light. The intensity of light scattering spectra depends upon the concentration of carbohydrates that bind with dye molecules (Zhang et al., 2008). In this method sodium alginate is used as standard for carbohydrate. Sodium alginate is a polysaccharide with acidic property, it shows negative charge at pH 4. It has a high binding property with the dye used to increase light scattering intensity of the solution.

The stock solution of 1000 mg/l was made by dissolving sodium alginate in water, and stored at 4 °C. The working solution with different concentration can be made by diluting stock solution in double distilled water. Neutral red is used as resonance light scattering probe for carbohydrate determination, it is dissolved in water to prepare the stock solution of 1×10^{-4} mol/l. The acidity was controlled using Britton–Robinson buffers (Zhang et al., 2008).

A 2 ml of sample solution and 3 ml of Britton–Robinson buffers solution were transferred into a test tube and rapidly mixed. Added 1 ml of neutral red solution into the test tube and mixed well. Subsequently, kept the test tube at room temperature for a pre-determined time before the analysis. The resonance light scattering spectrum of the sample solution is obtained by scanning the emission and excitation monochromators from 200–650 nm. The scanning speed was fixed to 500 nm/min and excitation and emission slits was set to 5 nm. The difference in resonance light scattering intensity between sample (i) and blank intensity (i_0) is referred to as $\Delta i = i - i_0$. The enhanced resonance light scattering intensity (I) under the optimum conditions of neutral red-carbohydrate complex at maximum scattering wavelength (350 nm) was measured, it can be used for the determination of carbohydrate content in the sample (Zhang et al., 2008).

Significance Carbohydrate has a vital role in the development of gluten-free breads. The simple sugar present in the dough undergoes fermentation by yeast to produce CO_2 and alcohol. The CO_2 produced during the fermentation provides desirable volume to the gluten-free breads. The damaged starch improves the water holding capacity of the dough. It also undergoes enzymatic degradation and gets converted to fermentable sugar. The starch content of the dough affects the texture, imparting more softness and improves the specific volume of the bread crumbs. Carbohydrate has a considerable role in the staling of breads also.

2.1.4 Protein Content

Digestion, distillation and chemical titration are the principal stages in the Kjeldahl's method of protein estimation. It involves heating of the protein sample with concentrated sulphuric acid for several hours (digestion), followed by distillation after cooling. During distillation, the ammonia gas evolved is collected by the acid, and subsequently titrated with standard acid.

To determine protein content, 0.2 to 1 g of sample is accurately weighed and transferred to digestion tube. To it is added 20 ml of sulphuric acid and a tablet containing 0.48 g of mercury oxide (catalyst) and 4.52 g of potassium sulphate. At the same time, the blank solution (without sample) containing these reagents was processed. The tubes are kept in the digestion block (preheated at 420 °C) for 30 min to 2 h. After digestion, the tubes are cooled to room temperature and added 30 ml of distilled water. Subsequently, the tubes are placed in the distillation–titration block. To it is automatically added 20 ml of sodium hydroxide solution and distilled for 6 min. The releasing ammonia was collected by 30 ml of receiving solution (1 g of boric acid, 10 ml of bromocresol green solution at 0.1% in methanol, 7 ml of methyl red solution at 0.1% in methanol, 0.5 ml of 4% sodium hydroxide, in 1000 ml of doubly distilled water), automatically titrated against standard hydrochloric acid (0.5 M) and colorimetric end point was detected (Marcó et al., 2002).

$$\text{Protein content} = \text{Nitrogen}\left(\text{N}\right) \times 6.25$$

The conversion factor used to estimate protein content of a given non-gluten sample is taken as '6.25' keeping in view the

$$16\%, \left(\frac{1}{0.16} = 6.25\right)$$

Significance Protein content has a considerable role in the water absorption capacity and strength of doughs. It also affects the flavour, texture, storage stability and nutritional value of finished product. Hence, protein has considerable role in the sensory characteristics and sensory acceptance of gluten-free bread. Higher protein content leads to the chewy texture of gluten containing breads. On contrary, lower protein content is desirable for the crisp and tender products such as snacks and cakes (Lassen & Skinhoj, 2004). Gluten-free cereals, pseudo cereals, legume flour and protein isolate from animal, plant and microbial origins are commonly used as protein sources in the gluten-free breads. The addition of non-gluten proteins increased elastic modulus by cross-linking and improved the rate of maillard reaction. It assists the development of flavour and colour in the gluten-free bread. Higher gelation of proteins also improved the foaming and structure of the gluten-free bread (Crockett et al., 2015). Proteins act as an antistaling agent in the gluten-free breads. It can be attributed to changes in water binding capacity and starch retrogradation (Ziobro & Witczak, 2013).

2.1.5 Ash Content

A porcelain ashing dish is ignited and cooled in a desiccator and weighed. Sample (2–5 g) is taken in a pre-weighed ashing dish and ignited at 550 ± 10 °C for 6 h. It is then cooled in air tight desiccators (having reignited CaO) and weighed as soon as it reaches the room temperature (AOAC, 1990). Ash content was calculated as:

$$\text{Ash }\left(\%\right) = \left(\text{Weight of the ash }\left(\text{g}\right) / \text{ weight of the ample }\left(\text{g}\right)\right) \times 100$$

Significance Ash content influences the quality of gluten-free breads. During heating at higher temperature (>550 °C) organic compounds such as starch, proteins, and lipids burn down leaving behind the ash. Ash content depends upon the presence of bran in the flour. The darker color of gluten-free breads is generally due to the higher ash content of flour. Some minerals like phosphorus also improve the pasting properties of dough (Lassen & Skinhoj, 2004).

2.2 Color Properties

Color values of crust and crumb of gluten-free breads can be determined using Hunter Lab digital colorimeter (Fig. 1). L^*, a^*, b^*, hue and chroma values are the color parameters. The L^* value indicates the lightness of a food sample. The value of L^* ranges from 0 (black) to 100 (white). The value of a^* indicates redness (+ve value) or greenness (−ve value) and b^* value indicates yellowness (+ve value) or blueness (−ve value). Hunter Lab colorimeter is standardized prior to measurement using white and black tiles (Sudheesh et al., 2019a). Following this, L^*, a^*, b^* values of food samples are measured. Hue and chroma are latter calculated using the equations given below:

$$\text{Hueangle} = \tan^{-1}\left(\frac{b}{a}\right)$$

$$\text{Chroma} = \left(a^2 + b^2\right)^{1/2}$$

Where, a^* represents redness or greenness and b^* value represents yellowness or blueness.

Hue value signifies the degree of discoloration and chroma value gives the intensity of color in different food sample.

Significance Color values of crumb and crust directly influence the consumer acceptance of the finished breads. Higher ash content of the flour is generally associated with the darker color (lower L^* value) of breads (Lassen & Skinhoj, 2004).

Fig. 1 Hunter lab digital colorimeter (Courtesy: Department of food science and technology, Pondicherry university

2.3 Texture Properties

Texture of bread samples can be examined using a Texture analyser (Fig. 2). For breads it is equipped with a load cell and PTFE (Polytetrafluroethylene) compression platens. The bread samples are sized to dimensions of 20 × 20 × 20 mm which generally means a bite sized piece and compressed using the 50% strain using a constant load rate at 0.1 mm/s with pre-test speed 1 mm/s; test speed 5 mm/s; post test speed 5 mm/s (Pongjaruvat et al., 2014). Textural parameters like hardness (N), adhesiveness (Ns), cohesiveness, springiness (mm), fractuarability (N), chewiness (N), gumminess (N) and stringiness (mm) are generally noted (Fig. 2b).

Significance Textural properties have important role in the quality of the gluten-free breads. These properties depend upon the protein and lipid content of breads and additives used such as emulsifying and stabilizing agent. The retrogradation properties of starch also have considerable role in the textural properties.

2.3.1 Hardness

Sensorial Definition Hardness is the force required for the compression of food between the molars.

Instrumental Definition It is the peak force of first compression cycle (Fig. 2b). First compression cycle is the phase in which food undergoes early deformation by the force. It is expressed in Newton (N). Higher retrogradation of starch leads to staling of breads which results in the higher hardness of bread crumbs.

Hardness of gluten-free bread crumbs depends upon their particle density and specific volume. Hardness is inversely correlated with particle density, specific volume and porosity of breads (Pongjaruvat et al., 2014). The additives like α- amylase-lipase enzyme formulation (Giannone et al., 2016), modified starch (Ziobro et al., 2012) and maltodextrins with higher DE value (Witczak et al., 2010) have been successfully used to reduce the hardness of gluten- free breads.

2.3.2 Adhesiveness

Sensorial Definition It is the work required to overcome the attractive forces between the food material and the other surfaces with which the food comes in contact such as tongue, teeth and palate of the oral cavity.

Instrumental Definition Adhesiveness is the force required to pull back the probe from the material placed in the texture profile analyzer. It may also be defined as the negative area for the first compression cycle (Fig. 2b).

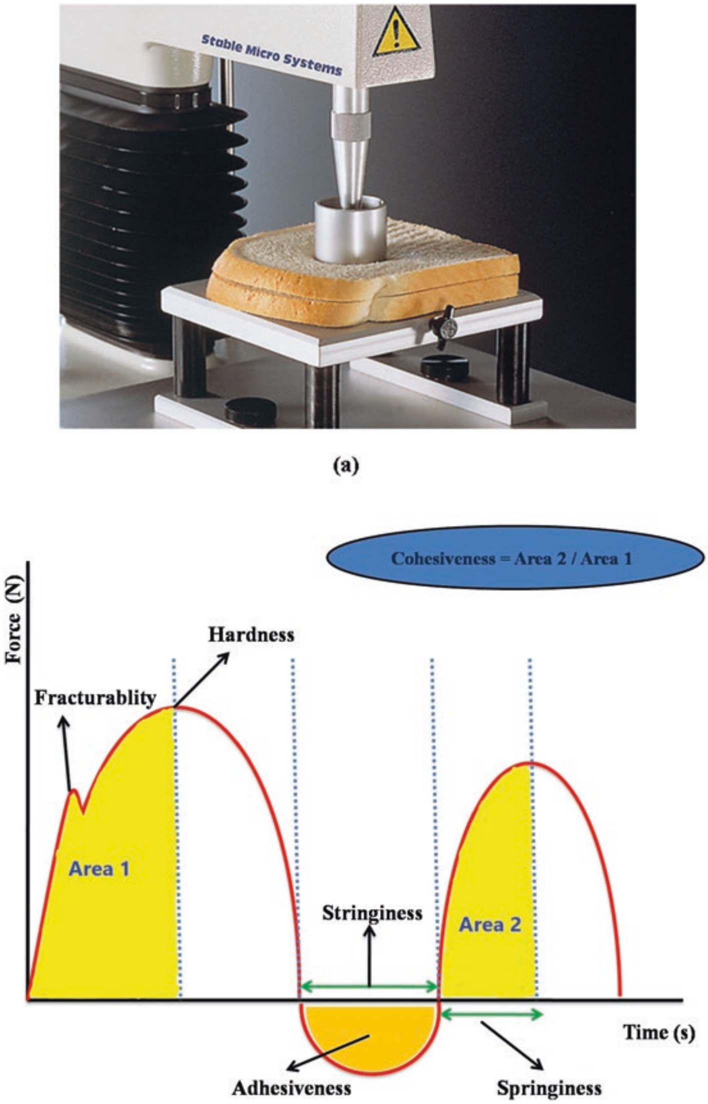


Fig. 2 (**a**) Texture profile analyser (TPA). (Courtesy: www.stable microsystom.com) (**b**) TPA curve

2.3.3 Cohesiveness

Sensorial Definition It is the strength of the internal bonds of the food product. Higher value of deformation of food product prior to breaking indicates the higher cohesiveness.

Instrumental Definition It is the ratio of positive force during the second compression curve to the first compression curve. Fried et al. (1963) defined cohesiveness is the ratio of energies expanded in the first and second compression cycles (Fig. 2b).

Higher cohesiveness of gluten-free breads reduces the separation from hand and break down in the mouth (Miñarro et al., 2012).

2.3.4 Springiness

Sensorial Definition Springiness is the rate at which a deformed food recovers back to its undeformed form, after the deforming force is removed.

Instrumental Definition It is the height that a deformed food product retains during the time elapsed between the end of the first compression cycle and start of second compression cycle (Fig. 2b).

2.3.5 Stringiness

Sensorial Definition When a food strongly adheres on the surface, it will stretch when it is pulled out (Trinh & Glasgow, 2012).

Instrumental Definition Stringiness is the distance upto which a food product is extended during decompression phase before separating from the probe (Fig. 2b).

2.3.6 Fractuarability (Brittleness)

Sensorial Definition It is an attribute related with the primary textural properties such as hardness and cohesiveness. Fracturablity is commonly defined as the ease with which a food material fractures, that is, when the first significant break appears in a fragile food.

Instrumental Definition It is the first significant break in food commodity during the first compression cycle (Fig. 2b).

2.3.7 Gumminess

Sensorial Definition Gumminess is a characteristic of the foods that have low hardness and high cohesiveness. It may be defined as the energy required to disintegrate a semi-solid food so that it is ready to be swallowed.

Instrumental Definition It is the product of hardness and cohesiveness.

$$\text{Gumminess} = \text{Hardness} \times \text{cohesiveness}$$

2.3.8 Chewiness

Sensorial Definition It is the energy required to masticate a solid food to a state such that, it is ready to be swallowed.

Instrumental Definition It is the product of hardness, cohesiveness and springiness of a food. In other terms we can also estimate the chewiness as a product of gumminess and springiness of a product.

$$\text{Chewiness} = \text{Hardness} \times \text{cohesiveness} \times \text{springiness}$$

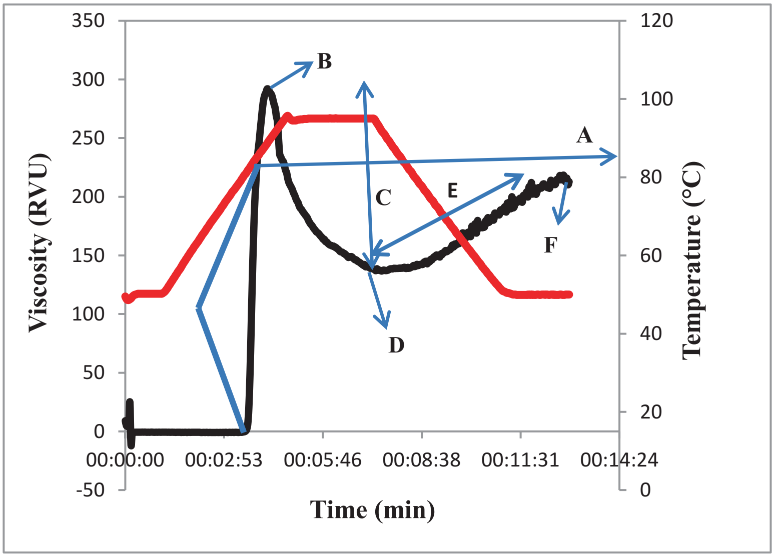
Moisture content and water activity inversely affects the chewiness of the gluten-free breads. Water has a plasticizing effect in the gluten-free breads. The lower water content increased the formation of hydrogen bonds among the starch chains and, between starch and proteins, it improved the chewiness of the gluten-free bread (Giannone et al., 2016).

2.4 Pasting Properties

Rapid Visco-Analyzer (Fig. 3a) is used to analyze the pasting properties of flour/dough. Near about 3 g of sample (flour/dough) are weighed in a canister. An aqueous dispersion of sample at 14% moisture basis is prepared by adding a specific amount of deionised water which depends upon the initial moisture content of the sample. The sample is generally equilibrated at 50 °C for 1 min, heated to 95 °C (at the rate of 12.2 °C/min) for 2.5 min and cooled back to 50 °C (at the rate of 11.8 °C/min) during the heating-cooling cycles of Rapid Visco analyser. The latter temperature is maintained for 2 min. A constant paddle rotational speed of 160 rpm is maintained by the rotor of device throughout the analysis, except for rapid stirring of 960 rpm for the first 10 s to disperse the sample (Sudheesh et al., 2019b, 2019c, 2019d). Properties such as pasting temperature, peak viscosity, breakdown viscosity, trough viscosity, setback viscosity and final viscosity are noted (Fig. 3b). The viscosity parameters can be expressed in RVU (Rapid Visco Unit) or cP (centi Poise) unit (1RVU = 12 cP).

Significance Pasting properties of dough have a significant role in determining the quality of gluten-free breads. The starchy flour suspension is converted to a viscous paste during heating. It acts as an excellent thickening agent and viscosifier within the dough and modifies the structure of gluten-free breads. Gluten-free doughs chiefly contain starch, water and yeast. One of the challenging attempts during the preparation of baked products is to retain the gas cells within the dough and settling

(a)



(b)

Fig. 3 (**a**) Rapid visco analyser (RVA) for pasting analysis. (Courtesy: Department of food science and technology, Pondicherry university (**b**) Pasting graph (A) Pasting temperature (B) peak viscosity (C) break down viscosity (D) trough viscosity (E) set back viscosity (F) final viscosity of yeast cells and ungelatinized starch granules, it poorly affects the development of gluten-free breads (Witczak et al., 2016). Yeast cells carry out the fermentation of dough to produce CO_2 and alcohol. CO_2 improves the volume of breads while the viscous starch paste formed during the gelatinization assists to retain the CO_2 within the dough (Witczak et al., 2016). It also prevents the settling of yeast cells and ungelatinized starch granules. In this way, the pasting properties of starch have a considerable role in the development of crumb structure of the gluten-free breads.

2.4.1 Pasting Temperature

The minimum temperature required to cook the starch suspension is expressed as pasting temperature. It is generally calculated in degree celcius (°C). Pasting temperature depends upon the strength of intermolecular forces and number of cross-links present in the starch granules (Sudheesh et al., 2020b). Starch granules with more number of cross-links and strong intermolecular forces have higher pasting temperature.

2.4.2 Peak Viscosity

Peak viscosity is the viscosity of starch at the point of equilibrium between the swelling of starch granules and polymer leaching. Higher peak viscosity improves the thickening ability of baked products at higher temperature. The factors affecting the peak viscosity are the degree of swelling of starch granules, amylose leaching, friction between the swollen granules, competition of leached amylose chains with ungelatinized granules for water molecules, amylose–lipid complexes and their crystalline and morphological properties (Sudheesh et al., 2019d).

2.4.3 Trough Viscosity

Trough viscosity is the value of decreased viscosity of starch paste due to breakdown of granules at higher temperature during RVA. It depends on the different susceptibility of starch granules during the shearing and heating (Shafie et al., 2016).

2.4.4 Break Down Viscosity

Break down viscosity is the value calculated from the difference of peak and trough viscosity. It indicates the thermal and shear force stability of starch granules. Higher break down viscosity indicates the lower stability of starch granules towards the heat and shear force which means that the dough with lower break down viscosity will exhibit a higher stability towards thermal and shear force (Sudheesh et al., 2020c).

2.4.5 Setback Viscosity

The difference between final and trough viscosity is expressed as setback viscosity. Setback viscosity is an indicative of the retrogradation and gelation properties of starch paste. Higher setback viscosity of dough can be attributed to higher staling effect of gluten-free breads which may lead to a harder crumb.

2.4.6 Final Viscosity

Final viscosity is the maximum viscosity of starch paste at the lowest temperature during the rapid visco analysis. It also indicates the retrogradation properties of starch paste (Sudheesh et al., 2019b, 2019c, 2019d).

Final and setback viscosities are mainly due to polymerization of leached out amylose and long linear amylopectin. Both viscosities signify the stability of the gluten-free breads at lower storage temperature. Higher setback and final viscosities indicates the higher retrogradation property of the gluten-free breads. Hence, dough with lower setback and final viscosities can be preferred for the development of gluten-free breads due to their anticipated softer crumb structure.

2.5 Rheological Properties

The rheological properties of dough can be examined using dynamic oscillatory measurement (Fig. 4). Dynamic oscillatory measurement requires only small sample size and helps to analyse at different frequency range without reloading the sample. It requires minimum time as compared with steady shear measurements

Fig. 4 Rheometer. (Courtesy; Anton Paar GmbH, www.anton-par.com)

and is feasible on both solid and liquid samples (Bafna, 1996). Analysis is done using a parallel plate system. The parallel plates with an average diameter of 25 mm and a clearance of 2 mm are used which consist of corrugated probe to prevent the slipping of dough. The temperature is adjusted to 35 °C using circulating water bath and controlled peltier system. The sample is loaded on the lower stationary plate and excess dough sample is trimmed off. The edge of the exposed sample is coated by a parafilm to avoid moisture loss during the measurement. The dough is kept undisturbed for 30 min before starting the experiment to equilibrate the stress. Finally the following tests are performed;

(1) Strain sweep test: It is a type of test in which the strain is varied from 0.01% to 100% at a constant frequency of 1 Hz.
(2) Frequency sweep test: It is a type of test in which the frequency is varied from 1 to 20 Hz at a constant strain of 0.05%.
(3) Temperature sweep test: It is a type of test in which the temperature is varied from 30–90 °C at a constant strain and frequency of 0.05% and 1 Hz, respectively at 4 °C/min (Mariotti et al., 2009). The storage modulus (G′) and loss modulus (G″) are recorded while the loss factor (Tan δ) is calculated from the ratio of loss modulus to storage modulus.

Significance Rheological analysis is mainly associated to the deformation of dough. It gives the information about the visco-elastic properties of the dough that have an important role in textural characters of the gluten-free breads. Rheological properties depend upon the factors such as addition of water, interaction between the components, enzymatic reaction and relaxation of stress induced during the mixing. Strain sweep test indicates the evaluation of visco-elastic region of dough. The properties of dough do not depend upon the magnitude of stress, strain or the rate of application of strain when the material is tested in the linear range. Frequency sweep curve indicates how the elastic and viscous property of the dough changes with the rate of application of stress or strain at constant amplitude. Temperature sweep test is identical to the first stages of amylographic test. It stands for physical and biochemical reaction taking place during the baking operation (Mariotti et al., 2009).

2.5.1 Storage Modulus (G′)

Storage modulus is the energy stored by the material per each deformation cycle. It indicates the elastic properties or solid like behaviour of dough. Higher G′ value represents higher elastic properties of the dough sample.

2.5.2 Loss Modulus (G′)

Loss modulus is the energy lost from the material as viscous dissipation in each deformation cycle. Loss modulus indicates the viscous nature or liquid like behaviour of the sample. Dough with higher loss modulus has a higher viscous nature (Sudheesh et al., 2020a).

2.5.3 Loss Factor (Tan δ)

Loss factor is the ratio of loss modulus to storage modulus. If the value of loss factor is greater than unity (Tan $\delta > 1$), it represents the viscous nature of the material (liquid like properties). On the other hand, a lower value of loss factor (Tan $\delta < 1$) indicates the elastic properties of the material, that is, the dominating solid like properties (Sudheesh et al., 2019c, 2019d).

The strength and ability of dough to expand and hold CO_2 and the extend of gelatinized starch determine the volume of gluten-free breads. Higher G′ and G″, and lower Tan δ values represent elastic behaviour of dough. The hardness of the dough can be attributed to the presence gluten proteins. Gluten-free dough generally shows a dominant liquid like behaviour as compared to the gluten containing dough. As a result, gluten-free dough has comparatively poor elastic behaviour. Both gluten containing and gluten-free doughs, however, exhibit the higher values of G′ than G″ which indicates a dominant elastic behaviour over the viscous behaviour (Magana-Barajas et al., 2012; Tsatsaragkou et al., 2014).

2.6 Stress Relaxation Test

Stress relaxation test is carried out using rheometer on parallel plate geometry in shear stress mode. In a way, similar to the rheological analysis, the average diameter of the plate used for this analysis is 30 mm and the clearance between the two plates is 2 mm. Dough sample is placed over the lower stationary plate of rheometer. The excess sample is trimmed off using razor blade and dough edges are coated with oil or parafilm to prevent dehydration. The sample is allowed to rest for 20 min prior to starting the test. Stress relaxation is determined at the interval of 0.1 s by applying optimum shear strain for 30 min at a constant temperature of 25 °C. Maximum stress at 15% shear strain and relaxation time (τ) are the two major parameters obtained from this test (Magana-Barajas et al., 2012). The stress relaxation curve is plotted between relaxation modulus (stress/strain) and the test time (s).

Significance Gluten containing and gluten-free doughs show different relaxation behaviour. Generally, gluten containing dough has a higher relaxation modulus and relaxation intensity (H_τ) than gluten-free dough (Li et al., 2003). The relaxation intensity can be calculated from the equation given below;

$$H_\tau = -\left(dG\left(t\right)/d\ln t\right)/t = \tau$$

Where, τ is the relaxation time. Relaxation time is associated with the process of flow occurring when dough is relaxed. It is the time required for the force to fall 1/e times, or by 36.8% of its original value (Magana-Barajas et al., 2012). Relaxation time is an important parameter in the expansion of gluten-free dough and less for strong dough as compared with moderately strong dough. Higher relaxation time of gluten-free dough indicates its more rigid and poor elastic property (Wu et al., 2012).

2.7 Gelatinization Properties

Gelatinization properties of samples are examined using differential scanning calorimetry (DSC) (Fig. 5a). A suspension is made by mixing the sample with water (in the ratio of 1:3) in an aluminium pan. The pan is hermetically sealed and held for 3 h to facilitate equilibration process so that sample is uniformly hydrated. Following this, the pan is heated within the Differential Scanning Calorimeter along with a similar empty pan which is taken as a reference. The samples are heated from 20 to 100 °C while heating at the rate of 10 °C/min (Sudheesh et al., 2019b, 2019c, 2019d). Gelatinization parameters such as onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and enthalpy of gelatinization (ΔH_{gel}) are calculated (Fig. 5b) during this analysis.

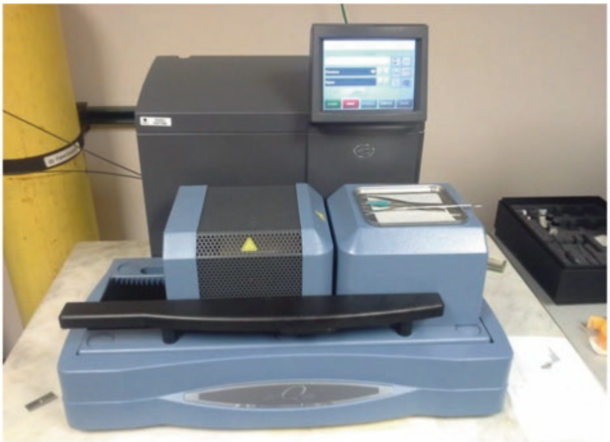
Significance When starch granules are heated in excessive amount of water, these absorb sufficient moisture and swell to maximum, which leads to rupturing of granules. Due to this, starch chains leach out and solubilise to form a viscous paste, the process being known as gelatinization. Melting of starch crystallites during the process of gelatinization leads to the loss of crystalline properties and birefringence pattern of granules (Witczak et al., 2016). Onset temperature, peak temperature, conclusion temperature and enthalpy are the gelatinization parameters of starch that are analyzed during its gelatinization under controlled conditions within a Differential Scanning Calorimeter.

2.7.1 Onset Gelatinization Temperature (T_o)

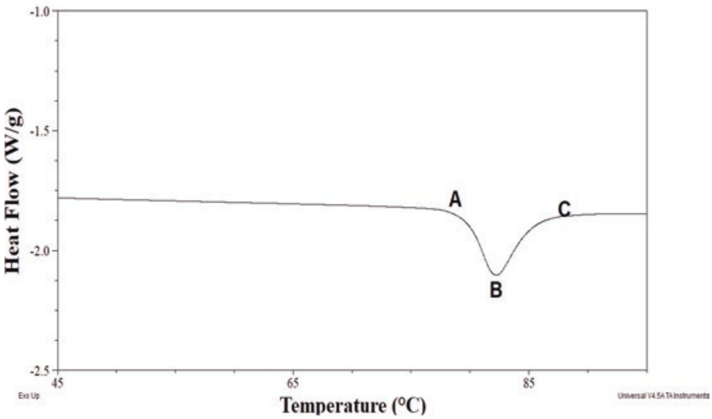
Onset temperature is the temperature required to disrupt weak starch crystallites during the process of gelatinization. In other words, it is the initial temperature of gelatinization process.

2.7.2 Peak Temperature (T_p)

Peak temperature is the temperature at which the maximum gelatinization of starch sample takes place.



(a)



(b)

Fig. 5 (**a**) Differential scanning calorimetry (DSC). (Courtesy: TA instruments, The University of Toledo) (**b**) DSC graph (A) onset temperature (B) peak temperature (C) conclusion temperature

2.7.3 Conclusion Temperature (T_c)

Conclusion temperature is the temperature required to disrupt strongest starch crystallites during the process of gelatinization. It appears towards the end of gelatinization process.

2.7.4 Enthalpy of Gelatinization (ΔH)

Enthalpy of gelatinization is the energy needed to convert the starch suspension to a thick viscous paste. It depends on both the quality and quantity of crystallites present within the granules.

2.8 Specific Volume

Specific volume measurement is a type of physical analysis of baked products. To measure the specific volume, bread loaves are subjected to analysis after 1 h of baking. Initially the volume of breads can be determined by rapeseed displacement method. The specific volume is latter calculated using the equation given below:

$$\text{Specific volume}\left(\text{cm}^3/\text{g}\right)=\frac{\text{Volume of the bread}}{\text{Weight of the bread}}$$

Significance Specific volume has an important role in determining the quality of breads. It is a kind of quantitative measurement of baking performance and affects the consumer preference of bread to a great extent. Dough with higher gas holding capacity helps in preparation of bread with higher specific volume. The volume of the bread loaves generally depends upon the type and amount of proteins present wherein, a good role is also played by the emulsifiers. The other factors that influence the specific volume of gluten-free breads include the degree of gelatinization, pasting properties, enzymatic susceptibility and loss of crystallinity of starch granules (Ziobro et al., 2012). It is the quantitative measurement of baking performance. Specific volume inversely affects the firmness of the breads. Higher specific volume indicates an enhanced porosity of the bread crumbs which is highly desirable.

2.9 Bake Loss

The bake loss also called moisture loss is an important parameter determining the quality of breads. It is calculated using following equation:

$$\textbf{Bake loss}=\frac{\text{Initial weight of the batter}-\text{weight of bread after cooling}}{\text{Initial weight of the batter}}$$

Significance Bake loss is an important physical parameter that influences the quality of breads. It plays a significant role in the development of firmness in breads rich in starch. Bake loss occurs due to the loss of water during baking. Higher bake loss results in breads with dry crust and increases their staling rate. Bake loss depend upon the water binding capacity of the dough and its ingredients. The incorporation of proteins, damaged starch and physically and chemically modified starch improves water binding capacity of the dough and reduces the baking loss.

2.10 Microstructure

Microstructure of gluten-free breads gives the information about their morphological properties such as surface uniformity, number of pores and pore size. The microscopic analysis of dough can be carried out using the technique like Scanning electron microscopy (SEM) and Laser scanning microscopy (CLSM) and others.

2.10.1 Scanning Electron Microscopy (SEM)

The micro structural study of breads/doughs is carried out using scanning electron microscope (Fig. 6). Double-sided carbon conductivity tape is used to mount the sample over the specimen stubs. A thin layer of gold/palladium coat is applied over the sample using an automated sputter coater. The sample is sputtered for 3 min and viewed under scanning electron microscope at different magnifications to produce scanning electron micrographs (Sudheesh et al., 2019b, 2019c, 2019d).

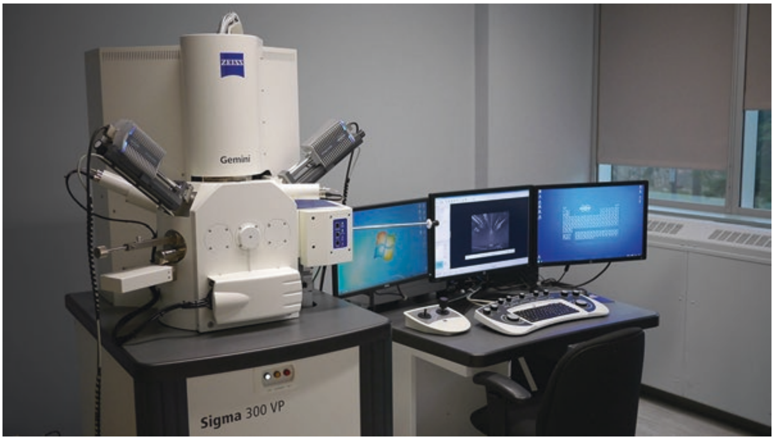


Fig. 6 Scanning electron microscopy (SEM). (Courtesy: Department of earth and atmospheric sciences, University of Alberta)

2.10.2 Confocal Laser Scanning Microscopy (CLSM)

After completion of baking, breads are dyed with the reagents like 2% of concanavalin, 0.2% Cell Mask and 10^{-9}% of Rhodamine B. Applying Triple dye assists to differentiate the starch, protein and yeast in the bread samples. A small piece (1 mm × 1 mm × 1 mm) is cut from the dyed bread samples and immersed in the oil (cedar tree oil). The sample is kept on the slide and overlaid with a glass cover slip. Finally, the sample is placed under the Confocal laser scanning microscope (Fig. 7) and scanned at different magnifications to obtain laser scanning micrographs (Miñarro et al., 2012).

Significance Micro structural study has a significant role in the volume and textural properties of the gluten-free breads. SEM analysis of breads gives the information about the number, size and shape of the pores present within the crumb. It also helps to analyse the type of pore distribution (heterogeneous or homogeneous). The pores properties are influenced by the moisture content. Hence, shrinkage of pores consistent with lower moisture content and water activity of the bread crumbs. More number of pores with uniform size improves the quality of the breads. It also indicates the good gas holding capacity and viscosity of the dough. The presence of ungelatinized starch granules after baking can be determined by the microstructure study. The protein, lipid, fibre and sugar present in the dough increases the gelatinization temperature of the starch (Giannone et al., 2016; Demirkesen et al., 2013). CLSM analysis gives the information about the structural compactness of bread crumbs (Miñarro et al., 2012) while the SEM analysis of dough gives details about the size and shape of starch granules present in the dough.

Fig. 7 Confocal laser scanning microscopy (CLSM). (Courtesy: Max Planck Institute For Plant Breeding Research)

2.11 Staling

After baking, breads are sealed in poly-ethylene bags and stored at 25 °C for 7 days (Haghighat-Kharazi et al., 2019). For each day, the bread samples are subjected to the following tests:

(i) Moisture content using hot air oven method is already discussed in Sect. 2.1.1.
(ii) Water activity (a_w) using water activity meter is already discussed in Sect. 2.1.2.
(iii) Hardness using texture profile analyzer is already discussed in Sect. 2.3.1.

Significance Staling of breads is due to the retrogradation properties of starch within the gluten-free breads. Amylose and amylopectin chains leached during the gelatinization process undergo reassociation. In this attempt of reassociation, amylose units are recrystallized and double helices of amylopectin units are formed. This leads to the development of firm texture of breads. Moisture has a plasticizing effect on the bread crumbs. Lower moisture content of the breads increases the crumb hardness. It also leads to shrinkage of crumb pores. Lower moisture content and water activity of the breads during the storage period also increases the staling of breads.

2.12 Sensory Evaluation

The sensory evaluation is carried out by 5–6 trained, 15 semi-trained or about 100 untrained panellists. After the preparation, bread samples are sliced and kept for sensory analysis. The bread slice from different formulations are served to each panellist and the whole bread is also presented to them to assess the external appearance. All the samples are given to panellist at same time. The sensory panellist assess the different attributes (crumb color, crust color, porosity, chewiness, hardness, mouth feel and flavour) using intensity scale and preference scale of 9 points commonly known as Hedonic Scale. In this way, the panellists noted most and least preferred samples.

The sensory analysis of fresh bread samples must be carried out within 4 h of baking. However, the sensory analysis of stored bread samples can be carried out within 24 h of baking (Milde et al., 2012).

Significance Sensory evaluation has important role in the assessment of quality of the gluten-free breads. Based on the sensory analysis score, the information about consumer acceptance of the gluten-free breads can be evaluated in a much easier way. Moreover, certain parameters of these breads including flavour and mouth feel can only be evaluated through the sensory mode of analysis.

3 Conclusion

The intent of this chapter was to start with quality parameters of gluten-free dough and bread and continue through the various tests for evaluating its quality. There are various tests followed for assessing the quality of gluten-free dough and bread. These include analysis of chemical composition, texture, pasting, rheology, gelatinization, morphology and retrogradation properties. Chemical compounds of dough/bread include moisture content influence on the textural and rheological properties of bread. Pasting and rheological properties have influence on the viscoelastic nature of gluten-free dough, higher pasting properties of the dough enhanced the volume of the gluten-free breads. Staling of bread can be described using moisture content and retrogradation property. Higher retrogradation rate and lower moisture content enhances the staling of gluten-free bread. Morphological analysis gives the information about the hardness and volume of the gluten-free bread crumbs. Sensory analysis have important role in the flavour and mouth feel of the bread.

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