Chapter 2
Nutritional Aspects and Health Implications of Gluten-Free Products

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Abstract Celiac disease (CD) is an autoimmune enteropathy arising from the peculiar immune response to gluten-derived peptide amongst the susceptible population. It is evidenced by the chronic inflammation of the mucosal surface and atrophy of the intestinal villi, resulting in abnormal absorption of nutrient. The pathogenesis of CD involves the molecular interaction between gluten peptides, intestinal epithelium, and T-lymphocyte cells, the activity of the latter being enhanced by transglutaminase located at the epithelial brush border. Gluten is a protein that attributes to the viscoelastic properties of dough and enhances the gas retention and structure of the baked products. It constitutes a composite of cereal storage proteins including prolamins and glutenins. The toxic prolamins in wheat, barley, and rye consist of gliadin, hordein, and secalin, respectively. These prolamins have a high amount of proline and glutamine that resists degrading in the gastrointestinal environment, which consequently agglomerate as large peptide fragments. The toxic protein fragments induce mucosal damage and activate the T-lymphocyte cells which in turn produces high levels of pro-inflammatory cytokines causing clonal expansion, thus depicting the hallmark of CD. The aim of the chapter is to discuss the idea of nutritional Aspects of Gluten-Free Products.

Keywords Enteropathy · Prolamines · Celiac diseases · Gluten-free

2.1 Introduction

As per the definition of Codex Alimentarius standards, the term gluten-free (GF) refers to foods comprising gluten under the permissible value of 20 ppm (Standard, 2007). The key aspect for safe consumption of the GF diet is the absence of gluten in natural or processed foods. Many of the gluten-containing cereals (wheat, barley, and rye) and their hybrids (spelt, triticale, semolina, malt, etc.) are restricted for the

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celiac population. Up to now, the only therapy to combat CD, is strict adherence to a GF diet. GF diet mainly consists of the consumption of GF cereals, pseudocereals, fruits, vegetables, pulses, meats, and specially produced GF products in which gluten is replaced by GF flours. Commonly used substitutes for the gluten-containing cereals include rice and corn, followed by sorghum and oats. The use of oats as a gluten substitute is questionable due to the presence of avenin (storage protein), however, some studies have confirmed that oats can be well digested by most of the celiac population, and improves the palatability and nutritional value (Lee, 2009). Other than that, less commonly grown cereals, also called minor cereals (such as teff, and millet) and pseudo-cereals which are small grain-like seeds (buckwheat, quinoa, and amaranth) represent another possibility (Alvarez-Jubete et al., 2010).

Gluten network engulfs the starch granules and prevents its easy access to the amylase, thus slowing down the starch hydrolysis rate in the small intestine. The removal of gluten from the products increases the postprandial blood glucose level in the body leading to obesity and metabolic disorders (Scazzina, 2015). Consequently, a major fraction of celiac patients has shown nutrient deficiencies including that of calorie/protein, dietary fiber, minerals, and vitamins (Bardella, 2000; Thompson, 2000; Barton et al., 2007). The incidence of iron and vitamin B12 deficiency in celiac patients varies from 12% to 69%, and 8% to 41%, respectively (Tikkakoski et al., 2007; Dahele & Ghosh, 2001). Several reports showed that GF diets are hyperproteic and hyperlipidic and do not provide an adequate amount of carbohydrates, calcium, iron, and fiber, resulting in overweight conditions amongst CD patient. Many of the GF products contain trans fatty acids and dietary lipids that trigger metabolic imbalance and risk of coronary heart disease (Lissner & Heitmann, 1995). At the same time, vitamin D deficiency may develop due to the avoidance of lactose from milk and dairy products, as the CD patients become secondary lactose intolerance due to the reduced lactase production by the damaged intestinal villi. However, the severity of the CD related nutrient deficiencies depends on the several factors such as degree of malabsorption, the severity of intestinal damage, and length of undiagnosed period for an active CD patient (Ojetti, 2005).

While several studies have suggested that adhering to the GF diet can resolve the nutrient deficiency (Hallert, 2002; Annibale, 2001), some authors have argued that following a strict GF diet can mitigate nutritional deficiencies. Henceforth, this chapter deals with the different aspects of nutritional characteristics of GF ingredients and the GF products keeping health perspective into consideration.

2.2 Nutritive Profile and Bioactive of Gluten-Free Ingredients

2.2.1 Major Gluten-Free Cereals

Currently, the most widely used cereal flours for making GF products include rice (*Oryza sativa*) and maize or corn (*Zea mays*) owing to their hypoallergenic character, bland flavor, and easy availability (Kadan, 2001). The nutritional composition and amino acid profile of gluten-containing, and GF grains suggest that that rice and corn have lesser protein content and total dietary fiber, while carbohydrate amount was considerably high. The rice proteins are insoluble due to their hydrophobic nature, due to which rice flour demonstrates inferior viscoelastic properties during the baking process. The carbon dioxide produced during fermentation escapes due to poor protein-starch network, as a result of which the product formed is compact and brittle with poor sensory qualities (Marco & Rosell, 2008). Maize is a high energy crop, but at the same time has low levels of essential amino acids such as tryptophan and lysine and lacks important minerals and vitamins (Foschia, 2016).

Sorghum (*Sorghum vulgare*), on the other hand, has higher protein content than rice and comparable to that of maize. At the same time, it consists of a comparatively lower carbohydrate than the two former cereals. The release of sugars from the starch matrix is relatively slower to other cereals, which makes it an ingredient of interest to diabetic and celiac people. Due to the poor starch and protein digestibility, a pretreatment process is usually carried out to release the components from the compact complexes (Correia, 2010). These processes include fermentation, malting, and enzymatic treatment. There has been increased attention in utilizing sorghum as a sole GF ingredient or in combination with other non-gluten flours. The major protein fraction present in sorghum consists of globulins and prolamins that are generally surrounded by starch granules (Marti & Pagani, 2013).

The use of oats (*Avena sativa*) as a GF ingredient is still controversial as it is believed that avenins (storage proteins in oats) can trigger up the immunogenic response. At the same time, others suggest that the immunogenicity depends on the cultivar consumed. Nevertheless, it has numerous health benefits such as high unsaturated fatty acids and dietary fibers mainly β-glucans (Lasa, 2017).

2.3 Minor Gluten-Free Cereals

2.3.1 Millet

Millet is characterized as small-seeded coarse cereal and is the most extensively studied cereal after rice, corn, and sorghum. They are categorized as the underutilized food in North America and Europe; however, their drought-resistant nature and low agricultural inputs make them a suitable crop for India, Africa, and China. The different varieties of millet grown today are Finger millet (*Eleusine coracana*), Foxtail millet (*Setaria italica*), Pearl millet (*Pennisetum glaucum*), Kodo millet (*Paspalum setaceum*), and Proso millet (*Panicum miliaceum*). Adding to these advantages, they have a relatively lower glycemic index and exceptional nutritional benefits (Saleh, 2013). Apart from being a gluten-free alternative, it has been helpful for the management of type II diabetes owing to their hypoglycemic properties (Annor, 2017). They suggested that the presence of lipids, proteins, α-amylase inhibitors, starch type, and phenolic compounds are the contributing factors to the hypoglycemic activity of millets. Most of the millet proteins contain a range of essential amino acids, with a relatively high quantity of methionine (Singh et al., 2012).

Pearl millet consists of around 69.1% carbohydrate, 11.4% crude protein, 4.8% crude fat, 2% crude fiber, and 2.2% ash. Finger millet has many health benefits, a few of which have been attributed to the presence of phenolics. The nutritional value of finger millet is marked as 71.52% carbohydrate, 7.44% crude protein, 3.6% crude fiber, and 3.38 mg/g of calcium. Foxtail millet, on the other hand, has a significant amount of lysine which makes them an additional protein source for most of the cereals (Ragaee et al., 2006). Kodo millet and little millet have fats mostly containing polyunsaturated fatty acids; are found to have 38% dietary fiber which is highest amongst other cereals (Hegde et al., 2005). Compared to the protein profile present in wheat, proso millet contains more essential amino acids such as methionine, leucine, and isoleucine (Kalinova & Moudry, 2006). The highest amount of protein and crude fiber is found in Proso millet, while finger millet possesses the highest amount of calcium suitable for fighting anemi (Chethan & Malleshi, 2007; Sripriya et al., 1997). Along with specific mineral and proteins, millets also contain dietary fibers such as resistant starch that are needed for the synthesis of short-chain fatty acids (butyrate) effective in preventing colon cancer. The in-vitro study on soluble polysaccharides (arabinose and xylose) have proved their prebiotic activity and wound dressing property (Mathanghi, 2012).

Speaking of micronutrients, millets are an excellent source of β-carotene and B-vitamins especially folic acid, niacin, and riboflavin. The amount of thiamine and niacin present in millet is comparable to that of rice and wheat. Besides, millet's bioactive constituents complement those present in fruits and vegetables. These include gallic acid, catechol, cinnamic acid, benzoic acid, ascorbic acid, ferulic acid, *p*-coumaric acid, vanillic acid, sinapic acid, chlorogenic acid, proto-catechuic acid, kaempferol, caffeic acid gentisic acid, salicylic acid, and syringic acid, however, the concentration of these bioactive compounds vary according to the cultivar and environmental conditions (Kumar, 2018).

2.3.2 Teff

Teff (*Eragrostis tef*) belongs to the Poaceae family, and is a minor GF cereal produced in Ethiopia and Eritrea (*Eragrostis teff*), and exhibit an excellent protein profile (Bultosa & Teff, 2016). Teff seeds are mostly distinguished based on color (white, red or brown, and mixed) for marketing purposes. The color of the hulled grains ranges from pale white to dark brown (Belay, 2009). The starch content in teff (73%) is relatively higher than most of the cereals which makes it a suitable alternative to wheat (Tatham, 1996). The protein profile of teff is similar to wheat and higher than rice, maize, sorghum, and millet.

The major storage proteins present in teff are glutelins (44.55%) and albumins (36.6%) and the rest constitute prolamins (11.8%) and globulins (6.7%) (Tatham, 1996). Since the teff flour consists of albumin, glutelin, and globulin as the major protein fractions, it is easily digestible relative to other gluten-free cereal such as sorghum and maize (Gebremariam et al., 2014).

The mean value of crude fat present in teff is 0.0238%, out of which 72.46% is unsaturated fatty acids, mainly consisting of oleic acid (32.41%) and linoleic acid (23.83%) (El-Alfy et al., 2012). The crude fiber content of teff is analogous to that of millets and higher than rice, wheat, maize, and sorghum. The reason for such high fiber content is its exceptionally small grain size due to which it is always milled as whole-grain flour.

Moreover, calcium which helps in bone building and prevention of colon cancer is present in high amounts in teff. To prevent the issues related to the low calcium intake, Roosjen suggested that flour should contain at least 150 mg/100 g of calcium (Roosjen, 2005). Except for teff and finger millet, most of the major cereals such as rice, wheat, maize, and sorghum fail to fulfill this requirement. Apart from that, teff has been found reasonable for the sparse occurrence of anemia in Ethiopia. Other than niacin, riboflavin, and thiamine; teff also contains vitamin B6 (0.482 mg/100 g), vitamin K (phylloquinone), vitamin A (9 IU), and α-tocopherol (0.08 mg/100 g) (wet basis) (Zhu, 2018).

Similar to millets, teff exhibits health-promoting effects due to the presence of a substantial amount of phenolics. The major phenolic compound present in teff includes ferulic acid (285.9 μg/g), with considerable amounts of cinnamic (46 μg/g), vanillic (54.8 μg/g), coumaric (36.9 μg/g), protocatechuic (25.5 μg/g), gentisic (15 μg/g), syringic (14.9 μg/g) acids (McDonough et al., 2000). These phenolics are responsible for the antioxidant activity, which helps in the prevention of cardiovascular diseases and cancer (Awika & Rooney, 2004).

2.3.3 Pseudo-cereals

In contrast to monocotyledonous cereals, Buckwheat (*Fagopyrum esculentum*), Amaranth (*Amaranthus* spp.), and Quinoa (*Chenopodium quinoa*) are dicotyledonous seeds under the family of *Poaceae*. The common term assigned to them is pseudo-cereals as they have similar structural and nutritional properties to that of the true cereals. These pseudo cereals present good opportunities for the production of GF products as they not only lack toxin prolamins; but are characterized by high macronutrients and micronutrients including the essential amino acids. Amaranth is a lens-shaped seed with a diameter varying from 1 to 1.5 mm and weighs around 0.6 to 1.3 mg (Bressani, 1994). The common amaranth species under cultivation are *Amaranthus hypochondriacus*, *Amaranthus caudatus, and Amaranthus cruentus.* Compared to Amaranth seeds, quinoa seeds are much bigger with a diameter of 1–2.5 mm (Taylor & Parker, 2002). The popular buckwheat varieties are common buckwheat (*Fagopyrum esculentum* Moench) and tartary buckwheat (*Fagopyrum tataricum*) (Oomah & Mazza, 1996). *The nutritional compositions of these pseudo-cereals from where it can be derived that amaranth and quinoa have the highest* *protein amongst all the cereals. The main advantage of using pseudo-cereals as the gluten-free ingredient is in the fact that they contain globulins and albumins as the main protein fractions, with negligible prolamins proteins, the latter being toxic to the celiac patients(Drzewiecki,* 2003*). The amino acid present in globulins and albumins fractions contains a lower amount of* proline and glutamic acid than prolamines (Gorinstein, 2002). These pseudo-cereals are a decent source of dietary fiber; therefore, infusion of these seeds with other gluten-free ingredients can help to alleviate the dietary fiber deficiency amongst the concerned celiac population (Alvarez et al., 2009)

Another important nutritional aspect of the pseudo-cereals is the presence of a high percentage of unsaturated fat such as linoleic acid (50% of the total fatty acid for amaranth and quinoa, and 35% for buckwheat), oleic acid (25% for amaranth and quinoa, and 35% for buckwheat), and palmitic acid (Bruni, 2001; Bonafaccia et al., 2003). The high α-linolenic content (3.8–8.3%) in quinoa seeds is responsible for the reduction of biological markers linked to common degenerative diseases namely, cancer, osteoporosis, cardiovascular, and autoimmune diseases (Ruales & Nair, 1993)

Furthermore, buckwheat consists of fagopyritols, a soluble carbohydrate which is a source of D-chiro-inositol, a compound that effectively controls the type II diabetes through glycemic index management. The range of fagopyritols present in buckwheat ranges from 269.4 to 464.7 mg/100 g (Steadman, 2000). The main phenolic compounds present in amaranth seeds are ferulic acid, caffeic acid, and p-hydroxybenzoic acid (Klimczak et al., 2002). Flavonoids such as glucosides of flavonol kaempferol and quercetin are abundantly found in quinoa seeds (Dini et al., 2004). For buckwheat, the main source of phenolics is glucosides of flavonol quercetin, accompanied by glycosides of the flavones luteolin and apigenin (Dietrych-Szostak & Oleszek, 1999).

2.4 Legumes

Besides pseudocereals, several attempts have been made to utilize legumes (pulses, in particular) to improve the protein profile and functional characteristics of GF products. Typically, pulses are categorized into 11 main classes: dry beans (*Phaseolus* spp.; *Vigna* spp.), dry peas (*Pisum* spp.), dry broad beans (*Vicia faba*), dry cowpeas or black-eyed peas (*Vigna unguiculata* (L.) Walp.), chickpeas or bengal grams (*Cicer aretinum* L.), pigeon peas (*Cajanus cajan* L. Mill sp.), bambara groundnuts or earth peas (*Vigna subterranean* L.), several varieties of lentils (*Lens culinaris* Medik.), vetch or common vetch (*Vicia sativa*), and lupins (such as *Lupinus albus* L. and *Lupinus mutabilis* Sweet), as well as minor pulses, including jack beans (*Canavalia ensiformis*), lablab or hyacinth beans (*Lablab purpureus*), winged beans (*Psophocarpuster agonolobus*), sword beans (*Canavalia gladiata*), yam beans (*Pachyrrizus erosus*), and velvet beans (*Mucuna pruriens* var. *utilis*) (Melini, 2017). These pulses contain a good amount of protein, complex carbohydrates, dietary fibers, and micronutrients. At the same time, they contain a high quantity of polyphenols demonstrating excellent antioxidant property, and other secondary metabolites including isoflavones, bioactive carbohydrates, polysterols, saponins, and alkaloids (Roy et al., 2010) Besides, pulses based GF products have low glycemic index.

The main protein proportion in pulses accounts for about 17–35% of the cotyledon weight (dry basis) (McCrory, 2010). Pulse proteins are categorized into four classes based on their solubility in several solvents i.e. (a) albumins (water-soluble), (b) globulins (soluble in a salt solution), (c) prolamins (soluble in an alcoholic solution of 70% concentration), and d) glutelins (dissolves in alkaline solution), of which globulins and albumins constitute the major proportion (60–80%) of protein fraction (Tiwari & Singh, 2012). Compared to cereal proteins, pulses possess higher levels of leucine, lysine, arginine, aspartic, and glutamic acid, however contain lower amounts of sulfur-containing amino acids such as cysteine, and methionine. Based on the protein concentration, pulses flours are available as pulse flour (<65%, db), protein concentrates (>65%, db), and protein isolates (>90%, db) (Schoenlenchner, 2010).

The carbohydrates in pulses are composed of starch, soluble sugars, and dietary fibers, which overall account for 55–65% of the whole pulse dry weigh (Boye et al., 2010). The starch in total constitutes of 22–45% of total carbohydrates, and generally contains up to 20–30% amylose and 70–80% amylopectin (Maaran, 2014). The resistant starch present in pulses attributes to the slow glucose release thus, controlling the glycemic and postprandial responses (Berrios et al., 2010). Along with that, they also represent a source of dietary fiber (soluble and insoluble, both), for instance, the insoluble fiber in chickpeas, lentils, and peas are 75%, 87%, and 89% (db) (Maaran, 2014).

Furthermore, they are a rich source of niacin, thiamine, riboflavin, pyridoxamine, pyridoxal, and pyridoxin. High amounts of folates are also present in pulses which are generally deficient in humans due to complex bonds with other biomolecules. As a relevant example, beans contain 400–600 μg/100 g of folates which covers 95% of the daily requirement. Chickpeas are also rich in folates and contain a higher amount than peas (150 vs 102 μg/100 g) (Campos et al., 2010). They also contain a good amount of minerals such as iron, zinc (highest in beans and lentils), potassium, and magnesium (highest in cowpea) (Oomah, 2011). Amongst secondary metabolites such as tannins, phenolic acids, and flavonoids, kidney beans and black grams contains the highest phenolic content. Chickpeas also contain a wide variety of bioactives including glucosides of flavone, flavonoids, and oligomeric cum polymeric proanthocyanidins.

2.5 Nutritional Interventions in GF Products

The production of baked products from gluten-free ingredients has several associated challenges. Firstly, the viscoelastic property of gluten is hard to mimic in order to develop a palatable GF baked product. During baking, gluten plays an important role in holding the CO_2 released during proofing, thus giving the product an excellent structure and acceptable volume (Drabinska et al., 2016). The absence of gluten impairs the dough structure, giving a liquid consistency batter and defected baked products (Gallagher et al., 2014). For instance, the removal of gluten prevents starch swelling during cooking of pasta, prevents the biological leavening, and hampers the texture of bread, whereas for biscuits it has an impact on the elasticity and cohesiveness of the dough (Di Cairano, 2018). Another challenge is to maintain an appropriate nutritional profile for these kinds of products. Frequently, the gluten-free products are characterized by a high amount of saturated fatty acids and sugars, and low levels of nutrients such as dietary fibers, calcium, iron, zinc, magnesium, vitamin B12, and folate. Moreover, GF products often depict a high glycemic index due to the presence of starchy ingredients. The high GI leads to several metabolic disorders such as obesity and diabetes (Jnawali et al., 2016; Naqash, 2017; Vici, 2016).

The most common cereals used as ingredients for GF products are rice and corn which have certain demerits associated such as poor viscoelastic properties of rice which hinders gas retention during baking, and inferior textural properties of GF product when developed using cornflour. On the other side, the protein and starch present in sorghum are not easily digestible. The high gelatinization temperature of sorghum flour results in poor quality bread with cracks and large holes in the crumb (Carcea, 2020). Therefore, substances such as hydrocolloids (guar and xanthan gums, alginate, carrageenan, hydroxypropyl methylcellulose, carboxymethyl cellulose), emulsifier, isolated proteins (from egg, legumes, or dairy products), or enzymes (cyclodextrin glycosyltransferases, transglutaminase, proteases, glucose oxidase, and laccase) have been added during the preparation of GF products to mimic the property of gluten (Matos & Rosell, 2015) Other interventions, sourdough technology, and high hydrostatic pressure being some of them, have been applied to improve the organoleptic qualities and nutritional properties, consequently (Capriles et al., 2016).

2.6 Baked Products

2.6.1 Biscuits and Cookies

The most commonly employed GF alternatives for biscuit preparation are maize starch and rice flour. However, these flours give high energy intake but lack many essential amino acids (lysine and tryptophan) and vitamins. Thus, to surpass the low nutrient challenge, different combinations of high-value ingredients have been used to enhance the nutritional properties, bioactive content, and glycemic index of GF biscuits. Rybicka and Gliszczynska-Swiglo (2017) suggested that biscuits prepared using buckwheat chickpea, oats, millet, amaranth, teff, and quinoa had higher mineral content than those prepared using potato, maize, rice, and GF wheat starch (Rybicka et al., 2019). The technological processes such as malting, fermentation, and germination can impact the overall quality of the biscuit. A relevant example of this was presented by Omoba et al. (2015) where they used sourdough technology to improve the nutritional profile of sorghum, pearl millet, and soy-based biscuits. They observed a moderate increase in phenolic content, consequently raising the antioxidant activity than the control and a reduction in the concentration of antinutritional phytate. At the same time, the addition of antioxidants, ash, and fibers rich ingredients can have a negative impact on the optical and sensory quality of GF biscuits, therefore, giving a darker colored product with a bitter aftertaste (Omoba et al., 2015).

In addition, the use of low fiber starch such as refined flour induces a high glycemic index in the developed product. It has been observed that the utilization of wholegrain flour from legumes or pseudo-cereals, and the high moisture thermal treatments (annealing) can reduce the glycemic index of the product (Rocchetti et al., 2018; Giubrerti & Gallo, 2018). For example, the biscuits prepared using tartary buckwheat presented a lower glycemic index (62.8) than that prepared using rice flour (110.2). Furthermore, the use of malted tartary buckwheat flour can boost up the antioxidant activity, with a further reduction in the glycemic index value to 57.6 (Molinari, 2018). Likewise, teff flour can also lead to the reduction of GI when compared with other conventional GF alternatives. The incorporation of soluble fibers, such as arabinoxylans, guar gum, high molecular weight β-glucan, or psyllium can significantly lower the glycemic index by delaying the gastric functioning (Scazzina et al., 2013). The carbohydrate present in a food is indicated by the glycemic load, which can be reduced by the increased concentration of non-digestible carbohydrates like resistant starch, as well as protein content.

Adebiyi (2017) demonstrated that fermentation and malting improved the nutritional characteristics of the pearl millet biscuits; which was pointed out by the increase in the amino acids, total phenolic and mineral content (Abediyi, 2017). Another study showed that using germinated flour blends of foxtail, kodo, and barnyard millets contained higher protein content, total antioxidant activity, and phenolic content than native blends (Sharma et al., 2016). Teff, on the other side, is characterized by high protein content, but it lacks gluten which impairs the biscuit quality. Oats can improve the nutritional properties of the conventional gluten-free biscuits by partial or full replacement of the GF flour. Incorporation of oats bran in oats biscuits increased the nutritive values and dietary fiber content (Duta & Culetu, 2015).

The most studied pseudo-cereal for GF formulations is buckwheat flour which has a peculiar phytochemicals present called rutin. Some studies have shown that the incorporation of buckwheat flour with rice or wheat flour has raised the DPPH radical scavenging activity, mineral content, total phenolic content, and rutin levels than those of control biscuits (Sedej, 2011).The addition of quinoa flakes (25%) and flour (30%) into base material (corn starch) lead to the increment in the dietary fiber content. The total fatty acids present in quinoa-based cookies were composed of polyunsaturated fatty acids (60.53%), monosaturated fatty acids (23.41%), and saturated fatty acids (17.45%). The essential amino acids identified were isoleucine, methionine, threonine, phenylalanine, and valine (Brito, 2015). Chauhan et al. (2015) compared the GF cookies prepared using native and germinated amaranth flour, wherein germinated GF biscuits exhibited higher antioxidant activity and total dietary fiber than raw amaranth flour biscuits (Chauhan et al., 2015).

At the same time, legume flour has been applied in the GF products to increase their nutritional quality. Sparvoli (2016) used a low anti-nutritional variety of common beans in combination with maize flour to make low glycemic index nutritive GF biscuits. The actual glycemic index value of the prepared biscuit was higher than the predicted one due to the presence of α-amylase inhibitors (Sparvoli, 2016).

2.6.2 Bread

A large section of the human population depends on bread as a staple meal which serves as a vital source of protein. However, the absence of gluten rendered low protein bread with poor sensory quality. The average protein value of GF bread is 4.4 g/100 g which is significantly less than that of conventional gluten-containing bread (10 g/100 g) (Do et al., 2014). Using protein isolates and protein-enriched flour are suitable options to augment the protein content of GF bread; however, they have an antagonistic impact on the texture and sensory characteristics of bread. Apart from gluten, the dough's viscoelasticity changes with the amylose content of the starch (Kaur, 2015). Rice flour is a common alternative for the replacement of conventional bread; however, they provide comparatively lower protein (6.14–7.30 g/100 g) and nutrition (Molina-Rosell & Matos, 2015). Leguminous flours (soybean, peas, lentils, chickpea, and beans), on the other hand, provide better protein content and nutritional profile than rice or maize flour. The addition of pea, chickpea, lentil, or pea flour with rice flour in a 1:1 ratio increased the protein content of the cake from 6.2 to 8.7 g/100 g. The addition of soy flour to starch of different origin (such as cassava, corn, and rice) improved the bread quality, sensory quality, as well as the nutritional characteristics of GF bread (Taghdir, 2017). However, the negative aspect of leguminous flour resides in their extremely high-fat content and presence of anti-nutritional compounds (Molina-Rosell & Matos, 2015).

Pseudo-cereals have similar protein profiles to that of glutinous flours, as a result of which, the bread developed using amaranth flour has better protein level and health benefits. According to Kaur (2015) the bread produced using buckwheat flour showed an optimal balance of amino acids and phenolics, however, the sensory quality was rated lower than wheat flour. The blend of rice and buckwheat showed the potential to increase the textural properties of bread, without the addition of hydrocolloids (Badiu et al., 2014). In addition, bread prepared using soy/egg/corn protein isolates (Foschia, 2016; Crockett et al., 2011) egg white solids (Nunes et al., 2009), whey protein isolates/concentrates were studied for their textural, sensory properties, and consumer acceptance.

Besides, the addition of dietary fiber to refined flour or starch lowers the glycemic index of the baked products due to improved water-binding ability. Soluble dietary fibers such as psyllium and guar gum are known to reduce the glycemic index of GF bread by 40 and 41%, respectively (Scazzina, 2015). Likewise, inulin reduced the glycemic index of GF bread from 71 to 48 (Segura & Rosell, 2011). Although these additives are advantageous for the reduction of GI, they concomitantly result in hard bread crumb with poor sensory quality. Another study used the blend of plantain-chickpea-maize to produce GF bread (Flores et al., 2015).

Apart from the ingredients used, the glycemic index of the baked product also depends on pre-processing technologies such as enzymatic treatment, germination, and sourdough fermentation. Enzymatic treatment reduces the susceptibility of starch to hydrolysis in presence of α-amylase (Dura & Rosell, 2016). It should be noted that the effect of sourdough technology varies with the type of substrate. For instance, the application of sourdough technology on sorghum and teff showed the expected decrease in GI, however using the same technique for quinoa and buckwheat resulted in a high glycemic index for the baked product (Wolter, 2014). Scazzina (2015) pointed out that white sourdough bread had higher fiber and protein, but a lower glycemic index (52.1) than normal white bread having a glycemic index of 61.2. Mixed sourdough dough (6% millet, 6% buckwheat, and 21% corn flour) was formulated and made into a multigrain puffed cake with a moderate glycemic index value of 66.7.

As far as the micronutrient availability of GF products is concerned, studies have evidenced that most of these products are crafted using highly refined substrates, which in result supplies very little nutrients (Wolter, 2014; Suliburska, 2013; Stantiall & Serventi, 2018). Several studies have suggested that the addition of buckwheat, amaranth, pearl millets, and flaxseeds have been found to increase the mineral content of the bread. The addition of buckwheat flour (10–40%) in GF bread composed of corn and potato starch enriched the micronutrient profile of the bread. The Fe content increased from 42.7 to 54.3 g/100 g and Zn content improved from 5.7 to 13 g/100 g; however, the major increase of 4 and 9 times was observed for Cu and Mn, respectively) (Rozylo, 2015). Although the nutritional quality of blends consisting of teff or pseudo-cereals (amaranth and quinoa) is much better than wheat flour; their baking properties and sensory characteristic were inferior to that of the conventional flour. As an example, it was reported that macronutrients such as fat and protein, as well as the mineral content such as Ca, K, Mg, Fe, Mn, and Zn were higher for teff, amaranth, and quinoa bread compared to what was produced using wheat (Rybicka et al., 2019)

2.6.3 Extrudates

Out of all the gluten-free products, pasta is the most popular product amongst the gluten-intolerant people (Gimenez, 2016). One of the main nutritional benefit of pasta is the low GI which controls the body weight, plasma lipid, and blood glucos. Gluten-free grains (replacing partially or totally) such as rice and corn have been used to target the specific group of the celiac population. Even though these conventional alternative grains are composed of many macro and micronutrients, they are not considered sufficient to fulfill all the requirements for essential micronutrients. Different types of GF flours (sorghum, rice, corn, and potato starch) have been used for spaghetti preparation, wherein the best results were obtained with a 40:20:40 ratio of sorghum, rice, and potato flour (Giacco et al., 2016; Demirkesen, 2010; Ferreria, 2016).

Different sources of proteins (egg white, whey, bovine plasma, cowpea, and lupine) have been incorporated in the GF products to improve their overall nutritional quality and texture (Kumar, 2019; Chapbell et al., 2016; Furlan et al., 2015). High protein legume (faba, lentil, and black gram) flours were used to develop the low glycemic index and highly nutritious pasta, with a reduction in the antinutritional compounds such trypsin, α-galactosides, and phytic acids during extrusion processing. Compared to the commercial cereal pasta, the legume pasta has 2.9–3.5 fold higher protein content, and 1.4–1.6 fold lower starch content. Moreover, legume flour has better lysine content, while cereal proteins contain more sulfur-containing amino acids. The anti-nutritional compounds such as trypsin inhibitors, α-galactosides, and phytic acids experience a decrease during cooking). In addition, the pasta prepared using soy flour in a combination of defatted almond flour demonstrated 3–5 times higher protein content (33.3–42.1%, db) in comparison to control pasta (2.8–13%, db) (Laleg, 2016; Martinez, 2017).

Incorporating biotechnological preprocessing such as fermentation and sprouting has been found to enhance free amino acids, minerals, and bioactives. Fermentation of black gram enriched rice evidenced the enhanced nutritional and functional properties with further improvement in total phenolic content and antioxidant activity after the extrusion. Another study focused on improving the nutritional characteristics of rice-based pasta by enrichment with fermented or sprouted sorghum flour. However, the results indicated a drop in protein and starch content after fermentation and sprouting. The limited starch breakdown in fermented sorghum enriched rice pasta does not lead to foul color or textural changes and appears to have a beneficial impact on the cooking properties. The decrease in protein content was attributed to the proteolysis of non-aggregated kafirins, thus, conserving the proteins necessary to form a stable network in the final product. During sprouting, starch cleavage by amylolytic activity alongside the peculiar protein breakdown rendered severe impairment in the cooking and nutritional properties of the enriched product (Rani, 2018; Marengo, 2015).

Satisfactory results have been obtained by blending pseudo-cereals (amaranth, buckwheat, and quinoa) with corn, soy, oats, and cassava to produce GF spaghetti (Mastromatteo, 2011; Chillo, 2009; Caperuto et al., 2001; Fiorda, 2013;

Gungormulsler et al., 2007). Milling treatment was observed to affect the nutritional profile of the quinoa used for the preparation of oat-quinoa spaghetti. The rise in amino acids including histidine, leucine, isoleucine, valine, methionine was observed, while the overall protein and lipid contents were reduced by the factor of 3.5 and 5.4, respectively. Nevertheless, the addition of quinoa guaranteed the improvement of the amino acid profile for corn protein. Moreover, the teff based pasta was reported to demonstrate similar textural properties to wheat flour; however, the overall sensory quality was inferior to the latter. Teff pasta had higher mineral, fiber content, as well as the low glycemic index (47) than the wheat-based pasta (65) (Hager, 2013).

On the other hand, using green banana flour-based pasta had inferior nutritional properties compared to standard pasta. However, the GF pasta prepared using banana-cassava composite flour has a more resistant starch content compare to semolina based pasta. Egg white and soy protein were incorporated in banana-cassava based pasta, wherein soy protein gave better protein content than egg protein powder. Furthermore, a study compared the rice, legume, and pseudo-cereal based pasta, which was shown to have satisfactory phenolics components. It was found that cooking via boiling reduced the bound to free ratio of phenolic compounds for all the GF pasta (Zandonadi, 2012; Rachman, 2020; Rocchetti, 2017).

2.7 Conclusions

In a nutshell, the demand for GF products has been increasing immensely which are majorly prepared using refined flour, however, at the same time their nutritional profile remains a major challenge. During milling, the minerals present in the bran and germs are lost which leads to poor nutritional quality in the end product. It has been observed that inferior micronutrient profile (minerals, vitamins, and bioactive content) can be improved using amaranth, buckwheat, and quinoa. Using a combination of different flours and incorporation of sources of protein and dietary fibres has shown to improve the micronutrients, amino acid profile, and bioactive compounds in the GF products. Different pre-treatments are cooking process have also been seen to impact the changes in the macronutrients and micronutrients of the GF products.

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