# **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 nongluten 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 viscoelastic 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  $\gamma$ -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  $\alpha$ - and  $\beta$ -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).  $\alpha$ -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  $\alpha$ -1,4 glyosidic starch linkages and also bonds between non-reducing and reducing ends, resulting in closed  $\alpha$ -,  $\beta$ -, or  $\gamma$ -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 (Guiral et al., 2003a). Guiral et al. (2003b) observed that addition of CGT enhanced loaf volume, reduced crumb hardness and staling rate. However,  $\alpha$ -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  $\alpha$ -amylase (Guiral 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-2lactylate) 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 pregelatinisation 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<sup>2</sup>. Analysis of crumb grain proved that the mean cell area of optimized GF bread (1.05 mm<sup>2</sup>) was equivalent to that of wheat bread (1.01 mm<sup>2</sup>). Besides, the gas cell walls of GF bread was thicker (22 cells/cm<sup>2</sup>) than those in control white bread (32 cells/cm<sup>2</sup>) (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 nonconventional 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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