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MULTI-APPROACH STRATEGY FOR THE NUTRITIONAL AND SENSORIAL IMPROVEMENT OF GLUTEN-FREE RUSKS AND OTHER PRODUCTS AIMED AT CONSUMERS WITH PARTICULAR NUTRITIONAL NEEDS

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Summary

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CHAPTER 1: INTRODUTION

1.*Celiac disease and other pathologies associated to gluten*

Celiac disease (CD) is an autoimmune enteropathy due to gluten and prolamins sensitization in genetically susceptible individuals (Houben et al., 2012; Ludvingsson et al., 2012). Recent epidemiological studies have shown that 1 in 100 people worldwide suffer from this disease. However, prevalence may be underestimated because the condition is often not diagnosed (Moreno et al., 2014).

The first studies of the disease date back to the first century after Christ, when some doctors described the disease, although they did not know the cause and identified it as "*Koliakos*", to indicate "those who suffer in the intestines". In 1856 Francis Adams, a Scottish doctor, translated the term into "coeliac" and it was only in the mid-20th century that it was made clear that this disease manifested in some people as a result of ingestion of certain wheat proteins, which damage the intestinal mucosa.

The food component that triggers CD is gluten, a complex protein contained in wheat and other cereals such as barley, rye and triticale (hybrid of wheat and rye). Gluten it is obviously also included in spelt, khorasan wheat (kamut®), einkorn, and emmer, that are ancient varieties of wheat.

Gluten is a viscoelastic structure that is formed as a result of the hydration of wheat flour during the kneading phase. Gluten is a complex mixture of water-insoluble proteins present in wheat and other cereals, that are distinguished by the degree of polymerization and level of sulphur-amino acids and are divided in two large groups: gliadins e glutenins. (Shewery et al., 1986). The gliadins are classified into 4 categories: α-gliadine, β-gliadine, ω-gliadine, γ-gliadine. They are prolamins with low molecular weight and are associated with each other by means of hydrogen bonds and hydrophobic interactions. Gliadins and glutenins during hydration and kneading of the dough establish covalent bonds with each other by forming disulphide bridges, hydrophobic interactions and non-covalent bonds, generating what we know as gluten with the viscoelastic characteristics capable of influencing the rheological qualities of wheat flour. (Cabras & Martelli, 2004).

Specifically, the allergic reaction is triggered by the prolamines that take specific names depending on the cereal: gliadine (in wheat), ordeine (in barley), secaline (in the Secale).

CD is a complex disease mainly due to the interaction between two factors: genetic and environmental. In the etiopathogenesis of this disease, the only known genetic factor at present is the presence of some human leukocytic antigens HLA-DQ2 and HLA-DQ8 (Kagnoff, 2005). Gliadin contains peptide sequences that are highly resistant to gastric, pancreatic and intestinal proteolytic digestion. This difficult degradation is due to the high content in gliadin of amino acids such as proline and glutamine, which many proteases are not able to split (Hausch et al., 2002).

The peptidic sequences that are not digested pass through the epithelium of the small intestine, penetrating the own lamina where the tissue transglutaminase (the enzyme identified as antigen) deamine the glutamine into glutamic acid, leaving highly reactive peptides for HLA-DQ2 and HLA-DQ8 molecules. The complex is thus recognizable by CD4+ T cells, which initiate the immunological response, leading to lesions typical of the mucosa of the small intestine (Briani et al., 2008)

This protein fraction is responsible for the toxic effect of CD (Niewinski, 2008).

Intake of these proteins, generates inflammation, atrophy, and hyperplasia of the celiac patient's intestinal crypts. As the disease progresses, the mucosa is permanently damaged and is no longer able to perform its natural function of nutrient absorption. This pathology not only causes deleterious effects in the intestine but also in, heart, brain, liver, skin, joints, and other organs (Comino et al., 2013). The manifestation of symptoms in this disease are extremely versatile: the subject may have gastrointestinal symptoms, extra-intestinal or even could be asymptomatic and therefore may not show symptoms. Frequently the clinical presentation of CD is comparable to an iceberg, also called "coeliac iceberg" (Figure 1), to describe that only a percentage of cases of CD is diagnosed and treated, although a good part is submerged and is not recognized and the symptoms manifested can be absent or atypical (Fasano e Catassi, 2005).

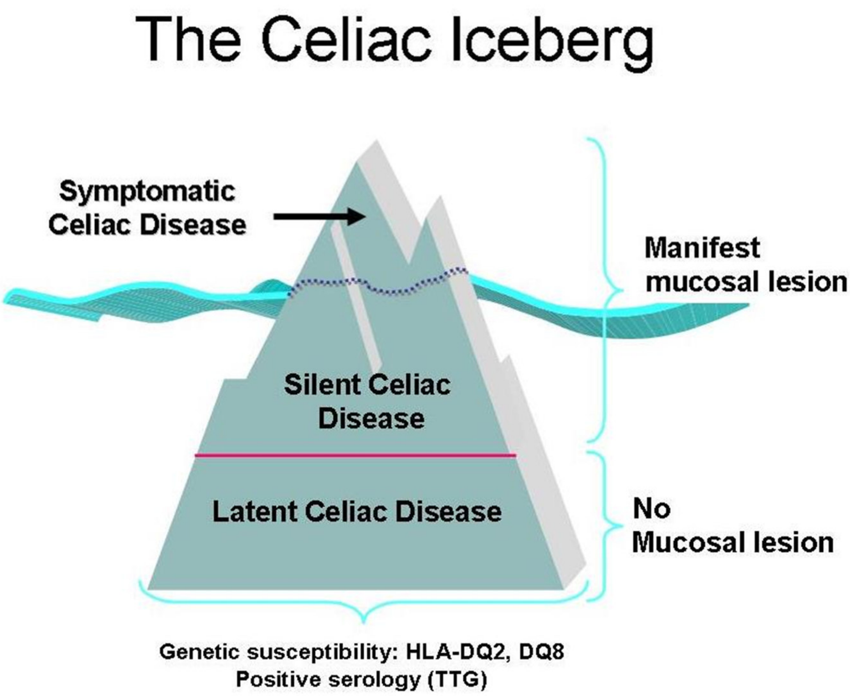

Figure1. Representation of celiac disease compared to an iceberg "Celiac iceberg" (Bozzola et al., 2014).

The diagnostic protocol provides for serological investigation with certain antibody markers followed, in case of positivity, by intestinal biopsy (Husby et al., 2012). Currently the only effective therapeutic treatment and solution for this disease is to follow, for life, a gluten-free diet (Gao et al., 2018).

Avoiding gluten-free cereals leads to a remission of symptoms and therefore to an improvement of the conditions of the intestinal mucosa that resumes to perform its normal absorbent function (Fasano and Catassi, 2001). A wide range of other conditions can be associated with the CD, like a type 1 diabetes, anemia and other associated diseases are for example autoimmune hepatitis and thyroids, and osteoporosis (Matthias et al., 2011).

Moreover, there are other disorders related to the intake of gluten, for example, allergy to wheat proteins, that is defined as an immune reaction and sensitivity to gluten (Sapone et al., 2012). Figure 2 shows the pathogenesis of wheat related disorders.

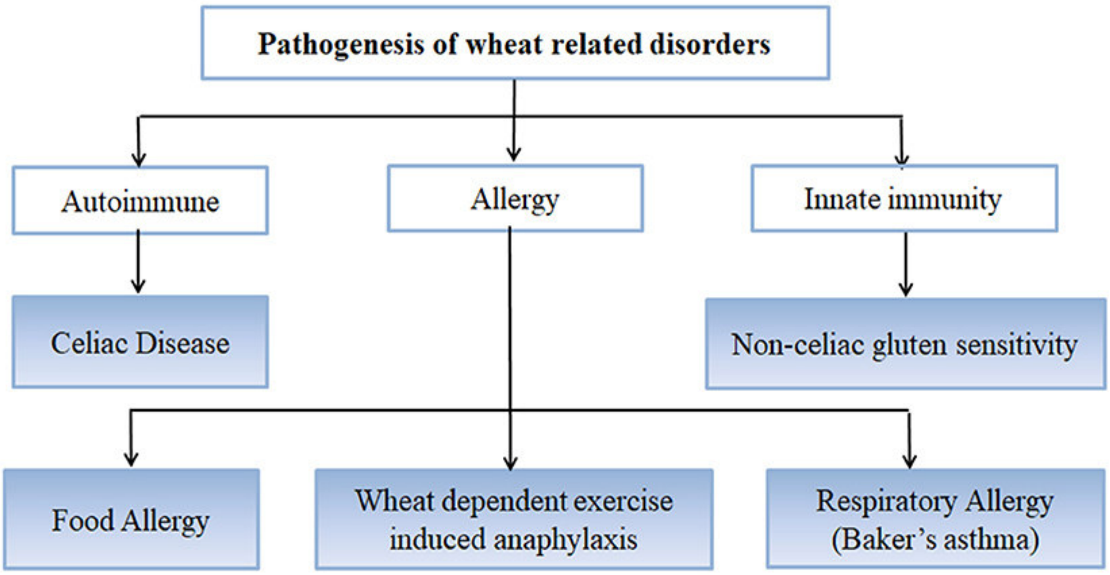

Figure 2. Immune reactions implicated in gluten-related disorders (Sharma et al.,2020).

Gluten-free diet is considered by celiac patients and those who suffer from disorders associated with gluten ingestion, as complex and restrictive, difficult to follow and expensive (Hall et al., 2009). In fact, the price you must "pay" to protect your own psycho-well-being physical is that of the constant and daily attention to diet both because gluten is a protein component widely used and widespread in the diet of the population and because there may be contamination (even minimal) which can be harmful to the patient.

2. Gluten free products

2.1 Raw materials used in the production of gluten-free products

During the last decade, several studies have been directed to the assessment of the impact of gluten-free diet on people with CD based on parameters such as weight, mass, metabolism, and lipid profile, to understand how this treatment can affect their health. According to the definition of gluten-free by Codex Alimentarius, only foods made from naturally gluten-free ingredients which could contain no more than 20 parts per million (ppm) gluten can be called gluten-free products (Codex Alimentarius Commission, 2007).

These products are distinguished by the logo with crossed-out spike present in the label. This symbol, today universally known, guides people with CD to choose safe, packaged foods that meet the specific needs of a gluten-free diet: allows immediate recognition of gluten-free products within the market offer.

A positive correlation has been found between the gluten-free diet and the increased incidence of overweight and obese people (Dickey et al., 2006). The gluten-free diet leads to a remission of the symptoms, as the villi and microvilli return to their original shape, promoting the assimilation of macro and micronutrients, which leads to weight gain in the celiac patient. Additionally, the low palatability of gluten-free foods may shift the consumer's preference towards more flavourful, but more caloric foods, resulting in an increase in daily calorie intake (Kupper, 2005). In addition to the most well-known clinical manifestations of gluten intolerance, there are also new morbid associations such as, with cardiovascular diseases. The scientific community questions the suitability of this diet from a nutritional point of view, in fact, the gluten-free diet is not always able to guarantee adequate nutritional contributions. This problem emerges from recent studies conducted in the last two decades that show how the population may not introduce the recommended amounts of certain basic nutrients such as minerals, vitamins and fiber (Thompson, 2005; Hopman, 2006; Dall'Asta et al., 2012). The inadequacy of these substances could lead to and predispose to the development of diseases such as obesity, constipation, and hypercholesterolemia (See end Murray 2006).

Following a gluten-free diet is undoubtedly difficult, has a strong impact on the quality of life, is expensive and presents numerous problems related to the quality of finished products that, are often less attractive and less compliant in technological, nutritional and sensory characteristics than the gluten containing counterparts (Lerner., 2010). In addition, keeping to a gluten-free diet is difficult due to the possible contamination that may occur during the production process and in the preparation of food, but also because gluten is present in many products as an additive/stabilising agent. However, the scientific medical progress of our day obtained thanks to the detailed knowledge of the pathogenesis of celiac disease is evaluating and studying different therapeutic strategies to gluten-free diet such as genetic manipulation of varieties of wheat to make gluten less toxic, enzymatic degradation, the use of permeability inhibitors and tissue transglutaminase inhibitors, the development of preventive vaccines and gliadin nanoparticles inducing gluten tolerance. (Catassi & Fasano, 2008; Lerner., 2010, Kelly et al., 2021).

As the adoption of genetic manipulation technique, is functional (detoxifies gluten), however, from a technological point of view, it leads to the loss of the viscoelastic characteristics needed in the production of bakery products; Furthermore, genetically modified organisms are often not accepted either by consumers or by the authorities.

To date, the gluten-free diet has proved to be the best and safest strategy in the treatment of coeliac disease. A traditional diet includes the use of cereals such as wheat, barley, spelt, greunker, kamut, rye and their derivatives. These are the main source of complex carbohydrates, but their consumption also guarantees protein, dietary fibre, minerals, vitamins and phytocompounds (Jones, 2006). In the production of gluten-free products, these cereals are replaced with cereal flours that do not contain gluten such as rice and maize, or with oilseed flours such as peanuts and soya and/or with different types of starches such as maize, potato, rice, cassava.

The conventional flours most used to produce gluten-free products are rice and maize. they have a low nutritional value because they contain a low percentage of protein, fibre, iron, folate compared to wheat flour (Hager et al., 2012). As far as starches are concerned, the ones most used in the production of bakery products are rice, potato, maize, and cassava (Houben et al., 2012). On the market, modified starches made from native starches and subjected to enzymatic, chemical, thermal and/or mechanical treatments are also spreading (Houben et al., 2012).

The key role of a starch in the production of gluten-free bread is to retain water, to influence the viscosity and the gelatinization temperature of the dough; they also act as thickeners, stabilize the structure of the dough, and can reduce the phenomenon of starch retrogradation (Houben et al., 2012).

In recent years, with the aim of improving the nutritional and technological aspects of gluten-free bakery products and due to the increasing spread of CD, intolerances and allergies to gluten, researchers and food business operators are being encouraged to respond to the needs of these people by offering a wide variety of commercial gluten-free foods made from alternative raw materials compared to conventional raw materials such as rice and maize.

Among the variants for coeliacs we find "minor cereals" such as fonio, teff, sorghum, millet, panic, chia, and pseudocereals such as buckwheat, quinoa, amaranth. Scientific studies show that both minor cereals and pseudocereals provide an excellent supply of carbohydrates, proteins and dietary fibers (Gutzmán - Maldonado & Paredes-Lopez 1999; Abdel-Aal & Hucl 2002). The quality of gluten-free dietary products is therefore overall improving, not only from an organoleptic point of view but from a technological and nutritional point of view thanks to the use of these new ingredients (De la Barca et al 2010; Alvarez- Juberte et al., 2009). It is of fundamental importance that the gluten-free diet is balanced and varied and for this reason you are always looking for new ingredients.

2.2 Conventional ingredients: Rice and Maize

Rice (*Oryza sativa* L.) is one of the widely cultivated and important gluten free grain for human consumptions in the world, it offers a staple food for more of world's people, mainly in the country of Asia. (Briffaz et al., 2013). It is predominantly composed of starch (78–80%) and protein (6–10%) (Jukanti et al., 2020).

After a series of processes were carried out on the grains of rice, the rice flour obtained is used in many products like as cake, biscuits, rice, crackers, bread etc. Rice flour is one of the most suitable ingredients for gluten-free (GF) bakery formulations due to the absence of gluten in its contents. Moreover, its hypoallergenic properties, low sodium and protein content, white colour, bland taste, and at the same time the presence of easily digested carbohydrates, are some of the benefits of this flour (Rosell, Barro, Sousa, & Mena, 2014). On a technological level, rice flour forms a weak gel network not able to retain gas bubbles during leavening process. Many strategies have shown to improve the rheological properties of the rice gel such asthe addition of hydrocolloids. (Lazaridou., 2007) proteins isolates (Crockett, Ie, & Vodovotz, 2011; Ziobro, Witczak, Juszczak, & Korus, 2013), emulsifiers

(Demirkesen, Mert, Sumnu, & Sahin, 2010), nutritionally relevant fibres (Ronda, Pérez-Quirce, Angioloni, & Collar, 2013 and enzymes (Amin et al., 2017; Renzetti & Rosell, 2016), dough acidification (Villanueva, Mauro, Collar, & Ronda, 2015; Ronda, Villanueva and Collar, 2014) the preparation of an emulsion gel (Yiano et al., 2017), and physical modification such as heat moisture treatment (Bourekoua, Benatallah, Zidoune, & Rosell, 2016; Puncha-arnon & Uttapap, 2013; Qin et al., 2016), heat-pressure processing (Cappa, Barbosa-Cánovas, Lucisano, & Mariotti, 2016; Xu et al., 2016), and application of microwave radiation (Villanueva, Harasym, Munoz, and Ronda 2019).

Maize or corn (*Zea mays* L.) is one of the most cultivated cereals in the world, second only to wheat per cultivated area and rice per total quantity produced. Other than rice, maize is considered the second basic safe ingredients most used in gluten-free bakery products.

The main components of maize are starch and proteins, ranging from 70-75 % and 9-10 % respectively. Among the proteins present, the most abundant are the zein (about 60%). The content of lipid (about 4%), slightly higher than that of rice, is principally constituted by polyunsaturated fatty acids and, especially, linolenic acid (Serna-Saldivar, 2018).

In addition, being a naturally gluten-free cereal, it is traditionally used, both in the form of starch and flour, as a basic ingredient in the production of gluten-free bakery products. However, its use is often associated with some technological limitations related mainly to its smell and the characteristic yellow colour. For this reason, the varieties most frequently used in the gluten-free baking process are those of white maize.

2.3 Alternative ingredients: Millet and Sorghum

Millet (*Panicum miliaceum*) is a cereal with small seeds ranging between 3.5-23 μm (Annor, Marcone, Bertoft, & Seetharaman, 2014a) and includes different variety such as proso millet, foxtail millet, pearl millet, finger millet, kodo millet etc. This cereal is important food in many underdeveloped countries because it has better ability to grow under adverse weather conditions like high temperature, limited rainfall, and infertile soils where other crops tend to fail (Punia et al., 2021). Cereals of this group as considered environmentally friendly because absorb more CO2 and convert it into O2, additionally, have high efficiency of water use, furthermore, have lower inputs required (Kumar, Tomer, Kaur, & Gupta, 2018). Millets are one of the more nutritious cereal comparable to rice and wheat (Saleh et al., 2013), they are non-glutinous, non-acid forming and easy to digest and are good source of energy, protein vitamins, minerals, fatty acids, dietary fiber and polyphenols (Thilagavathi et al., 2015). Regarding to its proteins content, the millet has a good amount of essential amino acids except for threonine and lysine but high quantity of sulphur amino acids content like as methionine. (Singh et al., 2012). These cereals release sugar slowly in the blood and diminish the glucose adsorption (Anderson et al., 1991). However, in addition to the nutritional benefits, it has health benefits effects like prevent cancer and cardiovascular disease (Saleh et al., 2013) There are several millet-based foods and beverages such as couscous, buns, porridge and alcoholic/non-alcoholic beverages (Saleh et al., 2013).

Sorghum (*Sorghum bicolor L.*) is the fifth most important cereal crop in the word after rice, wheat, maize and barley (CCCF,2011), it is used for human and animal consumptions. As in the case of millet also sorghum adapts well to unfavourable climates. It is produced in areas that are too hot and it is one of the currently cultivated cereals that have the best drought tolerance (Ramatoulaye, Mady, Fallou, Amadou et al., 2016).

Sorghum grains are rich in energy and non-energy nutrients. This cereal is utilized for the preparation of food such as pasta, and traditional beverages in industrialized countries. it is also used in the form of grain or fodder in animal feed and to produce bio ethanol. The sorghum demand increases more and more in many developing countries, particularly in West Africa (especially Nigeria, Ghana and Burkina Faso). This is linked both to population growth and partly, politics of these countries that aim to develop the industrial use of sorghum (brewery) to replace the barley malt (Khady et al., 2010).

Phenolic compounds (phenolic acids, flavonoids and tannins) are the most widely represented and ubiquitous secondary metabolites in the plant kingdom but among cereals, sorghum is the richest and can contain up to 6% (Karou et al., 2005; Awika et al., 2004).

Nowadays it is recognized that phenolic compounds are also involved in human health (Khady et al., 2010).

2.4. Alternative ingredients: Fonio (Digitaria exilis Stapf)

In this thesis we have investigated on the fonio (*Digitaria exilis* stapf,), one of the most ancient and important indigenous cereal of west Africa. This cereal also known as fonio or acha, is a naturally gluten-free cereal. Two species are distinguished: acha (*Digitaria exilis*) and iburu (*Digitaria iburua*) (Jideani &Jideani, 2011).

It is cultivated in various parts of Nigeria, Sierra Leone, Ghana, Guinea, Senegal, Togo, and Côte d'Ivoire (Jideani, 1999; Gyang and Wuyep, 2005). This cereal crop deeply contribute to food security in sub-humid and semi-arid areas of West Africa (Adoukonou-Sagbadja et al., 2010; Ibrahim Bio Yerima & Achigan-Dako, 2021) The fonio is not only an interesting gluten free alternative to the usual grains commercially available, various authors define it as a healthy (Roseboro, 2020), it is rich in phytochemicals useful in maintaining low cholesterol levels and linked to reducing cancer risk (Coulibaly et al., 2011).

Fonio is an alternative cereal to European diets, with the possibility, of generating in parallel a profit for local African farmers (Dury et al., 2007). Fonio contributes to the livelihoods of local peoples when other crops are not available (Ibrahim, 2001) and so represents an essential contribution in food security (Zhu, 2020). Biological and chemical properties and utilization foods helps the growth of fonio like a sustainable cereal (Zhu, 2020). This cereal can grow in marginal land, can survive in poor soil conditions, for example in sandy and acidic soils (Arendt & Dal Bello, 2011; Ayenan et al., 2018;).

The National Research Council describes it as an easily digestible cereal that can be employed in many recipes, this makes it an interesting ingredient for the well-being of peoples with gluten disorders, but also for children and management of diabetes. In addition, due to its small size, exceptionally tiny (weight 1000 seeds~ 400 to 500 mg) (Jideani & Akingbala, 1993) this cereal is consumed in its wholegrain form and is therefore suitable for people with constipation problems (Jideani and Akingbala, 1993; Jones, 2009, Ballogou et al., 2013). It has a good contentof fibres and mineral salts such as iron, magnesium, calcium, and zinc (Jideani,1999) and is also rich in sulfur aminoacids such as cystine and methionine (De Lumen et al., 1993; Lasekan, 1994). In West Africa, fonio is the tastiest of all cereals, with a mild flavor unlike other gluten-free alternative cereals such as teff and quinoa. Fonio is a source of protein, starch, and bioactive constituent, like fibres and polyphenols (Zhu, 2020; Chukwurah et al., 2016). In the different research conducted on fonio grains, were recorded significant variations in the proximate composition (e.g., carbohydrates, proteins, and dietary fibre) (Zhu, 2020). The major constituent of fonio grains is carbohydrates, the amounts ranged from 67.1 % (Serna Serna Saldivar,2003) to 91 % (Jideani and Akingbala ,1993), with an average value of about 80 % (Zhu, 2020). In fonio grain, starch is most abundant carbohydrate, about 68% (Crutz et al., 2011). Jideani and Akingbala (1993), determined the amyose content in fonio grain derived from a local Nigerian market. They extracted 28 % of amylase followed the method described by Robyt and Bemis (1967).

The content of proteins ranges from 5.1 % (Fulcher et al., 1981) to 11 % (Fliedel et al., 2004). Only more detailed research has been carried out by Jideani et al., (1994a) on the various Osborne fractions. The fonio grains owned the four-protein fraction principally albumin (3.5 %), globulin (1.8 %), prolamin (5.5 %) and glutelin (14%) (Salaudeen and Orhevba, 2021).

The contents of crude fibre for fonio grains were in order of 0.41% (Jideani and Akingbala (1993) and 11.3 % (Serna Saldivar 2003).

The total lipid content ranges from 1.3% to 5.2% and ash content were 1 to 6 % (Salaudeen and Orhevba, 2021).

Different variations in the compositions could be linked to differences in growing conditions, genetics factors, postharvest processing and experimental variables like a different measurement in the extraction methods.

The composition of this cereal grains is similar to that of white rice (Moreno et al., 2014).

For all these reasons fonio could be a valid alternative to conventional flours normally used in the gluten-free industry allowing to further vary the diet of people with CD. Scarce information is available regarding this cereal, especially regarding the technological quality.

In the common use, fonio is mainly limited to traditional food include porridge, couscous, and alcoholic beverages (Jideani ,1999), recently this ingredient has been used in different food formulations, for example in the preparation of bread, snacks and biscuits like coockies (Ibrahim Bio Yerima & Achigan-Dako, 2021) that could be useful for individual with gluten intolerance. Indeed, Olagunju et al. (2018), have used fonio flour and blanched Pigeon Pea (Cajanus cajan) in different ratios for the development of nutrient cracker. It was found that the high ratio of fonio added (70: 30 respectively) could help as a functional snack in the control of hyperglycaemia and prevention of degenerative pathology associated.

Ayo and Nkama (2003) have investigated the quality of wheat bread enriched with different level of fonio. The additions of more than 30 % of acha negatively affected the final product. Jideani et al, 2007, investigated the quality of bread made only with fonio flour and determined the consumer acceptability for the product. Fonio bread had a loaf comparable to wheat bread in terms of crumb texture and colour, and overall acceptability. Other applications of Fonio in food includes producing sprouts and malts (Ayo et al., 2019; Lasekan et al., 2010; Nzelibe et al., 2000), infant weaning and food for childrens (Agbede et al., 2019; Sosanya et al., 2018) and gluten free drinks (Badejo et al., 2017).

To improve the nutritional profile of fonio grain, a good strategy could be the use of germinated grains (Ayo et al., 2019). The amounts of proteins, lipids, fibre, zinc, iron, and vitamins B1 and B2 showed to increase with the increase of germination time accompanied by a decrease in the starch content and its pasting viscosity (Ayo et al., 2019). This fact could be linked to biochemical reactions and elevated activities of enzymes during the germination phase (Feng et al., 2019).

The European commission (2018) has approved fonio as novel food outside Africa (Zhu, 2020).

3. Technology importance of gluten in bakery products

The main ingredients used for preparing gluten-free (GF) bakery products are maize and rice. The products derived from these ingredients are similar in taste and thus offering a costumer's limited choice. The wide use of rice flour in gluten-free products is due to its bland taste and because it is naturally hypoallergenic. It provides a high amount of digestible carbohydrates but a low amount of proteins, thus indicating the need for other components to reinforce the batter matrix and the nutritional content of the final product (Mandala & Kapsokefalou, 2011). In the last twenty years has grown the interest for new formulations of gluten free bread and other bakery products, whose recipe mainly involve the incorporation of other non-gluten proteins such as dairy proteins, starches of different origin, hydrocolloids, and their combinations (Mariotti, Lucisano, Ambrogina Pagani, & Ng, 2009). The most used starches in GF bakery products are maize starch and potato starch, but also starches from tapioca and rice (Masure, Fieren, & Delcour, 2016). The production of baked goods is a complex process involving several steps from mixing of ingredients, molding and leavening of the dough, baking, and cooling.

The key factor in wheat breadmaking is the gluten protein matrix. Gluten becomes apparent when wheat flour is hydrated and subject to the energy of mixing, even by hand (Capriles and Arêas, 2014). Gluten contributes to the water absorption capacity of the dough, provides extensibility, elasticity, and cohesiveness to bread dough allowing the fermentation gas to be occluded and maintained in the liquid phase during the dough development, leading to well-developed high-grade loaves of bread (Wieser,2007).

The mixing step transforms the mixture of flour, water, and other ingredients into a homogeneous dough. When the dough is mixed the mechanical energy induces conformational changes in hydrated wheat proteins through rupture and formation of covalent (-SS-) and noncovalent (hydrogen and hydrophobic) bonds. The air bubbles are included in the dough to provide gas nuclei for the carbon dioxide generated by yeast fermentation (Sivam et al., 2010).

The leavening step comprises periods that can last from less than an hour to several hours. This phase is based on the fermentation reaction which will transform the glucose or other sugar into carbon dioxide and ethanol. The cooking steps involves the transfer of mass and heat, leading to the transformation of the dough into bread.

The elimination of gluten in baked products has a deleterious effect that is shown in nutritional characteristics of products, quality attributes, and consumer acceptance. Especially in breadmaking, the developed products, have deficient gas retention and the resulting low loaf volume with poor crumb softness. The lack of gluten involves a liquid batter instead of dough, which in turn results in baked bread with a crumbling texture, poor colour, and post baking quality defects (Naqash, Gani, Gani, & Masoodi, 2017). Hydrocolloids are increasingly used to fill these deficits in gluten-free products.

4. The role of hydrocolloids in gluten-free products

The several technological limitations linked to the use of gluten-free ingredients are frequently resolved by mixing them with structuring agents such as proteins isolates (vegetables or animals) and hydrocolloids, able to mimic the viscoelastic properties of gluten.

The hydrocolloids, known as gums, are high molecular weight polysaccharides of different origins and chemical structures and hydrophilic nature. Additionally, these substances are used as processing resources to provide dietary fibre or to impart specific functional properties to the products and improving all acceptability characteristics of the final products (Salehi, 2019a, 2019b).

When the hydrocolloids interact with water, form a network gel structure that increases the viscosity of the dough, reinforces the expanding edges of the alveoli and increases the gas retention capacity during fermentation, leading, in the final stage, to a bread with higher volume and better texture. Moreover, important characteristics are that these hydrocolloids have a neutral flavour and aroma, allowing them free access to food insertion. These gums are employed as anti-staling agents in bread and when are added in a formulation improve the self-life of the bakery products through moisture retention and prevention of syneresis (Ozkoc & Seyhun, 2015).

The classification of hydrocolloids provides for a distinction by their origin (natural or synthetic) or by the functionality they can exercise when included in a food system.

a) Classification by origin divides them into two large groups:

- Natural hydrocolloids, such as guar gum, psyllium fiber, pectins, β-glucans, carrageenan and gum arabic.
- Synthetic hydrocolloids, including synthetic cellulose derivatives such as methylcellulose (MC), carboxymethylcellulose (CMC) and hydroxypropylmethylcellulose (HPMC), and microbial biosynthesis substances like as xanthan gum and gellans.

b) Classification byfunctionalities:

- Thickening agents, which confer viscosity to the system but are not able to form suspensions capable of retaining other substances. These include, for example, guar gum, tara gum, xanthan gum, psyllium fibre, and gum arabic.
- Gelling agents form links intramolecular bonds, can form a three-dimensional lattice within which other substances present in the food matrix could be trapped. These include, for example, cellulose derivatives (MC e HPMC), xanthan gum, gelatin and carrageenan (Hoefler,2004).

Hydrocolloids, in addition to the primary thickening and gelling functions, are capable, when incorporated into a food matrix, of exhibiting other secondary functions which include the ability to stabilize foams, emulsions and dispersions, but also the capability to prevent formation of ice crystals and to regulate the release of the aromas. When these substances are added to a food system, not all behave in the same way. The type, percentage of use, but also the interactions with other substances in the formulation and the process parameters, may affect the effects they have on both the properties of the dough and the characteristics of the final product.

Many studies have been carried out exhibiting the potential use of hydrocolloids in bread- and cake-making including wheat bread, protein-fortified starch bread, rye bread, frozen bread dough, and gluten-free breads (Bourekoua et al., 2016; Gomez et al., 2007; Heinio et al., 2016; Ho & Noor Aziah, 2013; Salehi, 2017).

Hydrocolloids addition to bakery products significantly increased water absorption capacity (Kaur et al., 2015; Salehi, 2017), improved the water-holding capacity (WHC) of the starch aqueous systems and, increased emulsion activity in either combination or singly (Salehi,2019).

4.1 Guar Gum

Guar gum is a gel-forming galactomannan derived by the endosperm of the seeds of the *Cyamopsis tetragonoloba*, belong to Leguminosae family (Mudgil et al., 2014). This plant grown principally in India and Pakistan, it is a most important crop used as humans and animals' food. The world leader to produce guar is India followed by Pakistan, Brazil, Australia, South Africa and Southern part of the USA like Texas or Arizona.

The seeds are broken, and the germ is split up from the endosperm. The guar portion obtained are then treated and finished into powders, well-known as guar gum.

In polar solvent, guar gum swells and or dissolves and forms strong hydrogen bonds, while in nonpolar solvents, weak hydrogen bonds form (Mudgil et al., 2014). Guar gum exhibit pseudoplastic or shear-thinning behaviour in aqueous solutions which means reduction in viscosity with increasing shear rate as showed by many high molecular weight polymers (Torres et al., 2014).

Its capacity to hydrate quickly in cold water systems to confer highly viscous solutions, is the most important property of this gum (Mudgil et al., 2014)

Partial hydrolysis of guar gum increases the dietary fiber content of several food products like beverages without affecting the nutritional and sensory properties of the food products. In food application, guar gum is employed as a novel food additive in a myriad of food products for food stabilization and as fiber source (Morris 2010). It Is preferred by both maker and consumer for it is economical and natural additive. With hydrolysed guar gum it is possible decreased symptoms of irritable bowel syndrome, the laxative effect, incidence of diarrhea (Slavin and Greenberg 2003).

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A study conducted by Giannini et.al, 2006, demonstrated that the guar gum reduced the symptoms in both forms of irritable bowel syndrome i.e., constipation and diarrhea.

Glucose-lowering and cholesterol reduction effects are derived from gel-forming properties of guar gum and additionally, may influence weight loss by decreasing hunger and appetite (Butt et al., 2007). Corrected intake of dietary fiber with this gum improves control of diabetes, reduction of cholesterol, preservation of bowel regularity and prevention of constipation (Yoon et al., 2008).

Numerous studies have been done on animals to test for both harmful and beneficial effect of guar gum.

4.2. Hydroxypropyl methylcellulose

Hydroxypropyl methylcellulose (HPMC) known as E664, is most studied hydrocolloid in scientific research and used in commercial bread (Belorio., 2020). Is derived from chemical modification of cellulose, a major constituent of the many cell walls of plants. The modification make made, confer characteristic of the product unique and more stability during temperature changes and in different characteristics of hydration. (Sabanis and Tzia, 2011). HPMC like other hydrocolloids is a withe powder odourless or tasteless (Burdok., 2007). In the gluten free food systems are used such as stabilizer, emulsifier, suspending agent, thickener and fat substituted.

This hydrocolloid is widely used in a product like as bread. Study conducted on the rice dough containing HPMC had similar rheological properties obtained in wheat dough (Sivaramakrishnan et al., 2004). Gluten free bread (70% sorghum flour, and 30% potato starch) with addition of HPMC confers good quality characteristics (Shobert et al., 2007).

Additional study, conducted by Sabanis and Tzia (2011), suggest that with addition of HPMC, when compared to other hydrocolloids, is able to develop a greater volume in the breads, although these breads have a crumblier and dried texture. Its common combine HPMC with other hydrocolloids such as guar or xanthan gums because it has higher water retention capacities (Horstman et al., 2018).

4.3. Xanthan Gum

Xanthan gum, in the labels also known as E415, was first discovered in the 1960s and commercialized in the 1970s, is obtained by *Xanthomonas campestris*, a single-cell organism found in nature that produces the gum as a protective coating. Xanthan gum is sold as an off-withe powder, is widely employed in food production, for its uncommon characteristics (Hublick., 2012). Several of these properties are linked at its rigid structural conformation (Habibi et al., 2017).

Is a fast-hydrating water-soluble hydrocolloid that can be dissolved at room temperature. For powerful hydration, the xanthan gum particles must be well dispersed in the solvent. The ratio of hydration time decreased with increased the velocity of mixing. When the temperature rises, solution viscosity decreases but recovers almost entirely after cooling. Xanthan gum under unfavourable condition is an excellent stability, either when heated or in the presence of salts and/or acids, additionally exhibit strong synergies with locust bean gum (LBG), tara and guar gum. For its viscoelastic properties to form a weak gel is widely employed in liquid food like sauces, dressing and cake. Used in the formulation of cakes prevents sedimentation of fruit pieces or chocolate before and during baking, and increases volume, slows staling process, and extends freshness. It is used in dairy desserts to support gel.

In dairy desserts, addition of xanthan gum, help gel formers and reduce synaeresis. It is used also in dry mix products like as low-calorie products, beverages and soups. All animal studies conducted, and biochemical and toxicological examinations support its harmlessness.

4.4. Tara Gum

Tara gum, also known as Peruvian carob or E 417, is a powder obtained by grinding the endosperm of seed of the *Caesalpinia spinosa* tree, a plant that derived from Sud America. The major component of this legume gum's is galactomannan polysaccharides (Wu et al., 2015). Tara gum is included as a food additive in several countries, it functions principally as a thickener, stabilizer and gel-forming agent, whose rheological properties are crucial to product viscosity and consistency in the final product.

Tara can reach a high viscosity in water within a few minutes, in cold water is partially soluble whereas in hot water it is totally soluble. This hydrocolloid act synergistically with agar and xanthan to increase gel strength and make it less susceptible to syneresis (Wu et al., 2015). The rate of dissolution of tara gum rises with increasing temperature and with decreasing dimension of the particles. The rate of hydration decreases in the presence of increasing amounts of sugar and/or salt. Tara gum exhibit pseudoplastic or shear thinning behavior in solution. The degree of pseudoplasticity rises with increase concentration and molecular weight. Compared to the other hydrocolloids, tara is more viscous than LGB, but less viscous than guar gum at equal concentration. In addition, tara gum has intermediate characteristics between cold-insoluble LGB and cold-soluble guar, this gives it unique properties as a natural hydrocolloid. Is high-temperature stable, and it is widely used like a stabilizer, emulsifier and thickener in different food products like ice cream, yogurt and frozen dessert. Tara can be used like fat surrogate in low-fat and non-fat food in many food products, bringing obvious benefits.

4.5 Psyllium

Seeds from *Plantago ovata* Forsk, also known as psyllium, have fiber-rich husk which is valued for its nutrients and other properties. This plant is native to the Mediterranean region and nowadays the main global manufacturer of these seeds is India. There is also a significant production in Pakistan and exported worldwide.

The nutritional advantages of psyllium have been discussed by several authors and defined its effects in preventing constipation (Gelinas, 2013), diarrhea, inflammatory bowel disease–

ulcerative colitis, improving the symptoms the irritable bowel syndrome, prevention of colon cancer, hypercholesterolemia, and diabetes (Nazario-Franco et al., 2020).

Many physicians recommend that patients increase their intake of dietary fiber for the treatment of a chronic gastrointestinal disorder. The ingestion of psyllium decreases the risk of metabolic syndrome and cardiovascular disease, acting on the control of cholesterol, glycaemic index, and satiety (Belorio and Gomez, 2020). Additionally at advantaged linked to health body effects, this powder is employed as hydrocolloids for its functional characteristics like a hydrophilic powerful, gelling and emulsified attribute, and stabilizing suspending (Bahamani et al., 2016).

Psyllium is a polysaccharide with the ability to bind water and to generate viscous solutions, this is an important property in the food industry (Cui et al., 2013). This hydrocolloid finds the greatest utilization in the food industry, in bakery products, mainly in gluten-free breads. A study of 228 gluten-free commercial breads, showed that psyllium was present in formulations in 34% of cases and in 16% of as the main gluten substitute (Roman et al., 2019). This author indicated that psyllium to be the fourth most used hydrocolloid, behind only HPMC, xanthan gum and guar gum. However, the addition of psyllium in gluten-free breads is less studied than the other hydrocolloids and its uses are limited to its combination with HPMC (Mancebo et al. 2015). Other important study was conducted in the production of psyllium fiber-enriched biscuits and yogurt with low fat and calorie intake (Fradinho, Nunes & Raymundo, 2015; Ladjevardi, Gharibzahedi & Mousavi, 2015).

5. Gluten free bakery products

Several factors such as markets globalization's, climate-changing, ethics and ethnics affiliations and for healthy choices influence the rapid changes in food diet that, nowadays, are observing.

A specific indicator of new diets changes can be linked in the growing demand for gluten-free products such as people with coeliac, intolerant or allergic to gluten, people with gluten-related problems and people who deliberately choose the gluten-free diets. For that reason, in the last decade the quantity of products available on the shelf of the supermarkets increased exponentially.

Recently it is estimated that the market of gluten-free products (bakery products, pizza, pasta, snack, condiments etc.) will increase from USD 4.18 billion in 2017 to USD 6.47 billion in 2023 (Markets and Markets Research, Gluten-free products Market Global Forecast to 2023). The bakery and confectionery sectors are among the most actively revised by researcher and makers to offer products always more attractive trying for example to improving texture, reducing the calorie intake or use beneficial substances.

Among these, the most globally consumed cereal-based gluten-free foods, are bread and cookies (Jnawali, Kumar, & Tanwar,2016).

Alternatively, to bread, noodles, and pasta, chemically leavened gluten-free products, in particular biscuits, cookies, muffins, cakes, and crackers account for a large portion of bakery goods. A study carried out by Valutti et al. (2017) indicated that, among coeliac people, there is a higher consumption of gluten-free crackers and biscuits rather than gluten-free bread.

Gluten-free bakery goods like biscuits, cookies, muffins, and crackers are mainly prepared by using gluten-free flours like rice, maize, buckwheat, sorghum, lupin, quinoa, chickpea, and others (Di Cairano et al.,2018). There are many articles respect to main gluten-free ingredient, processing technology, and properties of resultant batter, dough and bakery products including rheological property, texture, structure, sensory property, shelf life. Moreover, there are also several articles on functional ingredients like hydrocolloids, emulsifiers, fibre, and proteins, employed to improve the baking quality of gluten-free biscuit, cookie, cake, and crackers.

To our knowledge, there are few articles on a wide requested product such as the rusks with gluten, and no information are available on gluten-free rusks. In 2000, Yaseen was the first to work on this type of product by developing a new formulation of rusks enriched with fibre for commercial scale production.

The investigation carried out by Gupta et al., (2011), is related to use of barley flour incorporation at different percentage in high-fiber rusks.

Punia et al. (2020) conducted a study to evaluate the phenolic acid composition, antioxidant potential and acceptance of rusk obtained by substitution of wheat flour with barley flour.

Lazaridou et al. (2018), have studied the impact of flour particle size and autoclaving on dough thermomechanical properties and rusk quality features and found that these processes are appropriate for the making of enriched-β-glucan rusks with promoted health benefits. Zeppa, Bertolino & Rolle (2011), conducted a study of texture analysis of different commercial rusks. The first study of the effect of the incorporation of 3 different flax-seed cultivars to wheat rusks are carried out by Kaur et al. (2017). That are found that the addition of 20% of barley flour produced sensory acceptable rusks.

More recently, another research, conducted by Lohan et al. (2020) has developed rusks enriched in fibre and essential fatty acids with flax seeds and Finger millet. Other research on rusks concerns their shelf life (Filipović et al., 2012; Marconi et al., 2020).

In this thesis we wanted to contribute to fill the lack of information that exists on gluten-free rusks. Using a basic formulation with conventional flours more widely used and testing different hydrocolloids in different percentages were made of rusks and were then evaluated based on the texture and colour.

References

Abdel-Aal and P. Hucl E.-S.M., and Hucl, P. (2002). Amino Acid Composition and In Vitro Protein Digestibility of Selected Ancient Wheats and their End Products. *Journal of Food Composition and Analysis*. 15 (6): 737-747.

Adoukonou-Sagbadja, H., Wagner, C., Ordon, F., & Friedt, W. (2010). Reproductive system and molecular phylogenetic relationships of Fonio millets (Digitaria Spp., Poaceae) with some polyploid wild relatives. *Tropical Plant Biology*, 3, 240–251.

Agbede, J.O., Omotoso, F.P., Oloruntola, O.D., Ayeni, O.A., & Aletor, V.A., (2019). A preliminary study of fonio-moringa seed meal-based complementary food in Wistar rats. *Journal of Food Biochemistry*, 43, e13010.

Amin, T., Naik, H.R., Hussain, S.Z., Rather, A. H., Murtaza, I., & Dar, B. N. (2017). Structural properties of high- protein, low glycemic index (GI) rice flour. *International Journal of Food Properties*, 20, 2793-2804.

Anderson J.W, Hamilton, C.C, Horn, J.L., Spencer, D.B., Dillon, D.W, Zeigler, J.A. (1991). Metabolic effects of insolubile oat fibre in lean men with type II diabetes. *Cereal chemistry*, 68 (5), 291-4.

Annor, G.A., Marcone, M., Bertoft, E., & Seetharaman, K. (2014 a). Physical and molecular characterization of millet starches. *Cereal chemistry*, 91 (3),286-292.

Anton, A. and Artfield, S. (2008) Hydrocolloids in Gluten-Free Breads: A Review. *International Journal of Food Sciences and Nutrition*. 59, 11-23.

Alvarez-Jubete L., Auty M., Arendt E. K. (2010). Gallagher E. Baking properties and microstructure of pseudocereal flours in gluten-free bread formulations. E*uropean Food Research and Technology.* 230: 437-445.

Arendt, E.K., Dal Bello, F. (2011). Gluten-free cereal, products and beverages. *Food Science and Technology*. Massachussett: Academic Press.

Aronsson, C.A., Lee H.S., Liu E., et al. (2015). Age at gluten introduction and risk of celiac disease. *Pediatrics*, 135, 239-245.

Ayenan, M. A. T., Sodedji, K. A. F., Nwankwo, C. I. K., Olodo, F., & Alladassi, M. E. B. (2018). Harnessing genetic resources and progress in plant genomics for fonio (Digitaria spp.) improvement. *Genetic Resources and Crop Evolution*, 65, 373–386.

Ayo, J.A., Nkama, I. (2003). Effect of acha (*Digitaria exilis* Staph) hrain flours on the physical and sensory quality of biscout. *Nutrition and Food Science*,33,125-130.

Ayo, J.A., & Ayo, V.A. (2018). Proximate composition, Physical and sensory quality of acha-moringa seed flour blend biscuits. *Asian Food Science Journal*, 5, 1-7.

Awika, JM, & Rooney, LW (2004). Sorghum phytochemicals and their potential impact on human health. *Phytochemistry*, *65*(9), 1199-1221.

Badejo, A.A., Olawoyin, B., Salawu, S.O., Fasuhanmi, O.S., Boligon, A.A., & Enujiugha, V.N. (2017). Antioxidative potentials and chromatographic analysis of beverage from blends of gluten-free acha (*Digitaria exilis*) and tigernut (*Cyperus esculentus*) extracts. *Food Measure*, 11, 2094-2101.

Bahamani, M., Mirhosseini, M., Fasihzadeh, S., Karimian, P., Rafieian-Kopaei, M. (2016). Plantago: A plant for internist. *Der Pharma Chemica*, 8(2), 84-91.

Ballogou, V. Y.; Soumanou, M. M.; Toukourou F.; Hounhouigan, J. D. (2013). Structure and nutritional composition of fonio (*Digitaria exilis*) grains: a review. *International Research Journal of Biological Sciences*, 2 (1), 73-79

Belorio M, Gómez M. (2020). Effect of Hydration on Gluten-Free Breads Made with Hydroxypropyl Methylcellulose in Comparison with Psyllium and Xanthan Gum. *Foods*, 9 11.

Belorio M, Gómez M. (2020). Psyllium: a useful functional ingredient in food systems. Crit Rev *Food Science and Nutrition*, 21,1-12.

Bourekoua, H., Benathalla, L., Zidoune, M.N., & Rossell, C.M. (2016). Developing gluten-free bakery improvers by hydrotermal treatment of rice and corn flours. Lebensmittel-Wissenschaft und_Technologie- *Food Science and Technology*. 73, 342-350.

Bozzola, Mauro & Meazza, Cristina & Maggiore, Giuseppe & Nastasio, Silvia. (2014). *Celiac disease*: An update.

Bravi, E., Sileoni, V., Perretti, G., Marconi, O. (2020). Accelerated shelf-life model of gluten-free rusks by using oxidation indices. *Food Chemistry*. 326, 126971.

Briani, C., Samaroo, D., & Alaedini, A. (2008). Celiac disease: From gluten to autoimmunity. *Autoimmunity Reviews*, Vol. 7, pp. 644–650.

Briffaz, A., Mestres, C., Matencio V, Pons, B., Dornier, M. (2013). Modelling starch phase transitions and water uptake of rice kernels during cooking. *Journal of Cereal Science.* 58, (3), 387-292.

Burdock, G.A. (2007) Safety assessment of hydroxypropyl methylcellulose as a food ingredient. *Food Chemical Toxicology Journal*; 45(12):2341-51.

Butt, M:S., Shahzadi, N, Sharif, M.K.,Nasir, M. (2007). Guar Gum: A Miracle Therapy for Hypercholesterolemia, Hyperglycemia and Obesity. *Critical Reviews in Food Science and Nutrition, 47, (4), 389-396.*

Cabras, P., & Martelli A. (2004) Chimica degli alimenti. Piccin Nuova Libreria s.p.a, Padova.

Cabrera-Chávez, F., and Calderon de la Barca, A. (2010). Trends in wheat technology and modification of gluten proteins for dietary treatment of coeliac disease patients. *Journal of Cereal Science*, 52, 337-341.

Cappa, C., Barbosa- Canovas, G.V., Lucisano, M., & Mariotti, M. (2016). Effect of high-pressure processing on the baking aptitude of corn starch and rice flour. *Lebensmittel-Wissenschaft und_Technologie- Food Science & Technology*, 73, 20-27.

Capriles, V. D., & Arêas, J. A. G. (2014). Novel approaches in gluten-free breadmaking: Interface between food science, nutrition, and health. *Comprehensive Reviews in Food Science and Food Safety, 13*(5), 871–890.

Catassi, C., & Fasano, A. (2008). Celiac disease. In Gluten-Free Cereal Products and Beverages (pp. 1–27).

Comino I., Moreno Mde L., Real A. , Rodríguez-Herrera A., Barro F. (2013). The gluten-free diet: testing alternative cereals tolerated by celiac patients. *Nutrients,*5, 4250-4268.

Conte, P., Fadda, C., Drabinska, N., Krupa-Kozak, U. (2019). Thecnological and nutritional challenges, and novelty in gluten-free breadmaking: a review. *Polish Journal and Nutrition Sciences.* p. 5-21.

Chukwurah, P. N., Uyoh, E. A., Usen, I. N., Ekerette, E. E., & Ogbonna, N. C. (2016). Assessment of intra and inter species variation in antioxidant composition and activity in marginalized fonio millet (Digitaria spp.). *Journal of Cereals and Oilseeds*, 7, 1−6.

Coulibaly, A., B. Kouakou and J. Chen. (2011). Phytic Acid in Cereal Grains Structure, Healthy or Harmful Ways to Reduce Phytic Acid in Cereal Grains and Their Effect on Nutritional Quality. *Americam Journal of Plant Nutrition and Fertilization Technology,* 1:1-22.

Crockett, R., Ie, P., & Vodovotz, Y. (2011). Effect of soy protein isolate and egg withe solids on the physicochemical properties of gluten- free bread. *Food Chemistry*, 129, 84-91.

Cruz J-F., Beavogui F. and Drame D. (2011). Le fonio, une céréale africaine. Agricultures tropicales en poche. Quae / Cta / Presses agronomiques de Gembloux. Versailles, France. 175p.

Cui S.W., Wu, Y., Ding H., (2013). The range of dietary fibre ingredients and a comparison of their technical functionality. *Fibre-Rich and Wholegrain Foods*, Jan A. Delcour and Kaisa Poutanen 96-119.

Dall'Asta, C., Scarlato, A.P., Galaverna, G., Brighenti, F. and Pellegrini, N. (2012). Dietary exposure to fumonisins and evaluation of nutrient intake in a group of adult celiac patients on a gluten-free diet. *Molecular Nutrition & Food Research,* 56, 632-640.

De Lumen, B.O., Thompson, S., Odegard, J.W. (1993). Sulphur amino acid rich proteins in acha (*Digitaria exilis*), a promising underutilized African cereal. *Journal of Agricultural and Food Chemistry,* 41,1045–1047.

Demirkensen, I., Mert, B., Sumnu, G., & Sahin, S. (2010). Rheological properties of gluten-free bread formulations. *Journal of Food Engineerig*, 96, 295-303.

Di Cairano, M., Galgano, F., Tolve, R., Caruso, M. C., & Condelli, N. (2018). Focus on gluten free biscuits: Ingredients and issues. *Trends in Food Science & Technology*, 81, 203–212.

Dickey, William M.D., Ph.D., F.A.C.G.; Kearney, Natalie B.Sc. (2006). Overweight in Celiac Disease, *American Journal of Gastroenterology* 101 (10) 2356-2359.

Dury S., Meuriot V., Fliedel G., Blancher B.G.F., Drame D. (2007). The retail market prices of fonio reveal the demand for quality characteristics in Bamako, Mali. In: Communication at 106th seminar of the Europian Association of Agricultural Economists. “Pro-poor development in low-income countries: food, agriculture, trade, and environment”. Montpellier, France.

European Commission. (2018). Fonio: EU novel food approval. *Official Journal of the European Union*, 61, L323.

Fasano A., Catassi C. (2001). Current approaches to diagnosis and treatment of celiac disease: an evolving spectrum. *Gastroenterology.* 120, 636–651.

Fasano, A., Catassi, C. (2005). Coelic disease in children. *Best Pract Res Clin Gastroenterol*. 19(3), 467-78.

Feng, H., Nemzer, B., & Devries, J. (2019). Sprouted grains: Nutritional value, production, and applications (1st ed.). Duxford, UK: Wood head Publishing

Filipović, N., Lazić, V., Filipovic, J., Gvozdenović, J., and Novaković, D. (2012). Packaging material characteristics contributing to shelf-life of rusk. *Romanian Biotechnological Letters.* Vol 17 (2).

Fliedel G., Ouattara M., Grabulos J., Dramé D. and Cruz J. (2004). Effet du blanchiment mécanique sur la qualité technologique, culinaire et nutritionnelle du fonio, céréale d'Afrique de l'Ouest. In: Brouwer Inge D., Traoré Alfred S. and Trèche S., (ed.) Voies alimentaires d'amélioration des situations nutritionnelles en Afrique de l'Ouest: Le role des technologues alimentaires et des nutritionnistes : actes du 2e Atelier international, Ouagadougou, 23-28 nov. 2003. Ouagadougou: Presses universitaires de Ouagadougou, Burkina Faso, 599-614.

Fradinho, P., Nunes, M. C., & Raymundo, A. (2015). Developing consumer acceptable biscuits enriched with Psyllium fibre. *Journal of Food Science & Technology*, 52(8), 4830–4840.

Fritz R.D., Chen Y. (2017). Kernel-based gluten contamination of gluten-free oatmeal complicates gluten assessment as it causes binary-like test outcomes. International *Journal of Food Science & Technology*, 52, 359-365.

Fulcher R.G., O'Brien T.P. and Wong S.I.(1981). Microchemical detection of niacin, aromatic amine and phytin reserves in cereal bran, *Cereal Chem*. 58, 130-135

Gao Y., Janes M.E., Chaiya B., Brennan M.A., Brennan C.S., Prinyawiwatkul W. (2018). Gluten-free bakery and pasta products: Prevalence and quality improvement. *Journal of Food Science & Technology*. 53 ,19-23.

Giannini, E.G, Mansi, C., Dulbecco, P., Savarino, V. (2006). Role of partially hydrolyzed guar gum in the treatment of irritable bowel syndrome. *Nutrition*, 22(3),334-42.

Gyang, J.D. and Wuyep, E.O. (2005). Acha: The grain of life. A Bi-annual Publication of the Raw Material Research and Development Council. Vol. 6 No. 1,39-41.

Gupta, M., Bawa, A.S. and Semwal, A.D. (2011), Effect of barley flour blending on functional, baking and organoleptic characteristics of high-fiber rusks. *Journal of Food Processing and Preservation*, 35,46-63.

Gupta N., Srivastava, A.K., PandeyV.N. (2012). Biodiversity and nutraceutical quality of some indian millets: Proceedings of the National Academy of Sciences, India section B*: Journal of Biological Science*, 82, 265-273.

Gutzmán-Maldonado, S. H., and Paredes-Lopez, O. (1999). Functional products of plants indigenous to Latin America: Amaranth, quinoa, common beans, and botanicals. Pages

293-328 in: Functional Foods. Biochemical and Processing Aspects. G. Mazza, ed. Technomic Publishing: Lancaster, PA.

Habibi, H., and Khosravi-Darani, K. (2017). Effective variables on production and structure of xanthan gum and its food applications: A review. *Biocatalysis and Agricultural Biotechnology*,10, 130-140.

Hager, A.S, Wolter, A., Jacob, F., Zannini, e., Arendt, E.K. (2012). Nutritional properties and ultra-structure of commercial gluten free flours from different botanical sources compared to wheat flours. *Journal of Cereal Science*, 56 (2), 239-247.

Hager, A. S., Wolter, A., Czerny, M., Bez, J., Zannini, E., Arendt, E. K., & Czerny, M. (2012). Investigation of product quality, sensory profile and ultrastructure of breads made from a range of commercial gluten-free flours compared to their wheat counterparts. *European Food Research and Technology*, 235(2), 333–344.

Hallert, C., Grant, C., Grehn, S., Grännö, C., Hultén, S., Midhagen, G., Ström, M., Svensson, H., Valdimarsson. T. (2002). Evidence of poor vitamin status in coeliac patients on a gluten-free diet for 10 years. *Alimentary Pharmacology & Therapeutics*,16(7):1333-9.

Hall, N.J., Rubin, G. and Charnock, A. (2009), Systematic review: adherence to a gluten-free diet in adult patients with coeliac disease. *Alimentary Pharmacology & Therapeutics*, 30, 315-330.

Heinio, R. L., Noort, M. W. J.,Katina, K.,Alam, S. A., Sozer, N., &de Kock, H. L.(2016).Sensory characteristics of wholegrain and bran-rich cereal foods - a review.*Trends in Food Science & Technology*,47,25–38.

Ho, L. H., &Noor Aziah, A. A. (2013). Dough mixing and thermal properties including the pasting profiles of composite flour blends with added hydrocolloids. *International Food Research Journal*,20,911–917.

Hoefler, A.C. (2004) Functions and Properties. Hydrocolloids, Eagan Press Ed. American Association of Cereal Chemist. St. Paul, Minesota, 27-41.

Hopman, E.G., le Cessie, S., von Blomberg, B.M., Mearin, M.L. (2006). Nutritional management of the gluten-free diet in young people with celiac disease in The Netherlands. *Journal of Pediatric Gastroenterology and Nutrition*; 43(1):102-8.

Horstmann, S.W, Axel, C., and Arendt, E.K. (2018). Water absorption as a prediction tool for the application of hydrocolloids in potato starch-based bread. *Food Hydrocolloids*, 81, 129-138.

Houben, A., Höchstötter, A., & Becker, T. (2012). Possibilities to increase the quality in gluten-free bread production: An overview. *European Food Research and Technology*, 235(2), 195–208.

Hujoel, I.A., Reilly, N.R., Rubio-Tapia, A. (2019) Celiac Disease: Clinical Features and Diagnosis. *Gastroenterology Clinics of North America*, 48(1),19-37.

Husby, S., Koletzko, S., Korponay-Szabó, I.R., Mearin, M.L., Phillips, A., Shamir, R., Troncone, R., Giersiepen, K., Branski, D., Catassi, C., Lelgeman, M., Mäki, M., Ribes-Koninckx, C., Ventura, A., Zimmer, K.P. (2012). European Society for Pediatric Gastroenterology, Hepatology, and Nutrition Guidelines for the Diagnosis of Coeliac Disease, *Journal of Pediatric Gastroenterology and Nutrition*: 54,136-160.

Ibrahim, A. (2001). Hungry rice (Fonio): A neglected cereal crop. *NAQAS Newsletter.1*(4), 4-5.

Ibrahim Bio Yerima, A.R., Achigan-Dako, E.G. 2021. A review of the orphan small grain cereals improvement with a comprehensive plan for genomics-assisted breeding of fonio millet in West Africa. *Plant Breeding, 140, 561-574.*

Jideani, I.A., Jideani, V.A. (2011). Developments on the cereal grains. *Digitaria exilis* (acha) and *Digitaria exilis iburua* (iburu). *Journal Food Science & Technology*, 48,251-259.

Jideani I.A. (1999). Traditional and possible technological uses of *Digitaria exilis* (fonio) and *Digitaria iburua* (iburu): a review, *Plant Foods for Human Nutrition*. 54, 363-374.

Jideani I.A. (1990). Acha, *Digitaria exilis*, the neglected cereal, *Agriculture Interntional*, 42, 132-134.

Jideani I.A., Akingbala J.O., 1993. Some physicochemical properties of acha (*Digitaria exilis*) and iburu (*Digitaria iburua*), *Journal of the Science of Food and Agriculture*, 63, 369-374.

Jideani, I.A., Owusu, R.K., Muller H.G., 1994a. Proteins of acha (*Digitaria exilis* Stapf): solubility fractionation, gel filtration and electrophoresis of protein fractions. *Food Chemistry*, 51:51–59.

Jideani V.A., Alamu R., Jideani I.A., 2007. Preliminary study into the production of non-wheat bread from acha (*Digitaria exilis*) *Nutrition and Food Science*, 37:434–441.

Jnawali, P., Kumar, V., & Tanwar, B. (2016). Celiac disease: Overview and considerations for development of gluten-free foods. *Food Science and Human Wellness*,*5*(4),169-176.

Jones, J.M. (2009). The /second C and E Spring Meeting and Third International Whole Grain Global Summit, *Cereals Foods World*. 54:132-135.

Jones, Richard, B., Robins, Gerry G., Howdle, Peter D. (2006). Advances in celiac disease. Current Opinion in *Gastroenterology*; 22 (2),117-123.

Jukanti, A.K., Pautong, P.A., Liu, Q., Sreenivasulu, N.,2020. Low glicemic-index rice a desired trait in starchy staples. *Trends Food Science & Technology*,106, 132-149.

Kagnoff, M. F. (2005). Overview and pathogenesis of celiac disease. *Gastroenterology,* 128 (41), S10–S18.

Karou, D., Dicko, M. H., Simpore, J., & Traore, A. S. (2005). Antioxidant and antibacterial activities of polyphenols from ethnomedicinal plants of Burkina Faso.*African Journal of Biotechnology*, *4*(8), 823-828.

Kasarda D.D., 2001. Grains in relation to celiac disease. *Cereal Foods World*, 46:209–210.

Kaur, R., Kaur, M., Purewal, S. P. (2017). Effect of incorporation of flaxseed to wheat rusks: Antioxidant, nutritional, sensory characteristics, and in vitro DNA damage protection activity. *Journal of Processing and Preservation*, 42: e13585.

Kaur, Maninder and Sandhu, Kawaljit and Arora, AmitPal and Sharma, Aruna (2015). Gluten free biscuits prepared from buckwheat flour by incorporation of various gums: Physicochemical and sensory properties. *Food Science & Technology*, 63, 628-632.

Kelly CP, Murray JA, Leffler DA, Getts DR, Bledsoe AC, Smithson G, First MR, Morris A, Boyne M, Elhofy A, Wu TT, Podojil JR, Miller SD; TAK-101 Study Group. TAK-101 Nanoparticles Induce Gluten-Specific Tolerance in Celiac Disease: A Randomized, Double-Blind, Placebo-Controlled Study. *Gastroenterology*, 2021 Jul;161(1):66-80 e8.

Khady, B., Tine, T., Destain, J., Cisse, N., Thonart, F. (2010). Comparative study of phenolic compounds, the antioxidant power of various Senegalese sorghum varieties and amylolytic enzymes of their malt. *Biotechnology, Agronomy and Society*. 14 (1),131-139.

Koh, L. W., Kasapis, S., Lim, K. M., Foo, C.W., 2009. Structural enhancement leading to retardation on in vitro digestion of rice dough in the presence of alginate. *Food Hydrocolloids*, 23(6), 1458-1464.

Kumar, A., Tomer, V., Kaur, A., Kumar, V., & Gupta, K., (2018). Millets: A solution to agrarian and nutritional challenges. *Agriculture & Food Security*, 7(1)31.

Kupper C. (2005). Dietary guidelines and implementation for celiac disease. *Gastroenterology*,128(4 Suppl 1).

Ladjevardi, Z. S., Gharibzahedi, S. M. T., & Mousavi, M. (2015). Development of a stable low-fat yogurt gel using functionality of psyllium (Plantago ovata Forsk) husk gum. *Carbohydrate Polymers*, 125, 272–280.

Lasekan, D.O., 1994. Chemical composition of acha (*Digitaria exilis*) flour. *Journal of the Science of Food and Agriculture*, 14:177–179.

Lasekan, O., Salva, J.T, & Abbas, K. (2010). Effect of malting conditions and quality characteristics of malt and roasted malt extract from 'acha' grains (*Digitaria exilis* Stapf). *Journal of the Science of Food and Agriculture*, 90,850-860.

Lazaridou, A.; Duta, D.; Papageorgiou, M.; Belc, N.; Biliaderis, C.G. (2007). Effects of hydrocolloids on dough rheology and bread quality parameters in gluten-free formulations. *Journal of Food Engineering*. 79, 1033–1047.

Lazaridou, A., Marinopoulou, a., Biliaderis, C.G. (2019). Impact of flour size and hydrothermal treatment on dough rheology and quality of barley rusks. *Food Hydrocolloids*. 87, 561-569.

Lerner, A. (2010). New therapeutic strategies for celiac disease. *Autoimmunity Reviews*, 9(3), 144–147.

Levent, H., & Bilgicli, N., (2011) Enrichment of gluten free cakes with lupin (*Lupinus albus* L.) or buckwheat (*Fagopyrum esculentum* M.) flours. *International Journal of Food Sciences & Nutrition*, 62(7),725-728.

Lohan, A., Kaushik, R., Bansal, V., Gandhi, K. (2018). Flax seeds and Finger Millet Enriched Functional Rusk. *International Journal of Food Studies.* Vol 9: 213-224.

Ludvingsson J.F., Leffler D.A., Bai J.C., et al. (2012). The Oslo definition for coeliac disease and related terms. *Gut*,62, 43-52.

Mancebo, C. M., San Miguel, M. Á., Martínez, M. M., & Gómez, M. (2015). Optimisation of rheological properties of gluten-free doughs with HPMC, psyllium and different levels of water. *Journal of Cereal Science*, *61*, 8-15.

Mandala, I., & Kapsokefalou, M. (2011). Gluten-Free Bread: Sensory, Physicochemical, and Nutritional Aspects. Flour and Breads and Their Fortification in Health and Disease Prevention, 161–169.

Marco, C., and Rosell, C., (2008). Breadmaking performance of protein enriched, gluten-free breads. *Euroean Food Research and Technology*, Vol 227, 1205-1213.

Martson, K., Khouryieh, H., & Aramouni, F. (2016). Effect of heat treatments of sorghum flour on the functional properties of gluten free bread and cake. LWT- *Food Science & Technology*,65, 637-644.

Mariotti, M., Lucisano, M., Ambrogina Pagani, M., & Ng, P. K. W. (2009). The role of corn starch, amaranth flour, pea isolate, and Psyllium flour on the rheological properties and the ultrastructure of gluten-free doughs. *Food Research International*, 42(8), 963–975.

Marquart L., Mclntosh G.H., Poutanen K., Reicks M. (2007). Whole grains and health. UK: *Blackwell Publishing*.

Matthias, T., Neidhöfer, S., Pfeiffer, S., Prager, K., Reuter, S., & Gershwin, M. E. (2011). Novel trends in celiac disease. *Cellular and Molecular Immunology,* 8(2), 121-125.

Moore M. M., Schober T. J., Dockery P., Arendt E. K. (2004). Textural comparisons of gluten-free and wheat-based doughs, batters, and breads. *Cereal Chemistry*, 81(5), 567-575.

Moreno, M.L., Comino I., Sousa, C. (2014). Alternative Grains as Potential Raw Material for Gluten-Free Food Development in The Diet of Celiac and Gluten-Sensitive Patients. *Austin Journal of Nutrition and Food Sciences*, 2(3), 1016.

Morris, J.B., (2010) Morphological and reproductive characterization of guar (Cyamopsis tetragonoloba) genetic resource regenerated in Georgia, USA, *Genetics Resources and Crop Evolution., 57*, 985-993

Mudgil, D., Barak, S., Khatkar, B.S. (2014). Guar gum: processing properties and food application –A Review. *Journal of Food Science & Technology*, 51, 409-418.

Naqash, F., Gani, A., Gani, A., & Masoodi, F. A. (2017). Gluten-free baking: Combating the challenges - A review. *Trends in Food Science & Technology*, 66, 98–107.

Nazario-Franco, E.A.N., Sanches-Silva, A., Ribeiro-Santos and Ramos de Melo, N. (2020). Psyllium (*Plantago ovata* Forsk): From evidence of health benefits to its food application. *Trends in Food Science & Technology.* 96, 166-175.

Niewinski, M.M. (2008). Advances in celiac disease and gluten-free diet. *Journal of the American Dietetic Association,*108(4),661-72.

Nzelibe, H.C., Obaleye, S., & Onyenekwe, P.C. (2000). Malting characteristics of different varieties of fonio millet (*Digitaria exilis*). *European Food Research & Technology*, 211, 126-129

Olagunju, AI, Omoba, OS,Enujuigha, VN,Aluko, RE.(2018). Development of value-added nutritious crackers with high antidiabetic properties from blends of *Acha* (*Digitaria* *exilis*) and blanched Pigeon pea (*Cajanus cajan*). *Food Science & Nutrition*, 6,1791–1802.

Öksüz, t., and Karakaş, B. (2016) Sensory and Textural evaluation of gluten-free biscuits containing buckwheat flour. *Cogent Food & Agriculture.* 2(1), 1178693.

Ozkoc, S.o. and Seyhun, N. (2015). Effect of gum type and flaxseed concentration on quality of gluten-free breads made from frozen dough baked in infrared-microwave combination oven. *Food and Bioprocess Technology*, 8(12), 2500-2506.

Pérez_Quirce, S., Collar, C., & Ronda, F., 2014. Significance of healthy viscous dietary fibres on the performance of gluten-free rice based formulated breads. *International Journal of Food Science & Technology,* 49, 1375-1382.

Puncha-arnon, S., & Uttapap, D., 2013. Rice starch vs rice flour: Differences in their properties when modified by heat-moisture treatment. *Carbohydrate Polymers*, 91, 85-91.

Punia, S., Kumar, M., Siroha, A.K., Kennedy, J.F., Dhull, S.B., Witheside, W.S. (2021). Pearl millet grain as an emerging source of starch: A review on its structure, physicochemical properties, functionalization, and industrial applications. *Carbohydrate Polymers,* 260, 117776.

Punia, S., Sandhu, K.S., Kaur, M. (2020). Quantification of phenolic acids and antioxidant potential of wheat rusks as influenced by partial replacement with barley flour. *Journal of Food Science & Technology,* 57 (10),3782-3791.

Qin, Y., Liu, C., Jiang, S., Cao, J., Xiong, L., & Sun, Q. (2016). Functional properties of glutinous rice flour by dry- heat treatment. *PLos One*, 11, e0160371.

Ramatoulaye, F. Mady, C., Fallou, S., Amadou, K., Cyril. D. (2016). Production and Use Sorghum: A Literature Review. *Journal of Nutrition Health & Food Science,* 4(1), 1-4.

Renzetti, S., & Rosell, C. M. (2016). Role of enzymes in improving the functionality of proteins in non-wheat dough systems. *Journal of Cereal Science*, 67,35-45.

Robyt J.F. and Bemis S. (1967). Use of the autoanalyser for determining the blue value of the amyloseiodine complex and total carbohydrate by phenol-sulfuric acid, *Anal Biochem*., 19 56-60

Roman, L., Belorio, M. and Gomez, M. (2019), Gluten-Free Breads: The Gap Between Research and Commercial Reality. Comprehensive Reviews in *Food Science and Food Safety*, 18, 690-702.

Ronda, F., Pérez_Quirce, S., Angioloni, A., & Collar, C. (2013). Impact of viscous dietary fibres on the viscoelastic behaviour of gluten-free formulated rice doughs: A fundamental and empirical rheological approach. *Food Hydrocolloids*, 32, 252-262.

Ronda, F., Villanueva, M., Collar, C. (2014). Influence of acidification on dough viscoelasticity of gluten-free rice starch-based dough matrices enriched with exogenous protein. LWT - *Food Science & Technology,* 59(1), 12-20.

Roseboro, K. (2020, May 2). Fonio: The tiny grain with big benefits. The Organic & Non-GMO Report. Retrieved from https://non-gmoreport.com/articles/terra-ingredients-tointroduce-african-ancient-grain-to-u-s-market/ Roseboro, K. (2020, May 2). Fonio: The tiny grain with big benefits. The Organic & Non-GMO Report. Retrieved from https://non-gmoreport.com/articles/terra-ingredients-tointroduce-african-ancient-grain-to-u-s-market/

Rosell, C.M., Barro, F., Sousa, C., & Mena, M. C. (2014). Cereals for developing gluten-free products and analytical tools for gluten detection. *Journal of Cereal Science*, 59, 12-20.

Sabanis D, Tzia C. Effect of hydrocolloids on selected properties of gluten-free dough and bread. *Food Science and Technology International*. 2011;17(4):279-291.

Saleh, A.S., Zhang, Q., Chen, J., & Shen, Q. (2013). Millet grains: nurtritional quality, processing, and potentisl health benefits. Comprehensive Review in *Food Science and Food Safety*, 12(3), 281-295.

Salehi, F. (2017). Rheological and physical properties and quality of the new formulation of apple cake with wild sage seed gum (*Salvia macrosiphon*). *Journal of Food Measurement and Characterization*,11(4),2006–2012.

Salehi, F. (2019). Improvement of gluten-free bread and cake properties using natural hydrocolloids: A review.*Food Science & Nutrition,* 7:3391–3402.

Salehi, F.(2019a). Characterization of different mushrooms powder and its application in bakery products: A review.*International Journal of Food Properties*, 22(1),1375–1385.

Salehi, F.(2019c). Effect of common and new gums on the quality, physical and textural properties of bakery products: A review.*Journal of Texture Studies*, 1–12.

Sapone A., Bai J.C., Ciacci C., Dolinsek J., Green P.H. (2012). Spectrum of gluten-related disorders: consensus on new nomenclature and classification. *BMC Medicine,* 10, 13.

Schober, T.J.; Bean, S.R.; Boyle, D.L. (2007). Gluten-free sorghum bread improved by sourdough fermentation: biochemical, rheological, and microstructural background. *Journal of Agricultural and Food Chemistry*, 55 (13), 5137–5146

Serna Saldivar S.O., (2003). Cereals/Dietary Importance. In: Caballero B, Trugo L and Finglas (Eds) *Encyclopedia of Food Sciences and Nutrition*: Academic Press, London, UK, 1027-1033

Sharma N., Bhatia S., Chunduri V., Kaur S., Sharma S., Kapoor P., Kumari A., Garg M. (2020). Pathogenesis of Celiac Disease and Other Gluten Related Disorders in Wheat and Strategies for Mitigating Them Frontiers. *Nutrition*, Vol 7, 6.

Shewery, P. R., Tatham, A. S., Forde, J., Kreis, M., & Miflin, B. J. (1986). The classification and nomenclature of wheat gluten proteins: A reassessment. *Journal of Cereal Science*, 4(2), 97–106.

Singh P, Raghuvanshi RS. (2012). Finger millet for food and nutritional security. *African Journal of Food Science*, 6(4),77–84.

Sivam, Anusooya S. and Sun-Waterhouse, Dongxiao and Quek, SiewYoung and Perera, Conrad O. (2010). Properties of Bread Dough with Added Fiber Polysaccharides and Phenolic Antioxidants: A Review. J*ournal of Food Science*, 75(8), 163-174.

Sivaramakrishnan, H.P.; Senge, B.; Chattopadhyay, P.K. (2004). Rheological properties of rice dough for making rice bread. *Journal of Food Engineering*, 62, 37–45.

See, J., Murray, J.A. (2006). Gluten-free diet: the medical and nutrition management of celiac disease. *Nutrition in Clinical Practice,* 21(1),1-15.

Slavin J., and Greenberg, N.A. (2003). Partially hydrolyzed guar gum: Clinical nutrition uses. *Nutrition*, 6, 19, 549-552.

Sollid L.M. (2002). Coeliac disease: dissecting a complex inflammatory disorder. *Nature Reviews Immunology,* 2,647-655.

Sosanya, M. E., Nweke, G.O., & Ifitezue, L. C. (2018). Formulations and evaluation of ready to use therapeutic foods using locally available ingredients in Bauchi, Nigeria. *European Journal of Nutrition & Food Safety*, 8-1-10.

Tebben, L., Shen, Y., Li, Y. (2018). Improvers and functional ingredients in whole wheat bread: A review of their effects on dough properties and bread quality. *Trends in Food Science & Technology*. 81,10-24.

Temple V.J, Bassa J.D. (1991). Proximate chemical composition of acha (*D. exilis*) grain. *Journal of Science & Food Agriculture*, 56,561–563.

Thilagavathi, T., Kanchana, S., Banumathi, P., Hemalatha, G., Vanniarajan, C., Sundar, M., and Ilamaran, M. (2015). Physico-chemical and functional characteristics of selected millets and pulses. *Indian Journal of Science &Technology*, 8 (S7), 147-155.

Thompson T., Dennis M., Higgins L.A, Lee A.R., Sharrett M.K. (2005). Gluten-free diet survey: are Americans with coeliac disease consuming recommended amounts of fibre, iron, calcium and grain foods? *Journal of Human Nutrition and Dietetics,* 18, 163-169.

Torres, M.D., Hallmark, B., and Wilson D.I. (2014). Effect of concentration on shear and extensional rheology of guar gum solutions. *Food Hydrocolloids*, 40, 85-95.

Valitutti, F., Iorfida, D., Anania, C., Trovato, C., Montuori, M., Cucchiara, S. (2017). Cereal consumption among subjects with celiac disease: A snapshot for nutritional considerations. *Nutrients*, 9(4), 396.

Wieser, H. 2007. Chemistry of gluten proteins. *Food Microbiology*, 24(2), 115– 119.

Wu, Y., Ding, W., Lirong, J., and Qiang, H. (2015). The rheological properties of tara gum (Caesalpinia spinosa). *Food Chemistry*, 168, 366-371.

Valitutti, F., Iorfida, D., Anania, C., Trovato, C. M., Montuori, M., Cucchiara, S., & Catassi, C. (2017). Cereal consumption among subjects with celiac disease: a snapshot for nutritional considerations. *Nutrients*, *9*(4), 396.

Villanueva, M., Mauro, R. R., Collar, C., & Ronda. F. (2015). Acidification of protein-enriched rice starch doughs: Effects on Breadmaking. *European Food Research and Technology*, 240, 783-794.

Villanueva. M., Harasym, J., MuNoz, J.M., Ronda, F. (2019). Rice flour physically modified by microwave radiation improves viscoelastic behavior of doughs and its bread-making performance. *Food Hydrocolloids,* 90, 472-481.

Xu, E., Pan, X., Wu, Z., Long, J., Li, J., Xu, X. (2016). Response surface methodology for evaluation and optimization of the process parameter and oxidant capacity of rice flour modified by enzymatic extrusion. *Food Chemistry*, 212, 146-154.

Xu, J., Wang, W., & Li, Y. (2019). Dough properties, bread quality, and associated interactions with added phenolic compounds: A review. *Journal of Functional Foods*. 52, 629-639.

Yaseen, A.A.E. (2000). Formulating a new high fiber rusk for production on commercial scale. *Nahrung*. 44(2), 110-113.

Yiano. H., Fuki, A., Kajiwara, K., Kobayashi. I., Yoza, K-I., Satake, A. (2017). Development of gluten-free rice bread: Pickering stabilization as a possible batter swelling mechanism. Lebensmittel-Wissenschaft and_Technologie- *Food Science & Technology,* 79, 632-639.

Yoon, S. J., Chu, D. C., & Juneja, L. R. (2008). Chemical and physical properties, safety, and application of partially hydrolysed guar gum as dietary fiber. *Journal of Clinical Biochemistry and Nutrition*,*42*(1), 1-7.

Ziobro, R., Witczak, T., Juszczak, L., & Korus, J. (2013). Supplementation of gluten-free bread with non-gluten proteins. Effect on dough rheological properties and bread characteristic. *Food Hydrocolloids*, 32, 213-220.

Zhu F. (2020). Fonio grains: Physicochemical properties, nutritional potential, and food applications. Compr Rev in *Food Science and Food Safety*, 19: 3365-3389

CHAPTER 2: RESEARCH OBJECTIVES

There is currently a significant increase in the demand for gluten-free products, which is driving both researchers and manufacturers to develop a wide range of bakery products that can compete with the quality characteristics of their gluten-containing counterparts.

Unfortunately, it appears that the majority of gluten-free products on the market have low sensory and nutritional quality, therefore it is important to find alternative gluten-free sources with higher sensory and nutritional qualities than conventional ones.

The Doctoral Thesis aims to identify an alternative gluten-free source, the use of fonio flour to produce gluten-free products and the development of a product such as rusk with different hydrocolloids.

This thesis investigates and evaluates the techno-functional and gelling properties of fonio flour in comparison to other commonly used gluten-free flours for industrial production. The potential of fonio flour as a new source of gluten-free starch in industrial applications is assessed, along with its suitability as a replacement for rice flour and/or maize starch in a gluten-free bread formulation.

A final focus of this thesis was the development of a product such as gluten-free rusk using different hydrocolloids. To achieve this objective, three studies were designed and conducted.

STUDY I. TECHNO-FUNCTIONAL AND GELLING PROPERTIES OF ACHA (FONIO) (*DIGITARIA EXILIS STAPF*) FLOUR: A STUDY OF ITS POTENTIAL AS A NEW GLUTEN-FREE STARCH SOURCE IN INDUSTRIAL APPLICATIONS.

Evaluation of the influence of fonio flour on the techno-functional properties and exploration of its suitability for the preparation of gel-like foods through the assessment of the gelling performance and characterisation of the rheological and textural properties of the gels compared to other major gluten-free cereals (rice, maize, sorghum and millet).

STUDY II. TECHNOLOGICAL EVALUATION OF THE ADDITION OF *DIGITARIA EXILIS* STAPF FLOUR IN A GLUTEN-FREE BREAD

The optimal proportion of *Digitaria exilis* Stapf flour to enhance the baking performance of gluten-free bread was identified. We evaluated the impact of various concentrations (25%, 50%, 75%, and 100%) of fonio flour on the rheological properties, pasting, fermentation, structural, and textural properties of fonio-based gluten-free bread and compared the findings with those of rice flour and maize starch controls.

STUDI III. EFFECT OF DIFFERENT HYDROCOLLOIDS ON THE RHEOLOGICAL AND STRUCTURAL CHARACTERISTICS Of GLUTEN FREE RUSKS.

Assessment of the effect of different hydrocolloids on the rheological and structural properties of a gluten free rusk.

CHAPTER 3: STUDY I

TECHNO-FUNCTIONAL AND GELLING PROPERTIES OF ACHA (FONIO) (*DIGITARIA EXILIS STAPF*) FLOUR: A STUDY OF ITS POTENTIAL AS A NEW GLUTEN-FREE STARCH SOURCE IN INDUSTRIAL APPLICATIONS

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Abstract: Fonio (*Digitaria exilis Stapf*) is an ancient African cereal that represents a rich source of carbohydrate, fat, fiber, vitamins, minerals, and sulfur-containing amino acids. Processing and utilization of fonio require adequate knowledge of its structural, chemical, and nutritional characteristics. The present work evaluates the structural, techno-functional, and gelling properties of fonio and compares them to other major gluten-free cereals (rice, maize, sorghum, and millet). Fonio flour presented significantly higher water absorption index and swelling power, while it scored a lower water solubility index than the reference flours. The pasting viscosity profile of fonio was similar to that of rice, with equivalent peak viscosity but a breakdown viscosity 24% lower than rice, indicative of higher stability and resistance to shearing and heating. Rheological properties demonstrated that fonio generates gels with remarkably strong structures. At 15% concentration, fonio gel withstood stress 579% higher than those observed in the reference flours without breaking its structure. Fonio flour presented the highest gelatinization enthalpy (11.45 J/g) and a narrow gelatinization temperature range (9.96 °C), indicative of a better-packed starch structure than the other analyzed flours. The texture of the gels made with fonio showed higher firmness over the evaluated period. These combined results suggest that fonio is a suitable ingredient for gel-like food formulations.

Keywords: fonio; gelation; gel texture; gel viscosity; rheological properties; thermal properties

1. Introduction

Over the last decade, the market for gluten-free (GF) products has grown considerably as a consequence of better diagnostic methods for identifying an increasing number of people suffering from celiac disease and other gluten-related disorders, and people who have eliminated gluten from their diet because they perceive it as a healthy improvement [1]. The nutritional composition of gluten-free products can be the most important cause of macro and micronutrient deficiencies in people with celiac disease [2]. For this reason, there are many concerns about the nutritional inadequacy of the gluten-free diet, often characterized by an excess of calories and a reduced intake of fiber, minerals, and complex carbohydrates [3,4].It is very important for gluten-free diets to be balanced and diverse, making it necessary for more gluten-free sources to be studied and applied in the development of novel food products with improved sensorial quality and nutritional value. *Digitaria exilis Stapf*, also known as fonio or acha, is a naturally gluten-free African cereal suitable for use in the diet of celiac patients [5]. Despite its low agronomic yield potential, fonio is gaining importance as a crop and food ingredient due to its superior nutritional characteristics compared with other cereals, the increased market interest in traditional food, and its suitability to be grown in tough conditions, such as in arid soil. Fonio flour has been mixed with other ingredients to improve the nutritional and textural quality of different food products such as malts, beverages, sourdough, bread, puddings, crackers, breakfast cereals, and biscuits [6,7]

Fonio has a high content of calcium and iron, compared to the other cereals indicated in the food composition table of Mali [8]. Potassium and magnesium appear to be the major mineral elements in fonio grains [6]. The protein content of fonio is like that found in withe rice [9,10], although it has a higher sulfur amino acid content (methionine and cystine [11]. Fonio has a high pentosan content, which gives it the capacity to absorb water to produce a very viscous solution, an attribute known for good baking operation [12]. Fonio has also been linked to health benefits such as the prevention and treatment of constipation, cardiovascular diseases, and hypertension [13]. The presence of polyphenols in fonio leads to antioxidant and free radical scavenging activities [6]. It is believed that fonio may have nutraceutical properties with a role in preventing and managing prediabetes and type 2 diabetes [5]. Sartelet et al. [14] have demonstrated the presence of apigenin and luteolin in fonio manifest strong anti-thyroid peroxidase (TPO) activities. Given these characteristics, fonio flour has the potential to be used as an ingredient to improve gluten-free nutritional profiles without compromising the taste and quality of products.

Despite the nutritional interest and health benefits of fonio flour, there is a lack of research on fonio compared to other major cereal grains [6]. An in-depth study into the techno-functional and gelling properties of fonio has not been covered in the available literature so far. The aim of this work is to evaluate the techno-functional, pasting, and thermal properties of fonio flour and the rheological and textural characteristics of the gels made from it. Other major gluten-free cereals, such as maize and rice (the two main gluten-free ingredients used in Europe), millet and sorghum (two warm-season grains that belong to the same family (*Poaceae*) and sub-family (*Panicoideae*) as fonio), were included in the study as reference flours.

2. Materials and Methods

2.1. Materials

Commercial fonio (*Digitaria exilis stapf*) flour was obtained from Obà Food (Roma, Italy). Maize (*Zea Mais* L.) flour was purchased from ADPAN (Asturias, Spain). Indica rice (*Oryza sativa* L.) flour was kindly provided by Herba Ricemills S.L.U. (Valencia, Spain). Sorghum (*Sorgum bicolor*) and millet (*Panicum miliaceum*) flours were kindly supplied by Salutef (Palencia, Spain).

2.2. Flour Proximate Composition

The flours' composition was determined following the 44–19 (moisture), 08–01 (ash), 30–25 (fat), and 46–11 (protein) AACC methods [15]. Carbohydrates were determined by difference to 100%.

2.3. Techno-Functional Properties

Foaming capacity (FC) and foam stability (FS) were determined as described by Abebe et al. [16]. The flour sample (2 g) was mixed with 40 mL of distilled water at 30 °C in a 100 mL measuring test tube. To produce foam, the suspension was shaken manually for 5 min. The volume of foam was measured after 0 min (V_0) and 60 min (V_{60}). FC was established directly from V_0, and FS was calculated as (V_{60}/V_0) 100.

Water absorption capacity (WAC), water absorption index (WAI), water solubility index (WSI), and swelling power (SP) were determined evaluating dispersions of 2 g of flour sample in 20 mL of distilled water using 50 mL centrifuge tubes, following the methods indicated by Abebe, Collar, and Ronda [16]. The WAC was determined by the centrifugation method. The dispersions were kept at room temperature for 30 min, then vortexed for 30 s and finally left to rest for 10 min. Immediately after, the tubes were centrifuged for 25 min at 3000×*g*. The supernatant was removed, and the sediment was weighed. Results were expressed as g H_2O retained/g of flour dry matter.

WAI, WSI, and SP were determined after cooking the flour dispersions for 15 min in a 90 °C water bath. The gels formed were cooled at room temperature for 1 h and centrifuged at 3000×*g* for 10 min. The supernatant was placed into an evaporating capsule to determine the soluble solid content (WSI (g of soluble solids/100 g flour dry matter) and SP (g of sediment/g insoluble solids flour dry matter)). The sediment was used to determine WAI (g of sediment/g of dry flour matter).

2.4. Least Gelation Concentration

The least gelation concentration (LGC) of the studied flours was determined with slight modifications of the method indicated by Joshi et al. [17]. Glass test tubes containing 2 mL of distilled water and the corresponding amount of flour to achieve a concentration of 2, 4, 6, 9, 10, 11, 12, 14, 16, 18, and 20 g/100 mL were kept for 1 h in a boiling water bath to form the gel. The tubes were cooled down by placing them under running water and stored at 4 °C for 2 h. LGC was determined as the minimum concentration where the gel did not drop or slip when the glass tube was inverted.

2.5. Pasting Properties

Pasting properties of flour samples were determined using a Kinexus Pro+ rheometer (Malvern Instruments Ltd., Malvern, UK) equipped with a starch cell and controlled by rSpace software. Each flour sample (3.0 g, dry basis) was transferred to the evaluation canister and mixed with 25 mL of distilled water. The sample was equilibrated at 50 °C for 60 s, heated to 95 °C at 6 °C/min, maintained at 95 °C for 300 s, cooled to 50 °C at 6 °C/min, and maintained at 50 °C for 120 s. The paddle speed rate was set at 160 rpm during the whole analysis. Parameters calculated from the pasting profile were pasting temperature (PT), peak viscosity (PV), trough viscosity (TV), breakdown viscosity (BV), final viscosity (FV), and setback viscosity (SV). Each sample was analyzed at least in duplicate.

2.6. Rheological Properties of Flour Gels

The gels of fonio, millet, sorghum, maize, and rice were analyzed by a dynamic oscillatory test, performed using a Kinexus Pro+ rheometer (Malvern Instruments Ltd., Malvern, UK) equipped with a parallel plate geometry (40 mm diameter) of serrated surface at a gap of 1 mm. Gel samples were obtained following the method described for the determination of flour pasting properties (Section 2.5). Dispersions of flour in water at different concentrations (6-8-10-12-15 g/100 g) were used to prepare the gels. Once the gel was prepared, it was placed in the plates and was left to rest for 5 min to allow relaxation. The temperature was stabilized at 25 °C. Strain sweeps were performed from 0.1 to 1000% at a constant frequency of 1 Hz to establish the maximum stress (τ_{max}) in the Linear Viscoelastic Region (LVR) and the stress at the cross point (G' = G") [18]. The limit of the LVR (τ_{max}) was identified as the sharp decrease of G' modulus, which coincided with the sudden increase of tan(δ). Frequency sweeps were made from 1 to 10 Hz at a constant strain of 1% of the LVR. The values G_1', G_1", and $\tan(\delta)_1$ were obtained from fitting the frequency sweeps experimental data to the power-law model as described by Villanueva, De Lamo, Harasym, and Ronda (2018) [19]; these variables represent the elastic and viscous moduli and the loss tangent, respectively, at a constant strain of 1%. The exponents obtained from the fitting (a, b, and c) quantify the dependence of the elastic and viscous moduli and the loss tangent to the oscillation frequency. The gels and the rheological tests were carried out in duplicate.

2.7. Thermal Properties

Thermal transitions (gelatinization, retrogradation, and amylose-lipid complex dissociation) were determined by differential scanning calorimetry (DSC) (DSC-822e, Mettler Toledo, SAE). Samples (~6 mg) were prepared at a 30:70 (*w/w*) flour:water ratio in 40 μL aluminum pans. The scan made for the evaluation went from 0 to 115 °C at a heating rate of 5 °C/min, using an empty pan as reference. Zinc and indium were employed to calibrate the calorimeter. The enthalpy change (ΔH, J/g dry basis) and the gelatinization temperatures [onset (To), peak (Tp), and endset (Te)] were recorded. Endothermic transitions of the retrograded starches were determined with a second scan made after 7 days of sample storage at 4 °C, following the same procedure described for the gelatinization evaluation. Each experimental sample was measured at least in duplicate.

2.8. Texture of Fresh Gels and Its Evolution with Time

The texture of gels was measured with a TA-XT2 Texture Analyser (Stable Microsystems, Surrey, UK) provided with the software "Texture expert exceed" version 2.63. The gels were prepared in 50 mL centrifuge tubes by heating 28 g of flour dispersion (at a concentration of 15 g/100 g) in a boiling water bath for 30 min, stirring the dispersion every 2–3 min until the boiling temperature was achieved. The tubes were cooled at room temperature and stored for 2, 6, 10, 24, 48, 96, and 192 h at 4 °C. Gels were left at room temperature for 15 min before the analysis of the texture. Measurements were done on gel cylinders of 2.7 cm diameter and 2 cm height. A "Texture Profile Analysis" (TPA) double compression test was performed using a 75 mm diameter aluminum probe (SMSP/75) to suppress 50% depth, at 1 mm/s speed test, with a 30 s delay between compressions.

Firmness (N), springiness, cohesiveness, and gumminess (N) were calculated from the TPA graphs.

2.9. Statistical Analysis

One-way ANOVA with parametric tests was used for the statistical analysis using Statgraphics Centurion XVIII software (Bitstream, Cambridge, MN, USA). The Levene test was used to check the homogeneity of variances. The normality of the studentized residuals was evaluated with the Kolmogorov–Smirnov test. Fisher's Least Significant Difference test at 95% confidence intervals ($p < 0.05$) was used to establish significant differences among means.

3. Results and Discussion

3.1. Flour Proximate Composition

The composition of fonio and the other studied gluten-free flours are presented in Table 1. It can be noted that the composition of fonio flour was very similar to rice flour. Both flours were the richest in carbohydrates and the poorest in proteins, along with maize. Fonio showed the lowest content of fat and ash among the studied flours. Analyzing the literature available for the fonio flour, large variations of the chemical and nutritional composition were found. Ballagoun [20] reported the following ranges in the main fonio flour components: 5.1–11% proteins, 1.3–5.2% fat, 1–6% ash. These differences can be attributed to genetic factors, geographical situation, environmental influences, agronomic characteristics, and analytical methods used in the determination.

Table 1. Proximal composition of fonio compared to the other gluten-free flours. Except for the moisture content, all presented values are referred to dry basis.

Flour	Moisture (%)	Ash (%)	Fat (%)	Proteins (%)	Carbohydrates (%)
Fonio	12.90 ± 0.01 e	0.45 ± 0.02 a	1.1 ± 0.2 a	6.6 ± 0.3 a	91.9 ± 0.9 c
Millet	10.47 ± 0.01 a	1.73 ± 0.09 e	4.2 ± 0.8 c	12.9 ± 0.5 d	81.2 ± 1.4 a
Sorghum	11.77 ± 0.01 b	1.35 ± 0.07 d	3.3 ± 0.6 b	10.0 ± 0.4 c	85.3 ± 1.1 b
Maize	11.96 ± 0.01 c	1.14 ± 0.06 c	4.4 ± 0.9 c	7.5 ± 0.3 b	87.0 ± 1.3 b
Rice	12.66 ± 0.01 d	0.57 ± 0.03 b	1.3 ± 0.2 a	7.8 ± 0.3 b	90.4 ± 0.6 c

Data are the mean (n = 2) ± standard deviation. Values with a letter in common in the same column are not significantly different ($p > 0.05$).

3.2. Functional Characteristics

Functional characteristics are summarized in Table 2. The studied flours have shown different values of foaming capacity (FC) and foaming stability (FS). The foaming capacity of flours is mainly related to proteins, which form a continuous, cohesive film around the air bubbles in the foam [21]. Fonio did not exhibit any foaming capacity, while millet and sorghum flours exhibited the highest FC and FS, probably due to their higher protein content. It has been reported that protein–carbohydrate interactions in fonio may contribute to the low solubility of fonio proteins [6], resulting in lower availability to interact with water and generate foam. No FS was measured in fonio, given that no foam was formed.

The remaining GF flours showed FS values above 50%, suggesting that the native proteins soluble in water are very surface active in these flours [21].

Table 2. Functional characteristics and pasting properties of fonio flour compared to other gluten-free flours.

	Fonio	**Millet**	**Sorghum**	**Maize**	**Rice**
			Functional properties		
FC (mL)	Nd	3.33 ± 0.34 b	4.99 ± 0.35 c	1.57 ± 0.17 a	1.74 ± 0.01 a
FS (%)	Nd	52 ± 9 a	82 ± 2 c	76 ± 8 b	50 ± 1 a
WAC (g/g)	1.12 ± 0.02 c	0.97 ± 0.01 a	1.09 ± 0.01 b	1.34 ± 0.02 d	0.98 ± 0.01 a
WHC (g/g)	1.8 ± 0.2 bc	1.8 ± 0.1 abc	1.85 ± 0.07 c	2.4 ± 0.1 d	1.65 ± 0.09 a
WAI (g/g)	7.7 ± 0.2 d	6.20 ± 0.07 b	6.9 ± 0.3 c	6.35 ± 0.06 b	5.77 ± 0.03 a
WSI (g/100 g)	0.7 ± 0.1 a	2.2 ± 0.1 cb	2.2 ± 0.1 c	4.7 ± 0.1 d	1.74 ± 0.03 bc
SP (g/g)	7.7 ± 0.2 d	6.16 ± 0.07 b	6.9 ± 0.3 c	6.26 ± 0.06 b	5.74 ± 0.03 a
			Pasting properties		
PT (°C)	82.2 ± 0.1 b	85.3 ± 0.1 d	91.6 ± 0.2 e	80.29 ± 0.02 a	82.72 ± 0.02 c
Pt (s)	562 ± 3 a	620 ± 1 c	682 ± 6 e	572 ± 5 b	633 ± 1 d
PV (Pa·s)	4.68 ± 0.03 d	2.11 ± 0.03 b	2.56 ± 0.03 c	1.580 ± 0.004 a	5.22 ± 0.09 e
FV (Pa·s)	5.15 ± 0.08 c	4.46 ± 0.02 b	5.4 ± 0.4 c	2.92 ± 0.02 a	5.22 ± 0.09 c
TV (Pa·s)	2.39 ± 0.02 d	1.32 ± 0.01 a	2.00 ± 0.02 b	1.270 ± 0.001 a	2.19 ± 0.04 c

BV (Pa·s)	2.29 ± 0.01 d	0.79 ± 0.04 c	0.56 ± 0.05 b	0.310 ± 0.005 a	3.03 ± 0.05 e
SV (Pa·s)	2.76 ± 0.07 b	3.14 ± 0.02 bc	3.4 ± 0.5 c	1.65 ± 0.02 a	3.03 ± 0.05 bc

FC: foaming capacity, FS: foaming stability, WHC: Water holding capacity (g H_2O/g flour dry matter, dm), WAI: Water absorption index (g sediment/g flour dm), WSI: Water solubility index (g soluble solids/100 g flour dm), WAC: Water absorption capacity (g H_2O/g flour dm), SP: swelling power (g/g insoluble flour matter). PT: Pasting Temperature, Pt: peak time, PV: peak viscosity, FV: final viscosity, TV: Trough Viscosity, BV: Breakdown viscosity, SV: Setback viscosity. Data are mean ± standard deviation. Values with a letter in common in the same row are not significantly different ($p > 0.05$).

Flour hydration properties varied significantly among GF flours, which could be explained by the different compositions of the flours, mainly protein, fiber, and starch [21]. In order to obtain good quality from alternative materials (such as novel gluten-free sources), it is necessary to know their hydration properties to balance formulations and adequate technological production processes to counteract changes in the rheological properties caused by the substitution of gluten [22]. It has been documented that hydration is a critical factor in many manufacturing processes of cereal-based products such as pasta, couscous, and bread [22]. The WHC, which quantifies the ability of a matrix to absorb and retain water without the influence of external forces, and the WAC, which depends on the source's susceptibility to form hydrogen bonds between starch, influenced by the hydrophilic parts in carbohydrates and proteins [23], followed a similar trend. Fonio showed average values for a GF flour in these two parameters, above millet, rice, and sorghum, which presented the lowest water binding capacities, and below maize flour. Fonio flour exhibited the highest WAI and SP and the lowest WSI among the other analyzed flours. This behavior denotes the ability of fonio starch to absorb the highest amount of water during gelatinization and swelling in excess water and retain it in the formed gel [24].

Swelling power (SP) followed an identical evolution as WAI, given that both parameters associate the weight of the formed gel with the number of insoluble compounds in the flour and the whole amount of flour, respectively. When the fonio flour gelatinizes, better interaction with water is exhibited, which could be due to the very small dimension of the starch granules, ~8 µm [25]. Said small size leads to a high specific surface area, with consequent higher interaction and absorption of water [26]. The very low solubility of fonio in comparison with the other GF flours (WSI value about 3 times lower than that of rice and 7 times lower than maize) is compatible with its very low mineral content; it also means a very low lixiviation and solubility of amylose during starch swelling [27].

3.3. Pasting Properties

The results obtained from viscometric tests are presented in Table 2 and Figure 1. Pasting temperature (PT) expressed the minimum temperature necessary to begin the cooking of the flour, identified as the temperature at which viscosity increases during the heating process [28]. Fonio flour showed an intermediate PT value (82.2 °C), similar to that of rice, higher than maize (80.29 °C), and significantly lower than sorghum (91.6 °C) and millet (85.3 °C). The highest value of PT was registered in sorghum flour, which could indicate the presence of a starch that is highly resistant to swelling and rupturing [29]. Fonio showed the shortest time to reach the peak viscosity (Pt) and a peak viscosity (PV) value significantly higher than millet, sorghum, and, especially, maize flour. The PV of fonio flour was only (slightly) surpassed by rice flour. The PV happens at the equilibrium point between swelling and rupturing of the starch [30]. It is obtained at the maximum swelling of starch granules, and it is linked to the water absorption index of the flour [31], as was shown in Section 3.2. Fonio flour also showed a higher trough viscosity (TV) value than millet, sorghum, and maize, similar to that of rice flour. Fonio showed a breakdown viscosity (BV) value 24% lower than rice flour, denoting higher paste stability and resistance against shearing and heating than one of the most used flours in GF production. Maize, followed by sorghum and millet, showed the lowest BV and the highest stability, although these flours presented significantly lower viscometric profiles (Figure 1), so lower values of BV were expected. The setback viscosity (SV) is an indicator of amylose's tendency to retrograde. In general, higher values of SV indicated a greater tendency of starch to retrograde [32]. The SV value of fonio was similar to that of rice and between those of millet and sorghum (the maximum) and maize (the minimum).

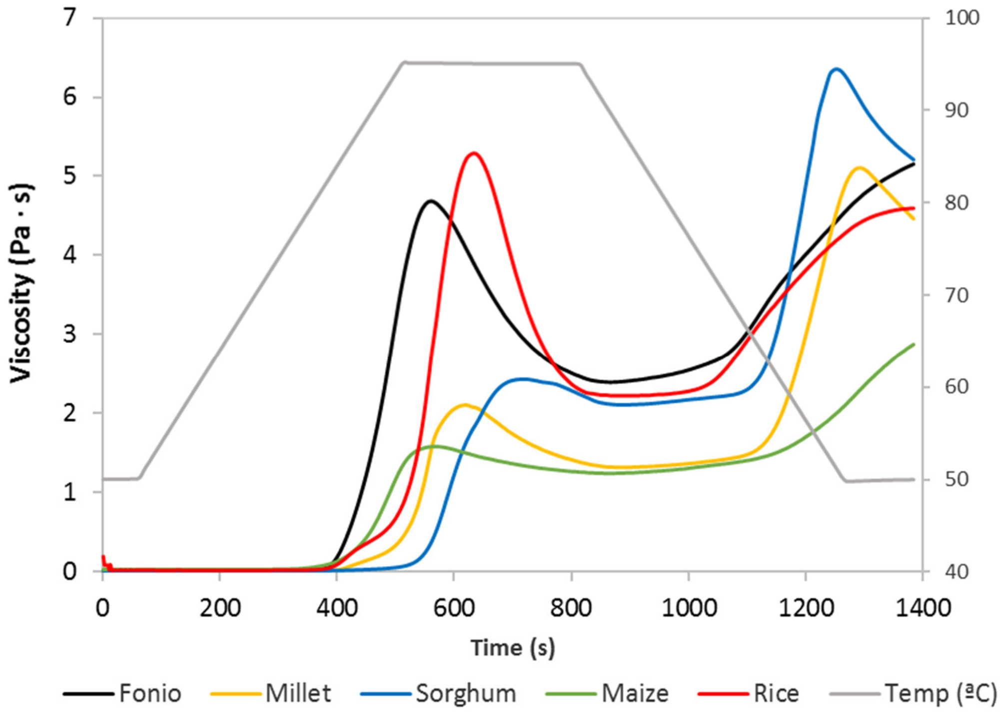


Figure 1. Pasting properties of the gluten-free flours studied.

In millet and sorghum, a second peak of viscosity appeared at the final cooling phase (50 °C) of the pasting curve. It is believed that this behavior could be due to the storage of the flours for a time equal to or greater than two months, given that a second peak during the holding stage was also found by Zhang and Hamaker [33] in sorghum flour when it had been stored for more than two months. The viscometric profile of fresh sorghum flour only showed one peak in the holding period (95 °C).

At the end of the pasting profile, during the temperature holding at 50 °C, the final viscosity (FV) was recorded. This value showed the capacity of the material to form a viscous paste that reflects the retrogradation of amylose [34]. The values registered are maximum for sorghum, fonio, and rice flours, and no significant differences are found among them. The lowest FV value was shown by maize flour.

3.4. Gel Viscoelastic Properties and Their Dependence on Flour Concentration

The viscoelastic properties of the gels were assessed by dynamic oscillatory tests at 25 °C (see Table 3 and Supplementary Figure S1). The samples were produced at different flour concentrations (from 6% to 15%) and analyzed with strain sweeps, allowing the establishment of the end of the linear viscoelastic region (LVR) and the identification of the maximum stress (τ_{max}) that samples could tolerate before the collapse of their structure. The effects of flour type and concentration on the value of maximum stress (τ_{max}) were statistically significant ($p < 0.05$), following an increasing trend with increasing concentration in all the studied GF flours. The strain sweep assays also provided the stress at which the gels passed from a solid-like to a liquid-like behavior (the crossing point of the curves where G' = G" and tan(δ) = 1) (Table 3). Results indicated that fonio formed gels with a remarkably stronger structure, being the sample that showed the highest values of τ_{max} and cross-over point in all studied concentrations. Differences were particularly marked with higher concentrations; the 15% fonio gel presented a τ_{max} and cross-over point 579% and 1788%, respectively, higher than sorghum (the sample with the lowest results).

Table 3. Rheological properties of fonio, millet, sorghum, maize, and rice flour gels at different concentrations.

Flour	Concentration (%)	G_1' (Pa)	A	G_1" (Pa)	B	tan $(\delta)_1$	C	Cross over (Pa)	τ_{max} (Pa)
	6	45 ± 4 aA	0.121 ± 0.006 eC	10 ± 1 aB	0.370 ± 0.007 dC	0.230 ± 0.007 cD	0.249 ± 0.001 abA	14.5 ± 0.4 aC	8.3 ± 0.1 aC
	8	385 ± 3 bD	−0.134 ± 0.006 aA	30 ± 1 bC	0.267 ± 0.001 cA	0.078 ±0.001 bA	0.401 ± 0.005 bD	574 ± 26 bD	548 ± 18 bD
Fonio	10	1242 ± 10 cC	−0.072 ± 0.002 bA	70 ± 1 cC	0.184 ± 0.001 aA	0.056 ± 0.004 aA	0.256 ± 0.003 abD	1347 ± 28 cD	1142 ± 4 cD
	12	1761 ± 66 dE	−0.003 ± 0.003 cA	95 ± 3 dD	0.201 ± 0.002 bC	0.054 ± 0.003 aA	0.204 ± 0.001 aB	1682 ± 102 dD	1387 ± 37 dD
	15	3106 ± 182 eE	0.0150 ± 0.0003 dA	169 ± 11 eBC	0.209 ± 0.005 bC	0.055 ± 0.004 aA	0.193 ± 0.005 aC	2535 ± 4 eD	2151 ± 76 eE
	6	66 ± 5 aBC	0.0320 ± 0.0001 aA	6 ± 1 aA	0.356 ± 0.004 dB	0.099 ± 0.002 cA	0.324 ± 0.003 cC	11.0 ± 0.5 aA	3.9 ± 0.3 aB
	8	167 ± 11 aA	0.064 ± 0.001 eB	17 ± 1 aA	0.290 ± 0.01 cB	0.105 ± 0.003 dB	0.230 ± 0.013 bBC	34 ± 2 aA	12 ± 3 abA
Millet	10	380 ± 36 bB	0.059 ± 0.003 dB	38 ± 3 bA	0.219 ± 0.014 bB	0.101 ± 0.002 cB	0.160 ± 0.010 aB	96 ± 14 bA	29 ± 2 bA
	12	833 ± 31 cC	0.047± 0.003 cB	71 ± 2 cB	0.181 ± 0.009 aB	0.086 ± 0.001 bB	0.133 ± 0.006 aA	204 ± 27 cA	95 ± 4 cA
	15	2213 ± 107 dC	0.0410 ± 0.0003 bB	176 ± 11 dC	0.189 ± 0.005 aB	0.079 ± 0.001 aB	0.148 ± 0.005 aB	526 ± 49 dBC	245 ± 11 dC

	6	59 ± 2 aBC	0.043 ± 0.006 aA	6 ± 1 aA	0.363 ± 0.001 dBC	0.101 ± 0.003 aA	0.321 ± 0.006 bB	9.0 ± 0.3 aA	2.0 ± 0.1 aA
	8	202 ± 5 bB	0.079 ± 0.003 dB	27 ± 1 bB	0.240 ± 0.007 cC	0.132 ± 0.001 dC	0.160 ± 0.010 aA	36.6 ± 0.5 bAB	11 ± 2 bA
Sorghum	10	458 ± 7 cB	0.068 ± 0.001 cBC	56 ± 1 cB	0.185 ± 0.003 bA	0.121 ± 0.003 cC	0.116 ± 0.004 aA	83 ± 1 cA	28.1 ± 0.2 cA
	12	1059 ± 19 dD	0.059 ± 0.002 bC	112 ± 4 dD	0.168 ± 0.001 aA	0.106 ± 0.001 bC	0.109 ± 0.001 aA	194 ± 12 dA	68 ± 6 dA
	15	2671 ± 4 eD	0.056 ± 0.001 bD	280 ± 5 eD	0.179 ± 0.004 bA	0.105 ± 0.002 bC	0.123 ± 0.005 aA	438 ± 6 eA	120.3 ± 0.3 eA
	6	54 ± 1 aB	0.086 ± 0.003 cB	10 ± 1 aB	0.311± 0.001 eA	0.187 ± 0.004 eC	0.225 ± 0.003 aA	13.8 ± 0.7 aB	3.6 ± 0.3 aB
	8	226 ± 6 bC	0.07 ± 0.02 bcB	36 ± 2 bD	0.242 ± 0.001 dC	0.158 ± 0.003 dE	0.170 ± 0.020 aAB	59 ± 3 bBC	34.6 ± 0.4 aB
Maize	10	407 ± 69 cB	0.062 ± 0.007 abBC	57 ± 11 cB	0.220 ± 0.004 cB	0.139 ± 0.003 cD	0.158 ± 0.005 aB	153 ± 2 cB	85 ± 6 bB
	12	673 ± 9 dB	0.051 ± 0.001 abB	812 ± 2 dE	0.209 ± 0.004 bC	0.121 ± 0.002 bD	0.158 ± 0.004 aA	364 ± 1 dC	292 ± 2 cC
	15	1492 ± 6 eB	0.044 ± 0.001 aC	155 ± 1 eB	0.193 ± 0.006 aB	0.104 ± 0.001 aC	0.149 ± 0.006 aB	759 ± 8 eC	454 ± 3 dD
	6	93 ± 1 aD	0.087 ± 0.001 cB	15 ± 1 aC	0.314± 0.001 bA	0.156 ± 0.003 bB	0.227 ± 0.001 bA	36 ± 2 aC	22.7 ± 0.7 aD
Rice	8	174 ± 3 bA	0.0530 ± 0.0001 aB	25 ± 1 bB	0.305 ± 0.001 aD	0.143 ± 0.001 aD	0.252 ± 0.001 dC	118 ± 1 bC	60.4 ± 0.7 bC
	10	196 ± 2 cA	0.072 ± 0.002 bD	32 ± 1 cA	0.321 ± 0.001 cC	0.164 ± 0.003 cE	0.249 ± 0.003 cC	206 ± 2 cC	97.3 ± 0.2 cC
	12	225 ± 4 dA	0.088 ± 0.003 cD	40 ± 1 dA	0.334 ± 0.001 dD	0.180 ± 0.001 dE	0.25 ± 0.02 bC	302 ± 4 dB	154 ± 1 dB

15	338 ± 5 eA	0.121 ± 0.001 dE	61 ± 1 eA	0.341 ± 0.001 eD	0.181 ± 0.001 dD	0.220 ± 0.002 aD	460 ± 9 eB	217 ± 4 eB

The power-law model was fitted to experimental results from frequency sweeps: $G'(\omega)= G_1'\cdot\omega^a$; $G''(\omega)= G_1''\cdot\omega^b$; $\tan\delta(\omega)= \tan\delta_1\cdot\omega^c$. G_1', G_1'' and $(\tan\delta)_1$: represent the elastic and viscous moduli and the loss tangent at 1% strain. The a, b, and c exponents quantify the dependence degree of dynamic moduli and the loss tangent with the oscillation frequency. τ_{max}: maximum stress that samples can tolerate in the LVR. Data are the mean ± standard deviation. Values with a letter in common in the same column for each flour are not significantly different ($p > 0.05$). Lowercase letters compare the effect of concentration in the same flour, and capital letters compare different flours at the same concentration.

The G_1', G_1", $\tan(\delta)_1$ and a, b and c exponents were generated from fitting the frequency sweeps data in the range of 1–10 Hz to the power-law model. The high value of R^2 (0.955–0.999) indicates to what extent the model adjusted to the studied systems. Elastic and viscous moduli significantly increased when the flour concentration increased. However, the rate of increase varied depending on the type of flour (see Supplementary Figure S1). Except for the lowest studied concentration (6%), fonio gel exhibited markedly higher elastic and viscous moduli than the other GF flours. The marked differences in gel rheological properties of the different gluten-free flours could be attributed to differences in their botanical origin, such as proteins, starch, and lipids contents [35–37].

The viscoelastic moduli of rice gels and the rate of increase of G_1' and G_1" with concentration were significantly lower than the gels made from the other GF flours, in agreement with previous studies [37].

All gels presented $G_1' > G_1''$, and consequently $\tan(\delta)_1 < 1$, in all the studied concentrations, indicating a solid-like behavior of the gels. This indicates that the gel structure was already formed at a concentration of 6%, regardless of the flour source. Except for the gels made from rice flour, $\tan(\delta)_1$ decreased with the increase in flour concentration (see Table 3 and Supplementary Figure S1). This behavior reveals a strengthening of their structure with concentration. The fonio gels showed the highest decrease in loss tangent with increasing gel concentration, in particular when increasing from 6% to 8%, reaching the lowest value at a concentration of 12% (0.0540). This value was between 50% and 66% lower than those obtained for the gels made with the other GF flours. In the case of rice gels, the loss tangent did not follow a decreasing trend with concentration, but rather the opposite, indicating that in this case, the increase in concentration enhanced the viscosity of the gel rather than its elasticity, resulting in a softer gel structure than that of the more diluted gels [38].

The low values of “a” exponents for all gels indicate that G’ was not dependent on the applied frequency and suggested a stable gel structure [30]. As can be seen in Table 3, the lowest values of “a” were obtained for fonio gels, while the highest ones were presented by rice gels. The dependence of the viscous modulus and loss tangent with frequency, evaluated from the “b” and “c” exponents, respectively, were higher than that of the elastic modulus (“a”) and similar among gels of different nature. Both exponents decreased with the concentration of flour in the gel, except for rice flour gels, where b increased with concentration. This impact of concentration on the stability of gels and batters has been reported in previous works [30–36].

The high dependence of storage modulus on the concentration allows gathering information of the gelation efficiency and the structure of the particle network of the gel [39]. Clark et al. [40] estimated the relation between concentration and storage moduli using a power-law equation. Power-law functions between concentration and G_1’ and G_1” were obtained for the dispersions: $G_1' = m * C^n$ and $G_1'' = p * C^q$, where m and p represent the G’ and G” moduli values at a gel concentration of 1% and a frequency of 1 Hz, and *n* and q, the exponents, quantify the dependence degree of the viscoelastic moduli to the concentration and reflect the nature of the association behavior in the gel and its network structure [41] (Table 4). The R^2 coefficients ranged from 0.9561 to 0.9997, indicating a good fitting of experimental results to the potential model. In the case of fonio and rice, the evolution of the elastic modulus with concentration would also be compatible with a linear model (see Supplementary Figure S1A). Linear correlations between G’ and concentration have been reported for potato, wheat, corn, and rice starch gels [42]. Therefore, the variation of G_1' with the concentration of millet, sorghum, and maize gels, is probably more related to the protein content than to the starch content of the flours, while in the case of fonio and rice, their naturally low protein content could explain the observed behavior, more similar to that of starch gels. However, to allow comparison among different flours, the potential equation was chosen to model the evolution of all GF gels viscoelastic moduli versus concentration. As can be seen (Table 4), the *n* and q values of fonio gels were 2.8 and 2.5, respectively. These values were greater in millet, sorghum, and maize flours, which reflects the formation of a more ordered gel matrix. Rice was the only one with significantly lower exponents (n = 1.26 and q = 1.53) than fonio. This denotes a higher increase in fonio gels' viscoelastic moduli with flour concentration and a higher modulation capacity of the gel's viscoelasticity by varying its concentration than rice gels. The exponents *n* and q of millet and sorghum were notably higher than that of fonio; however, their viscoelastic moduli at low concentrations (quantified by the m and p coefficients) were much lower than those obtained for fonio. The combination of a high consistency at low concentrations (compared to millet, sorghum, and maize) and a high increase in consistency with increasing concentration (compared to rice) makes fonio an interesting ingredient for the production of GF products of gel-like nature.

Table 4. Parameters obtained from fitting to the power-law model the experimental G_1' and G_1" data in function of the flour concentration in the gels ($G_1' = m \cdot C^n$; $G_1'' = p \cdot C^q$).

Parameter	Fonio	Millet	Sorghum	Maize	Rice
m (Pa)	2 ± 1 d	0.015 ± 0.004 a	0.030 ± 0.005 b	0.20 ± 0.08 c	11 ± 5 e
n	2.8 ± 0.3 b	4.4 ± 0.1 e	4.21 ± 0.06 d	3.3 ± 0.1 c	1.26 ± 0.17 a
R^2	0.979	0.9992	0.9997	0.9971	0.9561
p (Pa)	0.18 ± 0.09 b	0.005 ± 0.002 a	0.006 ± 0.001 a	0.15 ± 0.06 b	1.0 ± 0.2 c
Q	2.5 ± 0.2 b	3.9 ± 0.1 c	4.00 ± 0.07 c	2.6 ± 0.2 b	1.53 ± 0.09 a
R^2	0.989	0.9985	0.9995	0.9934	0.9914

The coefficients m and p represent the values of the G_1' and G_1" moduli at a concentration of 1%; *n* and q exponents inform about the dependence degree of both moduli on flour concentration. Values with a letter in common in the same row are not significantly different ($p > 0.05$).

3.5. Thermal Properties

Thermal properties of the gluten-free flours were evaluated using Differential Scanning Calorimetry (DSC). Gelatinization, retrogradation, and amylose-lipid complex data obtained from DSC are shown in Table 5. Thermograms of flour samples showed 2 wide endothermic transitions in the first scan; one due to starch gelatinization, which appeared at 73–77 °C, and the second peak at 93–98 °C, due to the dissociation of amylose-lipid complex [43]. The reversibility of this second endotherm indicates the presence of an amorphous amylose-lipid complex within these samples [44]. The different flour samples showed significant differences in gelatinization enthalpy (ΔH_{gel}). Fonio and rice gels presented the highest values (11.5 and 10.5 J/g, respectively), while maize presented the lowest (5.1 J/g). Higher values of ΔH_{gel} are indicative of a better-packed starch structure requiring more energy to fully gelatinize. Significant differences were also found among gelatinization temperatures. Ji et al. [45] discovered that higher crystallites perfection is linked to greater gelatinization temperatures required to melt them. Fonio presented a high value of onset temperature (To), statistically equal to that of rice, surpassed by millet and sorghum. Peak temperature (Tp), where the endothermic transition reaches a maximum, presented the highest value in millet flour (76.98 °C), while the lowest values were recorded in fonio and maize flours, being 73.5 and 70.07 °C, respectively. The width of the gelatinization temperature range (Te-To) was the highest in maize (17.3 °C), followed by rice (11.3 °C), sorghum (10.6 °C), fonio (9.96 °C), and millet (7.45 °C). Lower values of the gelatinization peak width indicate higher starch crystallites homogeneity and a better organized granular structure, requiring a shorter temperature range to fully hydrate. The formation of amylose–amylose linkages and amylose–lipid complexes within the starch granule and a more stable configuration have been associated with higher gelatinization temperatures [46]. The dissociation enthalpy of amylose–lipid inclusion complex obtained in the first run for fonio was equal to those of millet, sorghum, and rice.

A second scan was performed to assess the retrogradation properties of the flours after storage of the gelatinized samples in their corresponding pans at 4 °C for 7 days (Table 5). Two visible peaks were detected from this scan. The first peak, which appeared at a temperature around 50 °C, was linked to the melting of the recrystallized amylopectin (ΔH_{ret}) during the gel staling. All values determined for ΔH_{ret} were lower than their corresponding ΔH_{gel} value, but showed the same trend, with fonio presenting the highest value. The width of this first peak was observed to be higher in fonio flour, while maize presented the lowest value. The second peak registered was related to the reversible amylose–lipid complex dissociation and appeared approximately at the same temperature as it did in the first scan. The enthalpies of the amylose–lipid complex were increased in the second scan with respect to the first scan. Eliasson [44] indicated that the increased values during the second scan are due to better conditions for complex formation after the first heating. This is related to the leaking of amylose out of the granules that occur at temperatures above the gelatinization temperature range.

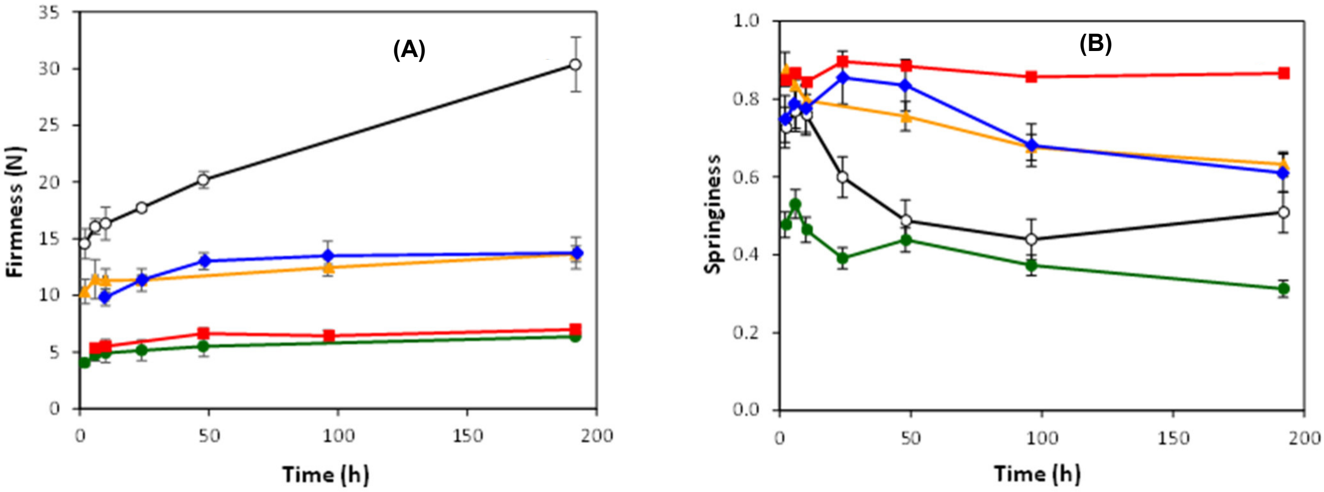
Table 5. Thermal properties of fonio, millet, sorghum, maize, and rice flours.

Times	First Scan						Second scan					
Samples	ΔH_{gel} (J/g)	To_{gel} (°C)	Tp_{gel} (°C)	Te_{gel} (°C)	$\Delta H_{am\text{-}lip}$ (J/g)	$Tp_{am\text{-}lip}$ (°C)	ΔH_{ret} (J/g)	To_{ret} (°C)	Tp_{ret} (°C)	Te_{ret} (°C)	$\Delta H_{am\text{-}lip}$ (J/g)	$Tp_{am\text{-}lip}$ (°C)
Fonio	11.5 ± 0.3 e	68.92 ± 0.03 b	73.5 ± 0.1 b	78.88 ± 0.08 b	0.91 ± 0.01 b	97.0 ± 0.2 bc	7.9 ± 0.3 b	32 ± 6 a	50.5 ± 0.3 a	68 ± 4 b	1.3 ± 0.2 a	97.8 ± 0.1 c
Millet	7.0 ± 0.1 c	73.45 ± 0.01 d	76.98 ± 0.02 d	80.9 ± 0.1 d	0.94 ± 0.05 b	93.3 ± 0.1 a	4.5 ± 0.4 a	38 ± 1 a	52.1 ± 0.1 b	63.5 ± 0.3 ab	3.1 ± 0.2 bc	92.6 ± 0.3 a
Sorghum	6.5 ± 0.4 b	69.8 ± 0.1 c	74.7 ± 0.1 c	80.4 ± 0.2 cd	0.99 ± 0.06 b	98.1 ± 0.2 c	5.1 ± 0.6 a	36 ± 2 a	51 ± 1 a	64.2 ± 0.3 ab	3.6 ± 0.7 c	95 ± 1 b
Maize	5.1 ± 0.5 a	61.1 ± 0.1 a	70.07 ± 0.01 a	78.4 ± 0.3 a	0.16 ± 0.03 a	97 ± 1 bc	4.3 ± 0.1 a	38 ± 1 a	50.7 ± 0.8 ab	62.6 ± 0.2 a	2.0 ± 0.2 a	91.6 ± 0.6 a
Rice	10.5 ± 0.2 d	68.9 ± 0.2 b	74.68 ± 0.06 c	80.2 ± 0.2 c	0.98 ± 0.05 b	96.5 ± 0.2 b	5.2 ± 0.6 a	39 ± 1 a	50.4 ± 0.1 a	62.8 ± 0.5 ab	2.3 ± 0.4 ab	98.0 ± 0.1 c

ΔH_{gel}, $\Delta H_{am\text{-}lip}$, and ΔH_{ret}: Enthalpy associated to starch gelatinization, dissociation of amylose lipid complex and melting of the recrystallized amylopectin; To_{gel}, To_{ret}: onset temperature of gelatinization and retrogradation peaks; Tp_{gel}, Tp_{ret}, $Tp_{am\text{-}lip}$: Peak Temperature of gelatinization, retrogradation, and amylose-lipid complex dissociation peaks; Te_{gel}, Te_{ret}: endset temperature of gelatinization and retrogradation peaks; First scan: Scan carried out on native (un-gelatinized) sample. Second scan: scan carried out on gelatinized samples after 7 days of storage at 4 °C. Means values with different letters for the same parameter imply significant differences between means at $p < 0.05$.

3.6. Texture of Fresh Gel and Its Evolution with Storage Time

The texture properties of the gels made from the studied flours and their evolution during a period of 192 h of storage at 4 °C, depicted in Figure 2 and Table 6. Fonio gels presented the highest values of firmness in the studied range, in agreement with the information obtained from gel's rheology. As could be expected, the firmness of gels increased with storage time regardless of the type of flour. As can be seen in Figure 2A, except for the fonio gel, all gels reached a constant/asymptotic firmness value after 24–48 h of storage. In the case of fonio, however, a constant increase was obtained during all 192 h, reaching a value of 30.37 N, representing an increase of 109% with respect to the initial firmness. This change over storage periods was mainly related to amylopectin recrystallization [47], hence highly dependent on the starch composition and botanical origin of the sample.



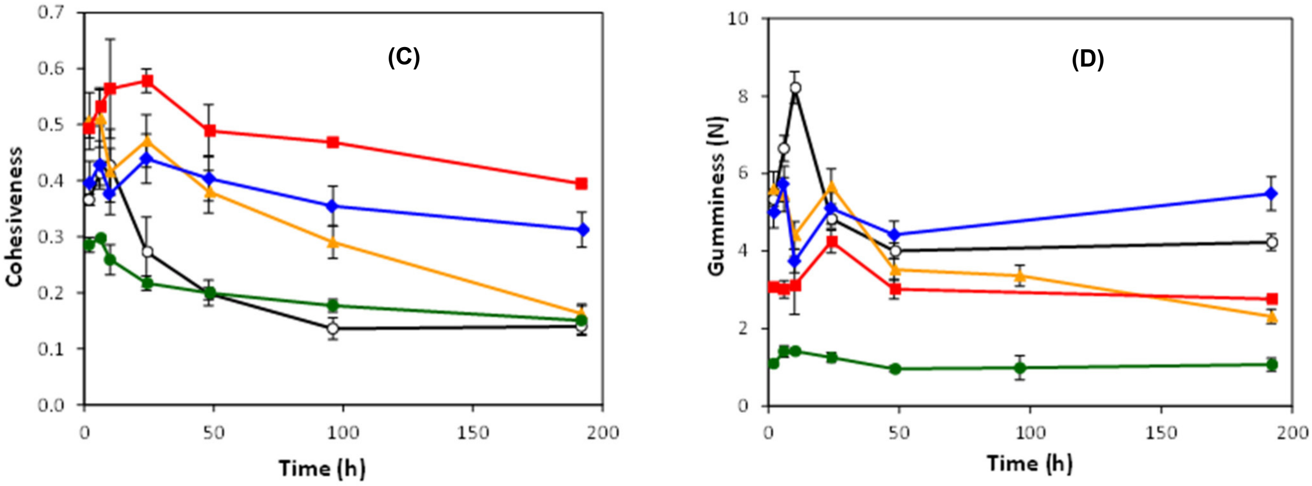


Figure 2. Evolution of firmness (**A**), springiness (**B**) cohesiveness (**C**) and gumminess (**D**) of gels made from fonio (○), millet (▲), sorghum (◆), maize (●) and rice (■) flours at 15% concentration with the storage time at 4 ± 2 °C. The error bars represent the standard deviation.

Springiness is a mechanical textural attribute related to the rapidity and degree of recovery from a deforming force [48]. The initial springiness of fonio gel was lower than those gels made from the remaining flours, except for maize. Gels' springiness decreased significantly ($p < 0.05$) with storage time, except for rice gel which showed a very stable springiness value during the whole studied period. Fonio was the only sample to show some springiness recovery after 100 h, with the value corresponding to 192 h being higher than those determined at 48 and 96 h. In general, the cohesiveness of all gels showed a similar trend to that obtained for springiness, with decreasing values during the studied storage time.

The value of gumminess increased after 6 h and decreased at longer times. The highest value was recorded in fonio and millet gels, but after 192 h, the fonio gel had only lost 21% of this maximum value, while millet lost 59%. This parameter is often used to characterize the necessary energy to disintegrate semisolid foods and is adequately correlated to sensory evaluation by a trained panel [49].

Table 6. Textural parameters of fresh gels (15% flour concentration) and their evolution with storage at 4 °C for 192 h (8 days).

Parameter	Fonio	Millet	Sorghum	Maize	Rice
Firmness (N)					
P_0	15 ± 1 d	11 ± 1 c	12.7 ± 0.4 cd	3.9 ± 0.3 a	6.2 ± 0.1 b
ΔP (%)	109 ± 17 c	27 ± 6 a	9 ± 11 a	65.0 ± 0.6 b	12 ± 4 a
Springiness					
P_0	0.73 ± 0.01 b	0.88 ± 0.01 c	0.75 ± 0.03 b	0.48 ± 0.05 a	0.85 ± 0.01 c
ΔP (%)	−30 ± 10 a	−28 ± 13 a	−7 ± 16 ab	−34 ± 11 a	−2 ± 1 b
Cohesiveness					
P_0	0.37 ±0.01 b	0.51 ±0.04 c	0.40 ± 0.03 b	0.29 ± 0.01 a	0.49 ± 0.02 c
ΔP (%)	−62 ± 4 a	−68 ± 4 a	−21 ± 21 b	−47 ± 9 ab	−20.0 ± 0.7 b
Gumminess (N)					
P_0	5.3 ± 0.6 c	5.60 ± 0.06 c	5.0 ± 0.5 c	1.10 ± 0.03 a	3.08 ± 0.08 b
ΔP (%)	−21 ± 2 ab	−59 ± 8 a	−13 ± 32 b	−13 ± 15 b	−10 ± 2 b
Resilience					
P_0	0.17 ± 0.01 b	0.36 ± 0.05 d	0.21 ± 0.02 bc	0.09 ± 0.01 a	0.23 ± 0.02 c
ΔP (%)	−57 ± 11 a	−75 ± 4 a	−13 ± 33 b	−41 ± 17 ab	−40 ± 5 ab

$\Delta P = 100 \cdot (P_8 - P_o)/P_o$, P= textural parameter, P_8= textural value after 8 d of storage, P_0 = initial textural value. Values with a letter in common in the same row are not significantly different ($p > 0.05$).

4. Conclusions

Fonio flour demonstrated good performance when comparing its properties to other gluten-free sources, showing significant differences in techno-functional, pasting, rheological, and gelling properties. Fonio presented a higher ability to absorb water during gelatinization and swelling in excess water, resulting in the highest determined values of WAI and SP, believed to be influenced by its small starch granule size and high specific surface area. This greater ability to interact with water was also verified in the evaluated gel properties. Fonio had a higher pasting viscosity, as well as high shearing and heating stability and resistance, consistent with a better-packed starch structure than the other analyzed GF flours, as shown by the thermal analysis. Rheological properties revealed that fonio flour generated gels with a remarkably strong structure, particularly at high flour concentrations (12% and 15%). Fonio gels also exhibited notably higher elastic modulus and firmness values than the rest of the GF flours. These combined results lead to the conclusion that fonio has the potential to be used in food, especially in preparations such as porridge, where better gelling properties are appreciated. Therefore, it is suggested that fonio is a promising starch source to compete with other commercially important flours, such as rice, in industrial applications.

References

1. Foschia, M.; Horstmann, S.; Arendt, E.K.; Zannini, E. Nutritional therapy-Facing the gap between coeliac disease and gluten-free food. *Int. J. Food Microbiol.* 2016, *239*,113–124.
2. Alvarez-Jubete, L.; Auty, M.; Arendt, E.K.; Gallagher, E. Baking properties and microstructure of pseudocereal flours in gluten-free bread formulations. *Eur. Food Res. Technol.* 2010, *230*, 437–445.
3. Sollid, L.M. Coeliac disease: Dissecting a complex inflammatory disorder. *Nat. Rev. Immunol.* 2002, *2*, 647–655.
4. Bernardo, D.; Peña, A.S. Developing strategies to improve quality of life of patients with gluten intolerance in patients with and without celiac disease. *Eur. J. Intern. Med.* 2012, *23*, 6–8.
5. Jideani, I.A.; Jideani, V.A. Developments on the cereal grains *Digitaria exilis* (acha) and *Digitaria iburua* (iburu). *J. Food Sci. Technol.* 2011, *48*, 251–259.
6. Zhu, F. Fonio grains: Physicochemical properties, nutritional potential, and food applications. *Compr. Rev. Food Sci. Food Saf.* 2020, *19*, 3365–3389.
7. Abrouk, M.; Ahmed, H.I.; Cubry, P.; Šimoníková, D.; Cauet, S.; Pailles, Y.; Bettgenhaeuser, J.; Gapa, L.; Scarcelli, N.; Couderc, M.; et al. Fonio millet genome unlocks African orphan crop diversity for agriculture in a changing climate. *Nat. Commun.* 2020, *11*, 4488

8. Barikmo, I.; Outtara, F. Protein, carbohydrate and fibre in cereals from Mali- How to fit the results in a food composition table and database. *J. Food Compos. Anal.* 2004, *17*, 291–300.
9. Temple, V.J.; Bassa, J.D. Proximate chemical composition of acha (*D. exilis*) grain. *J. Sci. Food Agric.* 1991, *56*, 561–563.
10. Jideani, I.A.; Akingbala, J.O. Some physicochemical properties of acha (*Digitaria exilis*) and iburu (*Digitaria iburua*). *J. Sci. Food Agric.* 1993, *63*, 369–374.
11. Ayo, J.A.; & Nkama, I.;Effect of acha (*Digitaria exilis*) grain flour on the physico-chemical and sensory properties of bread. *Int. J. of Food Prop*. 2004, 7(3),561-569.
12. Lasekan, D.O. Chemical composition of acha (*Digitaria exilis*) flour. *J. Food Sci. Agric.* 1994, *14*, 177–179.
13. Kamran, M.; Saleem, N.; Umer, Z.N. Ready-to-eat (rte) wheat bran breakfast cereal as a high-fiber diet. *J. Food Processing Preserv*. 2008, 32, 853–867
14. Sartelet, H.; Serghat, S.; Lobstein, A.; Ingenbleek, Y.; Anton, R.; Petitfrare, E.; Aguie-Aguie, G.; Martiny, L.; Haye, B. Flavonoids extracted from fonio millet (*Digitaria exilis*) reveral potential antithyroid properties. Elvisier Science Inc. *Nutrition* 1996, *12*, 100–106.
15. American Association of Cereal Chemists (AACC), 2000. Approved Methods of the AACC Method 44-19, 10 th ed. The Associatiomn, St.Paul,MN.
16. Abebe, W.; Collar, C.; Ronda, F. Impact of variety type and particle size distribution on starch enzymatic hydrolysis and functional properties of tef flours. *Carbohydr. Polym.* 2015, *115*, 260–268.

17. Joshi, D. C.; Sood, S.; Hosahatti, R.; Kant, L.; Pattanayak, A; Kumar, A; & Stetter, M. G. From zero to hero: the past, present and future of grain amaranth breeding. *Theoretical and Applied Genetics*. 2018,*131*(9), 1807-1823.
18. Ronda, F.; Pérez-Quirce, S.; Villanueva, M. Rheological Properties of Gluten-Free Bread Doughs: Relationship with Bread Quality. In *Advances in Food Rheology and Applications*; Ahmed, J., Ptaszek, P., Basu, S., Eds.; Elsevier: Cambridge, UK, 2017; Volume 12, pp. 297–334.
19. Villanueva, M.; De Lamo, B.; Harasym, J.; Ronda, F. Microwave radiation and protein addition modulate hydration, pasting and gel rheological characteristics of rice and potato starches. *Carbohydr. Polym.* 2018, *201*, 374–381.
20. Ballagoun, V.Y. Sistemes post-récolte, transformation, qualité du fonio et produits dérivés au nord du Bénin. In *Thèse de Doctorat Unique. Faculté des Sciences Agronomique*; Université d'Abomey-Calavi: Bénin, 2013; p. 152.
21. Solaesa, A.; Villanueva, M.; Vela, A.J.; Ronda, F. Protein and lipid enrichment of quinoa (cv. Titicaca) by dry fractionation. Techno-functional, thermal and rheological properties of milling fractions. *Food Hydrocoll.* 2020, *105*, 105770
22. Boucheham, N.; Galet, L.; Patry, S.; Zidoune, M.N. Physicochemical and hydration properties of different cereal and legume gluten-free powders. *Food Sci. Nutr.* 2019, *7*, 3081–3092.
23. Gani, A.; Ashwar, B.A.; Akhter, G.; Shah, A.; Wani, I.A.; Masoodi, F.A. Physico-chemical, structural, pasting and thermal properties of starches of fourteen Himalayan rice cultivars. *Int. J. Biol. Macromol.* 2017, *95*, 1101–1107.

24. Marson, W.R.; Hoseney, R.C. Factors affecting the viscosity of extrusion cooked wheat starch. *Cereal Chem.* 1986, *63*, 436–441.

25. Carcea, M.; Acquistucci, R. Isolation and physicochemical characterization of fonio (*Digitaria exilis* Stapf) Starch. *Starch/Stärke* 2006, *49*, 131–135.

26. Solaesa, A.; Villanueva, M.; Beltrán, S.; Ronda, F. Characterization of quinoa defatted by supercritical carbon dioxide. Starch enzymatic susceptibility and structural, pasting and thermal properties. *Food Bioprocess Technol.* 2019, *12*, 1593–1602.

27. Cornejo-Ramírez, Y.I.; Martínez-Cruz, O.; Lizette Del Toro-Sánchez, C.; Javier Wong-Corral, F.; Borboa-Flores, J.; Javier Cinco-Moroyoqui, J. The structural characteristics of starches and their functional properties. *CyTA-J. Food* 2018, *16*, 1003–1017.

28. Singh, S.; Singh, N.; MacRitchie, F. Relationship of polymeric proteins with pasting, gel dynamic-and dough empirical- rheology in different Indian wheat varieties. *Food Hydrocoll.* 2011, *25*, 19–24.

29. Kaushal, P.; Kumar, V.; Sharma, H.K. Comparative study of physicochemical, functional, antinutritional and pasting properties of taro (*Colacasia esculenta*), rice (*Oryza sativa*) flour, pigeon pea (*Cajanus cajan*) flour of their blends. *Food Sci. Technol.* 2012, *48*, 59–68.

30. Acevedo, B.A.; Avanza, M.V.; Cháves, M.A.; Ronda, F. Gelation, thermal and pasting properties of pigeon pea (*Cajanus cajan* L.) dolichos bean (*Dolichos lablab* L.) and jack bean (*Canavalia ensiformis*) flours. *J. Food Eng.* 2013, *119*, 65–71.

31. Ballet, S.; Guelpa, A.; Fox, G.; Manley, M. Rapid Visco Analyser (RVA) as a tool for measuring starch- Related Physicochemical Properties in cereal: A Review. *Food Anal. Methods* 2019, *12*, 2344–2360.
32. Grace, C.F.; Henry, C.J. The Physicochemical Characterization of Uconventional Starches and Flours Used and Flours Used in Asia. *Foods* 2020, *9*, 182.
33. Zhang, G.; Hamaker, B.R. Sorghum (*Sorghum bicolor* L. Moench) Flour Pasting Properties Influenced by Free Fatty acids and Protein. *Cereal Chem.* 2005, *82*, 534–540.
34. Shafie, B.; Cheng, S.C.; Gani, A.; Ashwar, B.A. In-vitro digestibility, rheology, structure, and functionality of RS3 from oat starch. *Food Chem.* 2016, *212*, 749–758.
35. Rao, M.A. Rheology of food gum and starch dispersions. In *Rheology of fluid and Semisolid Foods Principles and Applications*; Rao, M.A., Ed.; Spinger Science + Bussines Media: LLC, New York, NY, USA, 2007; pp.153–214.
36. Ronda, F.; Oliete, B.; Gòmez, M.; Caballero, P.; Pando, V. Rheological study of layer cake batters made with soybean protein isolate and different starch sources. *J. Food Eng.* 2011, *102*, 272–277.
37. Abebe, W.; Ronda, F. Rheological and textural properties of tef Eragrostis tef (Zucc.) Trotter] grain flour gels. *J. Cereal Sci.* 2014, *60*, 122–130.
38. Weipert, D. The benefits of basic rheometry in studying dough rheology. *Cereal Chem.* 1990, *67*, 311–317.
39. Renkema, J.M.S.; van Vliet, T. Concentration dependence of dynamic moduli of heat induced soy protein gels. *Food Hydrocoll.* 2004, *18*, 483–487.

40. Clark, A.H.; Ross-Murphy, S.B.; Nishinari, K.; Watase, M. Shear modulus concentration relationships for biopolymer gels. Comparison of independent concentration and cooperative crosslink descriptions. In *Food Polymers, Gels, and Colloids*; Burchard, W., Ross-Murphy, S.B., Eds.; Elsevier: London, UK, 1990; pp. 209–229.
41. Avanza, M.V.; Puppo, M.C. Rheological characterization of amaranth proteins gels. *Food Hydrocoll.* 2005, *19*, 889–898.
42. Biiliaderis, C.G.; Juliano, B.O. Thermal and mechanical properties of concentrated rice starch gels of varying composition. *Food Chem.* 1993, *48*, 243–250.
43. Solaesa, A.; Villanueva, M.; Munoz, J.M.; Ronda, F. Dry- Heat Treatment vs. Heat_Moisture Treatment assisted by microwave radiation: Techno-functional and rheological modifications of rice flour. *LWT Food Sci. Technol.* 2021, *141*, 110851110851.
44. Eliasson, A.C. Interactions between starch and lipids studied by DSC. *Thermochim. Acta* 1994, *246*, 343–356.
45. Ji, Y.; Ao, Z.; Han, J.A.; Jane, J.L.; BeMiller, J.N. Waxy maize starch subpopulations with different gelatinization temperatures. *Carbohydr. Polym.* 2004, *57*, 177–190.
46. Lewandowicz, G.; Jankowski, T.; Fornal, J. Effect of microwave radiation on physico-chemical properties and structure of cereal starches. *Carbohydr. Polym.* 2000, *42*, 193–199.
47. Jacobson, M.R.; Obanni, M.; BeMiller, J.N. Retrogradiation of starches from different botanical sources. *Cereal Chem.* 1997, *74*, 511–518.

48. Di Monaco, R.; Cavella, S.; Masi, P. Predicting sensory cohesiveness, hardness and springiness of solid foods from instrumental measurements. *J. Texture Stud.* 2008, *39*, 129–149.

49. Truong, V.D.; Walter, W.M.; Hamann, D.D. Relationship between instrumental and sensory parameters of cooked sweet potato texture. *J. Texture Stud.* 1997, *28*, 163–185.

CHAPTER 4: STUDY II

TECHNOLOGICAL EVALUATION OF THE ADDITION OF *DIGITARIA EXILIS STAPF* FLOUR IN A GLUTEN-FREE BREAD

Abstract: In recent years, the market for gluten-free bakery products has expanded due to the continuing increase in the incidence of celiac disease and food hypersensitivity. The African cereal *Digitaria exilis* Stapf (also known as acha, fonio or hungry rice) has sparked worldwide interest as a potential raw material for gluten-free bakery products. The objective of this study was to determine the optimal percentage of *Digitaria exilis* Stapf flour to enhance the baking performance of gluten-free bread. The research evaluated the impact of increasing concentrations (25, 50, 75 and 100%) of fonio flour on the rheological, pasting, fermentation, structural and textural properties of gluten-free bread. The viscosity profile of the fonio flour was lower than that of the control, both in the first and in the second part of the analysis. The capacity of fonio dough to rise was significantly reduced as the amount of fonio flour incorporated increased. Breads containing medium to high levels of fonio (50, 75 and 100%) were harder and less elastic than the control. Only the 25% substitution samples showed to be a good alternative to traditional gluten-free bread formulations.

Keywords: Fonio, rheological properties, texture, gluten-free, bread, celiac disease.

1. Introduction

The consumption of gluten, a protein component of wheat consisting of a broad group of prolamins (gliadins and glutenins), triggers an immune-mediated enteropathy known as celiac disease (CD) in genetically susceptible individuals (Ludvingsson et al., 2012). CD is one of the most prevalent food intolerances with recent epidemiological studies estimating its global prevalence to be 1 in 100 individuals (Rubio-Tapia et al., 2009). The ingestion of wheat or related cereals, such as barley and rye, by celiac disease patients leads to an immunological response localised in the small intestine, destroying the villi and microvilli present on the surface of the small intestine (Armstrong et al., 2012; Aronsson et al., 2015; Fritz and Chen, 2017). Currently, the only treatment for celiac disease is a life-long, strictly gluten-free diet (Gao et al., 2018).

In recent years, there has been a considerable increase in the market for gluten-free products, with a growing number of people being diagnosed with celiac disease and other gluten-related disorders, as well as people purchasing gluten-free (GF) products as part of a 'healthier' lifestyle. As a result, the production of GF products has considerably increased, particularly in the bakery industry (Villanueva et al., 2021). Bakery products frequently have nutritional imbalances and poor sensory quality due to the use of corn and rice starches and flours (Sollid, 2002; Bernardo and Peña., 2012). As a result, researchers have focused their attention on finding alternatives to these raw materials.

In this context, an undervalued and underutilised gluten-free cereal like Fonio, which is a member of the Gramineae family, the subfamily Panicoideae, and the Digitaria genus and originates from the West African Sahelian countries (Portères 1976), may be a candidate to replace conventional GF raw materials due to its nutritional properties (Diop et al., 2018). White Fonio, also known as Digitaria exilis Stapf or acha, and Digitaria iburua Stapf or ibura as black Fonio, have been approved as a novel food in the European Union and as a healthy grain in the United States (European Commission, 2018). Fonio is considered one of the most nutritious and tasty grain in Africa and is now mostly consumed as a whole grain due to its small size (Jideani 1990). Additionally, Fonio has a relatively low amount of free sugars and a low glycemic index, which makes its consumption a good alternative for diabetics and lactating women (Temple and Bassa, 1991). Of particular interest are its high levels of methionine and cysteine, which are similar to those of the main grains, wheat, sorghum, barley, rye, and corn (Vietmeyer et al., 1996; Fliedel et al., 2004), and phosphorus and calcium (Jideani, 1999). Fonio flour has a high content of pentosans, which gives it the ability to absorb water and produce highly viscous solutions (Lasekan, 1994; Olapade & Oluwole, 2013). Currently, there is limited research on the use of fonio flour in the production of gluten-free bakery products. Igyor (2005) found that replacing wheat flour with fonio flour up to 25% (w/w) resulted in decent bread, while a previous study found that higher substitution values reduced fonio bread acceptability (Ayo and Nkama, 2004). The possibility of producing consumer acceptable acha bread using carboxymethylcellulose (CMC) and potato starch was demonstrated by Jideani (2007) and Jideani et al (2008).

The aim of this study was to further investigate the effect of fonio on the structural, textural, technological, and nutritional properties of gluten-free bread.

2. Materials and Methods

3.1. Preliminary trials to define the water content in gluten-free batters

Preliminary analyses were carried out to evaluate the impact of replacing rice flour and maize starch with fonio flour in a gluten-free bread formulation, with an attempt to obtain doughs with the same consistency by modulating the amount of water added.

The study analysed the resistance to deformation of dough under different water content levels, following Nunes et al.'s (2009) method used to observe the impact of adding low lactose dairy powder on gluten-free batters and bread quality properties. This method provided the same consistency as the 90% water control for every formulation tested. A rheological analysis was conducted using a rotational rheometer MCR 102 (Anton Paar MCR, GmbH, Inc., Graz, Austria). Three measurements of the complex modulus (G*) were taken in triplicate for the control sample with varying water content (70, 75, 80, 85, 90, 95, 100, 105, and 110%). The complex modulus reflects the degree of resistance to deformation. Dough samples for small deformation rheological evaluation were prepared without yeast and allowed to rest for eight minutes before testing. Subsequently, the doughs were subjected to an applied stress oscillating between 1 and 100 Hz, and the complex modulus (G*) was assessed at 10 Hz. The average G* values obtained for each water level (WL) were plotted against WL (%) to show the rheological behaviour of the dough with increasing WL. The plotted curve was evaluated and the corresponding regression equation and r2 value were determined using the regression curve. The rheological behaviour of the batter was described by the following equation: G*= 6 × 1019×WL-8.3086 (Nunes et al .2009).

Single frequency tests were performed on the dough control sample. A WL of 90% was used in these sample, according to the control formulation.

The value of G* obtained with each fonio-sample was introduced in Eq.1 and a theoretical WL was fixed. The adjust WL was determined by direct comparison between the G*/WL of the control and G*/WL of the fonio-containing samples. The adjusted WL was then determined by a direct comparison between the G*/WL of the control and that of the samples containing fonio. By aligning the theoretical WL with the control dough (90% WL), the amount of water required to change the G* of each ingredient was determined, thus facilitating the prediction of new amounts of water to ensure uniformity across all samples. Due to variations in rheological behaviour in each formula, minor adjustments of the theoretic WL are required to attain a similar level of G* in all samples.

3.2. Raw materials

Commercial maize starch, rice flour, *Psyllium* fiber and guar gum were supplied from Chimab Campodarsego (PD, Italy). Fonio flour was obtained from Obà food group (Roma, Italy). Fresh compressed yeast, sunflower oil, salt and sugar were purchased from a local supermarket.

3.3. Flour viscometric properties

The viscometric properties of the samples F25, F50, F75, F100, C, R75 and R100 were obtained using a Rapid Visco Analyser (RVA-4, Newport Scientific, Warriewood, Australia) according to AACC method 76–21 (AACC, 2000). Three grams of each dough formulation were dispersed in 25 mL of distilled water into an aluminium canister.

The initial temperature for the test set at 50 °C and stirred at a rotation speed of 960 rpm, for 5 min until complete homogenization. Afterwards, holding time of 1 min at 50 °C, heating to 95 °C for 3 min 42 s, holding at 95 °C for 2 min 30 s, cooling to 50 °C for 3 min 48 s; and in the end, holding at 50 °C for 2 min.

Using the software program Thermocline for Windows were measured the following parameters: Pasting Temperature (°C), Peak Time (when peak viscosity resulted), Peak Viscosity (highest hot paste viscosity), Holding Viscosity (lowest hot paste viscosity), Breakdown (minimum hot paste viscosity), Setback (final viscosity minus holding strength), and Final Viscosity (end of the test after cooling to 50 °C). The parameter of viscosity is indicated in mPa s. Each measurement was performed in triplicate.

3.4. *Alpha-amylose activity of flour samples*

In accordance with Method 22-08.01, α-amylase activity was determined using a Rapid Visco Analyser (RVA-4, Newport Scientific, Warriewood, Australia). To liquefy the sample, it was mixed in a flour-water suspension and simultaneously heated to a constant speed of 95°C for 180 seconds. The enzyme activity was assessed through the stirring number (SN) which is defined as the apparent viscosity, and the value was measured in rapid visco units. Each measurement was carried out three times.

3.4. Dough formulations

A conventional gluten-free formula based on 50% rice flour, 50% maize starch, 1.5% psyllium fiber, 1.5% guar gum, 5% sunflower oil, 90% water, 1.8% salt, 3% sugar and 3% yeast (Conte et al., 2018), was using for prepared a gluten-free control sample.

Fonio-enriched GF breads were prepared by replacing rice flour and maize starch with increasing amounts of fonio at four different levels (25%, 50%, 75% and 100%), as shown in Table 1. Additionally, two other rice-based GF formulations (R75, R100) were prepared to compare the characteristics of Fonio-enhanced GF breads to those of other popular GF products.

The gluten-free dough samples were prepared by dissolving yeast, sugar, and salt in warm water (26°C), followed by the addition of pre-mixed dry ingredients.

The suspending components and sunflower oil were combined using a Kitchen Aid mixer (Model 5KPM50, St. Joseph, MI, USA) equipped with a flat beater. The mixer operated at speed 1 for 5 minutes, followed by 8 minutes at speed 2. The resulting doughs were divided into 600-gram loaves and shaped into baking pans. The samples were then left to rise in a climatic chamber with a temperature of 30°C and a relative humidity of 90%. These steps were performed according to Conte et al. (2018).

The samples were then baked in an electric oven (Europa, Molina di Malo, VI, Italy) at 200°C for 70 min, after which the gluten-free bread was cooled at room temperature for 2 h. The bread was then sliced into 2 mm pieces using a bread slices (GRAEF GmbH, Donnerfeld 6, D-59757 Amsberg, Germany) and the central slices of each loaf were used for testing.

Table 1. Composition of the formulation used in experimental design.

SAMPLE CODE	RICE FLOUR (%)	MAIZE STARCH (%)	FONIO FLOUR (%)	WATER LEVEL (%)
F25	25	50	25	88,3
F50	0	50	50	88,5
F75	0	25	75	97
F100	0	0	100	110
C	50	50	0	90
R75	75	25	0	98
R100	100	0	0	110

Abbreviations: F25; fonio 25%; F50, fonio 50%; F75, fonio 75%; F100, fonio 100%; C, control; R75, rice 75%; R100, rice 100%.

3.5. Dough rheological measurements

3.5.1. Small deformation tests

Rotational rheometer MCR 102 (Anton Paar MCR, GmbH, Inc.,Graz, Austria) was utilized to perform rheological measurements by means of an oscillation test. For these measurements, dough samples were prepared without yeast and, prior to starting the analysis, were left to rest for 10 min. Doughs samples were analysed using a parallel plate geometry, consisting of a 25 mm diameter corrugated probe and plate.

The thickness of the sample was adjusted to 2 mm with the lowering of the upper geometry and the excess was trimmed off. On the edge of the samples was applied a thin layer of vaseline oil to prevent moisture losses during the test.

The test used was a frequency sweep test, conducted over the range 0.1–100 Hz at 20°C with a target strain of 0.01%. Data were obtained in such a way as to ensure that the results obtained in the sample would always fall within the linear viscoelastic region (LVR).

The Values of storage modulus (G'), loss modulus (G''), and loss tangent (tan δ) were recorded at a frequency of 10 Hz. Three repetitions on each sample were made.

3.5.2. Leavening properties of fonio-enriched doughs

The rheofermentometric test was conducted to understand the behaviour of the dough during leavening, allowing to evaluate the development in height of the dough and the production and retention of carbon dioxide. A Rheofermentometer F4 (Chopin, Villanueve- La-Grenne, France) was used to measure: maximum height of dough (Hm (mm)), development of dough height at the end of the test (h (mm)), (Hm-h)/Hm, that is a percentage decrease in dough development at the end of the test compared to the maximum recorded development and T1 (min) time corresponding to the maximum development of the dough. The total amount of CO_2, both released and retained in the dough samples, were also measured.

A dough piece (315 g) was placed in a movable container of the gas meter with a cylindrical weight, and the cover of the container was fitted with an optical sensor. The test was conducted at 30 °C for 180 min. The analyses were done in duplicate.

3.6. Bread measurements

3.6.1. Specific volume

The volume of the bread samples was measured using the rapeseed displacement method in accordance with AACC 10-05.01 (AACC, 2005), followed by evaluation of the specific volume (in mL/g) by calculating the ratio of the volume of the bread with its weight.

3.6.2. Bread texture

To assess the mechanical properties of bread, texture analysis was performed using a TA-XT2 Plus texture analyser from Stable Micro Systems, UK, equipped with a 30kg load cell and a 36mm diameter cylindrical aluminium probe. Slices of bread 2 cm wide were used. They were compressed at a constant rate of 1 mm/s with a 30 second interval between compressions. The force required to compress the samples to 50% of their original width was determined from the force versus time curve. For the texture measurements, three slices from two loaves of bread were used for each formulation on the same baking day.

3.6.3. Colour determinations

Colour measurements were determined for the crumb and crust on a baking day using a Minolta colorimeter (Minolta CR-300, Konica Minolta Sensing, Osaka, Japan). The Hunter Lab color space was employed to express the results of colorimetric data as L values for lightness, ranging from 0 (black) to 100 (white), and a coordinate for redness (+) to greenness (−), and b coordinate for yellowness (+) to blueness (−) (CIE L*a*b* colour system). Bread crust colour measurements were taken at three points in the central and two distal areas of six loaves, while crumb colour was measured at three points of three central slices from two loaves.

3.7. Statistical analysis

Statistical analysis was conducted using STATISTICA software, version 6.0 (Statsoft, Inc., Tulsa, OK, USA). The experimental data was assessed through one-way analysis of variance (ANOVA) and nonlinear regression analysis. Fisher's least significant differences (LSD) test was applied to establish differences between each pair of means with 95% confidence.

4. Results and Discussion

4.1. Viscometric properties of gluten-free flour sample

The viscometric properties of flour have a significant influence on the quality and structure of the bread (Bollaìn et al., 2006). Table 2 shows the influence of different levels of fonio flour substitution on the viscometric properties of the sample.

Alpha-amylase activity plays an important role in determining the peak viscosity: when this enzyme is present at high levels, the starch in the flour is hydrolysed to a greater degree. This value is also a predictor of the shelf life of bread (Balet et al., 2019). A decrease in peak viscosity is linked to a reduction in both starch content and degree of swelling in the starch granules (Leon et al., 2010).

The sample with fonio addition showed a lower value of peak viscosity compared to the C sample, with F25 demonstrating the lowest value followed by F50, F75 and F100. In contrast, the R75 and R100 samples yielded higher peak viscosity values.

The peak time occurred earlier in the samples made with fonio flour compared to both the controls and the rice-based samples, regardless of the substitution percentages used, and is probably due to the low alpha-amylase activity of the fonio starch granules.

Additionally, when the starch's viscosity begins to increase, the temperature is recorded as the pasting temperature (Liang and King, 2003). The temperature mentioned above is the minimum cooking temperature that is required for the flour to be cooked (Sandhu et al., 2007). When compared to the control and rice-based samples, this value was lower in all fonio enriched samples due to their structural characteristics, such as the organization and amount of amylose and amylopectin (Chen et al., 2017). The breakdown value represents the point of lowest viscosity during the holding period and is determined by calculating the difference between the peak and trough viscosities. The breakdown viscosity of sample R100 was higher than the breakdown viscosity of the other samples, indicating greater degradation of swollen systems and orientation of amylose and other linear components in shear direction. The samples incorporating high percentages of fonio flour (F75 and F100) exhibited enhanced thermostability and reduced shear thinning and disintegration of swollen systems compared to the remaining samples.

A setback parameter, obtained by calculating the variance between peak viscosity and final viscosity, is noted when the temperature declines to 50°C. Retrogradation of the starch is enabled by temperature cooling, causing an increase in viscosity. In this stage, the amylose and amylopectin chains realign to form a more crystalline structure (Balet et al., 2019). The reduced setback of fonio flour samples, when compared to the C, R100 and R75, is associated with retrogradation and indicates that fonio flour retrogrades to a lesser extent than other samples.

At the end of the pasting cycle, final viscosity was recorded, indicating the flour's ability to form a viscous paste during cooking and cooling, mainly reflecting amylose retrogradation (Leon et al., 2010). The experimental data obtained showed that the final viscosity decreased with increasing the amount of fonio used and increased with the percentage of rice flour used (R100 > R75) compared to C.

4.2. Alpha-amylase activity of gluten-free flour sample

The activity of alpha-amylase is quantified by the Stirring Number (SN), as shown in Table 2. This hydrolytic enzyme causes a decrease in viscosity as the SN increases. A low SN indicates weak amylase activity, which ultimately results in poor bread quality, such as underdeveloped bread with a dry and compact crumb. In the experimental samples, it was found that the fonio added sample had a lower SN than the control and rice-based samples.

Table 2: Viscometric properties of flour samples at different percentages of fonio flour Mean values ± standard deviation. Different letters within the column mean significant differences among the dough samples according to LSD test ($p < 0.05$).

4.3 Fundamental rheological features of gluten-free doughs

Parameters Samples	Peak Viscosity (mPa· s)	Peak time (min)	Pasting Temp (°C)	Trough Viscosity (mPa· s)	Breakdown (mPa· s)	Final Viscosity (mPa· s)	Setback (mPa· s)	Stirring Number
F25	3243 ± 80^{d}	5.47 ± 0^{c}	79 ± 42 ^{cd}	2454 ± 98 ^{d}	709 ± 60 ^{c}	4160 ± 0 ^{d}	1706 ± 1 ^{d}	2521± 90 ^{d}
F50	2307 ± 16^{e}	5.14 ± 0^{d}	79 ± 36 ^{cd}	1616 ± 139 ^{e}	692 ±140 ^{c}	2941± 0^{ef}	1325 ± 1 ^{e}	2222 ± 26 ^{e}
F75	1959 ± 16 ^{f}	5.20 ± 0^{d}	82 ± 7 ^{b}	1451 ± 10 ^{f}	509 ± 1 ^{d}	2856 ± 0 ^{f}	1406 ± 0 ^{e}	2248 ± 7 ^{e}
F100	1981 ± 8 ^{f}	5.14 ± 0^{d}	77± 22 ^{d}	1461 ± 218 ^{f}	521 ± 203^{d}	3142 ± 0 ^{e}	1681± 0^{d}	2022 ± 7 ^{f}
C	3702 ± 20^{c}	5.73 ± 0^{b}	79± 18 ^{c}	2917 ± 66 ^{c}	786 ± 28 ^{b}	5210 ± 0^{c}	2293± 0 ^{c}	2974 ± 39^{c}
R75	4098 ± 67 ^{b}	6.17 ± 0^{a}	83 ± 23 ^{ab}	3390 ± 25 ^{b}	790 ± 116 ^{b}	6112 ± 0 ^{b}	2722 ± 1^{b}	3389 ± 0. ^{a}
R100	4475 ± 75 ^{a}	5.73 ± 0^{b}	84 ± 18 ^{a}	3557 ± 86 ^{a}	919 ± 6 ^{a}	7493 ± 0 ^{a}	3937 ± 0^{a}	3240 ± 41 ^{b}

The storage modulus (G') and loss modulus (G") of dough samples with 90% water addition are detailed in table 3.

An analysis of fundamental rheology in oscillatory movement, the frequency Sweep test, was used to evaluate the viscoelastic properties of the mixtures. This allowed determination of the values for the elastic component (G') and the viscous component (G") of the experimental samples.

Table 3 reveals a notable difference in both G’ and G’’ of control, rice-based and fonio-enriched dough samples. The elastic component (G’) in all samples was higher than the viscous component (G’’), indicating a solid, elastic behaviour across all dough samples. As a general trend, the increase in fonio flour substitution led to a rise in both modules, G’ and G’’. There was no significant difference in the value of G' between the control sample and samples with low substitution of fonio flour, F25-F50. The highest value of G' was recorded by the R100 sample, followed by the F100 sample. Similarly, a comparable trend was observed for the G" parameter.

In order to obtain a more precise evaluation, we calculated the Loss Factor or Tangent Delta (TAN δ), which describes the ratio of the two components of the viscoelastic behaviour (G"/G'). All formulations examined showed viscoelastic properties (TAN $\delta<1$). The highest value for this factor is observed in F50 and C, with no statistically significant differences ($P < 0.05$) observed between F25 and F75.

Table 3. Rheological properties in the gluten-free doughs control, rice-based dough, and fonio-enriched batter samples.

Parameters	F25	F50	F75	F100	C	R75	R 100
G'	6909± 425.61 e	6072 ± 378.17 e	9918 ± 351.40 d	20879 ± 1514.89b	6774 ± 87.24 e	12035 ± 969.38 c	23690 ± 1404.60 a
G''	1820 ± 40.72 d	1706 ± 70.21 d	2615 ± 79.97 c	5045 ± 285.61 b	1857 ± 27.25 d	2930 ± 219.07 c	5414 ± 311.71 a
TAN δ	0.26 ± 0.011 b	0.28 ± 0.006 a	0.26 ± 0.007 b	0.24 ± 0.004 c	0.27 ± 0.001 a	0.24 ± 0.002 c	0.23 ± 0.002 d

Mean values ± standard deviation of G', Storage modulus; G'', loss modulus; TAN δ, tan delta. Different letters within the column mean significant differences among the dough samples according to LSD test ($p < 0.05$).

4.4. Leavening properties of fonio-enriched doughs

Tracking the expansion of bubbles during fermentation can establish a link between the incorporation of air into the dough during mixing and the aerated texture of the baked loaf (Chiotellis and Campbel, 2003). The influence of fonio-enriched dough and other gluten-free samples on dough development, as detected by rheofermentometry, is displayed in Figure 1 and Table 4. All doughs prepared by substituting rice flour and/or maize starch with fonio flour demonstrated development curves that were significantly different from those of the control and rice-based samples.

The dough development curves showed that increasing the amount of fonio flour proportionally decreased the maximum dough development (Hm) compared to the C sample. This resulted in final breads with an inferior weight/volume ratio.

With the exception of F25, the samples enriched with fonio flour showed a much faster and more pronounced development than both the control and the rice-based samples. The progression was observed in terms of the maximum dough development during rising (Hm) and its maintenance during the final phase of the test (h), with statistically significant differences ($p < 0.05$).

From the data shown in Table 4, as regards the height of the dough, it can be observed that the addition of more than 25 % voice prevented a correct measurement of this parameter (h) by the instrument. Unfortunately, in all the tests carried out on samples F50, F75 and F100 the probe of the instrument was incorporated by the sample.

When evaluating the parameter related to the reduction of the final volume of the dough, expressed as a percentage of the maximum height value (Hm-h)/Hm, it was observed that the samples containing fonio had a higher percentage of this parameter. Consequently, the final reduction of the dough in the fonio samples was significantly greater compared to the control and the two other rice-based formulations. The preservation of the macrostructure of the dough during the fermentation process may be related to the least favourable combination of gas production and microstructural properties (Xu et al., 2018).

Gas retention capacity relates to the ability of the dough to retain and release produced gas, but also significantly affects the final product's quality (Hackenberg et al., 2017). The porous quality of the dough linked to the gluten network is responsible for this characteristic (Marti et al., 2017). However, when considering the parameters for gas release, it is noteworthy that the total gas production in almost all fonio-enriched doughs was similar or even higher than that observed in the control and R-based samples. Nonetheless, the final volume of CO2 retained by the fortified doughs was only similar to that of the control dough in the case of the F25 sample. These findings suggest that the use of fonio flour as a substitute in gluten-free dough negatively impacted its ability to rise and maintain gas, with the exception of F25 samples.

Table 4. Rheofermentometer parameters of fonio-enriched doughs, control e rice-based doughs.

Samples	F25	F50	F75	F100	C	R75	R100
Hm (mm)	62.50 ± 0.85 [b]	43.30 ± 0.42 [e]	32.45 ± 0.35 [f]	26.95 ± 0.07[g]	68.55 ± 0.64 [a]	58.90 ± 1.13 [c]	50.60 ± 0.85 [d]
h (mm)	0.25 ± 0.07[d]	0.00 ± 0.00 [d]	0.00 ± 0.00[d]	0.00 ± 0.00 [d]	49.30 ± 1.98 [a]	15.05 ± 1.77 [b]	10.35 ± 2.76 [c]
T1 (min)	103.30 ± 0.00 [b]	76.30 ± 0.00 [d]	61.30 ± 0.00 [e]	57.00 ± 0.00 [f]	114.00 ± 4.24 [a]	101.15 ± 1.20 [b]	85.30 ± 0.00 [c]
CO2 TOT (mL)	1414 ± 11.31 [a]	1299 ± 9.19 [d]	1348 ± 5.66 [c]	1385 ± 5.66 [b]	1348 ± 19.80 [c]	1370 ± 2.38[bc]	1378 ± 12.73 [b]
CO2 REL (mL)	132.00 ± 1.41 [d]	152.50 ± 2.12 [c]	199.50 ± 9.19[b]	226.00 ± 1.41 [a]	68.00 ± 7.07[e]	130.00 ± 5.66 [d]	164.50 ± 6.36 [c]
CO2 RET (mL)	1282 ± 11.31 [a]	1147 ± 7.07 [d]	1149 ± 2.83 [d]	1159 ± 3.54 [d]	1280 ± 12.73 [a]	1239 ± 7.78 [b]	1214 ± 7.07[c]

Mean Values ± standard deviation. Within columns, values with the same letter do not differ significantly from each other according to LSD test ($p < 0.05$). Abbreviations: Hm (mm), maximum height of dough, T1, time of maximum development of the dough (min), CO_2 TOT, total gas production; CO_2 REL, gas released by the dough; CO_2RET, gas retained by the dough.

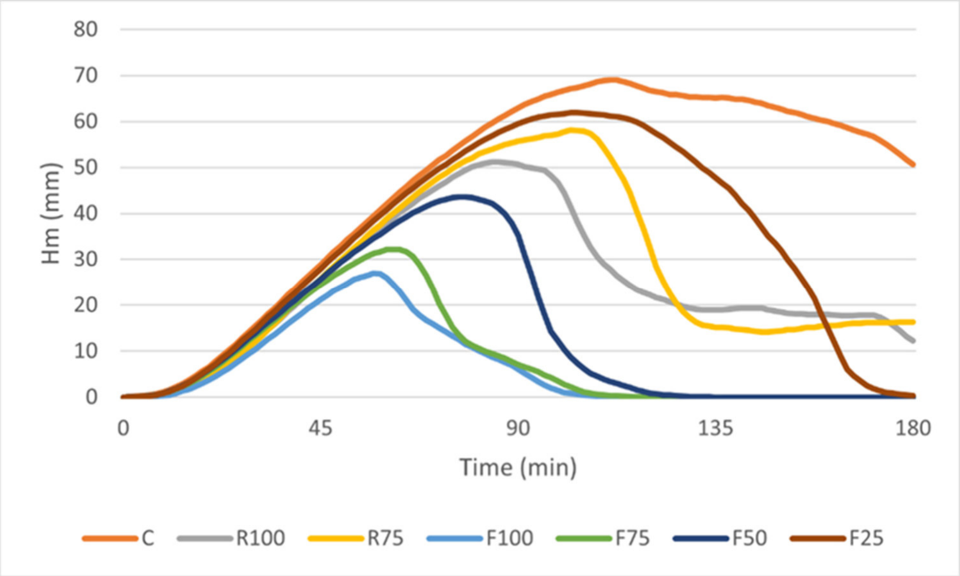


Figure 1: Dough development curves of the gluten free control, rice-based and fonio-enriched dough at different percentages.

4.5. Fresh bread characteristics

The technological aspects of baking bread impact the consumer's preferences (Conte et al., 2018). In this study, specific volume, crumb and crust colour and textural parameters were analysed to compare fonio-enriched bread with control and rice-based bread.

4.5.1. Specific Volume of breads loaves

Bread aeration characteristic is linked with high ratio of volume per weight (Conte et al.,2018). Fonio-enriched loaves GF breads showed a specific volume, obtained by dividing the final weight of the loaves by their volume, from 1,65 to 2,27 mL/g. The sample F25 is not statistically significant different from the control sample, instead, the sample F50 is significantly higher than the GF breads control (2,27 mL/g). Additions of more than 50% of fonio significantly decrease the volume when compared to the control samples (F75, F100 and R100).

Table 5. Specific volume of the gluten free control, rice-based and fonio-enriched dough at different percentages.

Samples	Specific volume (mL/g)
C	2.20 ± 0.05 b
F25	2.21 ± 0.06 b
F50	2.27 ± 0.04 a
F75	1.90 ± 0.03 d
F100	1.65 ± 0.02 e
R75	2.21 ± 0.04 ab
R100	2.12 ± 0.05 c
p value	***

4.5.2. Crust and Crumb colour

Fonio flour substitution have affected the visual features of the experimental GF breads, significantly influenced the crumb and crust colour (Table 6).

regarding the crust colour, F50 showed the highest value of L*, followed by F25 and C samples. Instead, with respect to gluten-free fonio- enriched breads prepared with the high level of fonio substitution, a lower lightness L*compared to R75 and R100 and F100 was registered.

There were no significant differences between the F25 and F100 bread samples and the control with regard to the redness coordinate (a*); however, these samples differed from the rice-based breads, which had the highest value. The F50 sample showed the lowest score. Similar differences to the a* parameter were observed in the b coordinate of colour crust.

As for crumb colour features, the L* value was highest in the control and rice-based bread samples, while the fonio-enriched bread samples with the least lightness were F25 and F100, respectively. All samples showed a significant statistical difference ($p < 0.05$) with respect to the b* coordinate. The value of fonio-enriched bread increased generally through an increase in fonio flour substitution (See Table 6). A lower whiteness index and more coloured crumb with redder (a* positive) colour values were found in all rice-based breads compared to the control and fonio-enriched breads. Additionally, high levels of fonio (F75-F100) resulted in a higher yellow (b* positive) value in comparison to the control and rice based bread.

	F25	F50	F75	F100	C	R75	R100
Crust Colour							
L*	71.58 ± 0.23^{b}	78.47 ± 0.058^{a}	64.83 ± 1.32^{c}	62.98 ± 0.63^{d}	72.31 ± 0.20^{b}	61.91 ± 1.50^{d}	54.34 ± 0.69^{e}
a*	5.29 ± 0.09^{c}	1.78 ± 0.27^{e}	4.01 ± 0.28^{d}	5.13 ± 0.22^{c}	5.04 ± 0.16^{c}	8.90 ± 0.52^{b}	12.82 ± 0.14^{a}
b*	24.61 ± 0.78^{c}	17.98 ± 1.11^{f}	20.54 ± 2.17^{e}	22.47 ± 0.71^{de}	22.95± 1.20^{cd}	30.70 ± 0.45^{b}	34.06 ± 0.22^{a}
Crumb Colour							
L*	60.11 ± 0.47^{bc}	50.26 ± 2.12^{e}	57.01 ± 2.32^{d}	58.73 ± 1.80^{cd}	63.70 ± 0.75^{a}	61.29 ± 0.44^{b}	62.14 ± 1.36^{ab}
a*	0.84 ± 0.10^{bc}	0.78 ± 0.17^{c}	2.10 ± 0.16^{a}	2.33 ± 0.17^{a}	0.76 ± 0.11^{c}	0.29 ± 0.16^{d}	1.04 ± 0.23^{b}
b*	6.39 ± 0.33^{bc}	6.66 ± 0.47^{b}	10.31 ± 0.16^{a}	10.88 ± 0.25^{a}	5.84 ± 0.14^{c}	6.06 ± 0.49^{bc}	6.69 ± 0.79^{b}

Table 6: Crust and crumb colours of gluten-free bread samples.

Mean Values ± standard deviation. Within columns, values with the same letter do not differ significantly from each other according to LSD test ($p < 0.05$).

4.5.3. Textural properties

To assess the quality of the bread and its mechanical properties, we evaluated its hardness, springiness, cohesiveness and elasticity. Table 7 shows that the replacement of rice flour and/or corn starch by over 50% of fonio flour significantly increased the final hardness of the bread compared to both the control and the rice samples. Sample F25 showed a similar behaviour to that of the control samples, all of which had similar values of hardness. With the substitution of 50-75-100% of fonio flour, the elasticity value of the crumb is considerably reduced, which means that the crumb is less able to return to its original state after compression, which is shown by the lowest elasticity value observed in this sample compared to the control and other fonio and rice breads.

The parameter of the cohesiveness of the crumb, which indicates the extent to which the structure of the food can be modified before it breaks up, increased significantly in all the experimental breads containing up to 25% fonio flour (see Table 7). However, the tendency for the fonio-enriched breads to be less cohesive than the control and other rice-based breads is an undesirable characteristic specific to gluten-free breads, which typically have a high tendency to break or crumble (Onyango et al., 2011). Overall, the cohesiveness parameter increased in the fonio-enriched bread samples as the percentage of fonio flour substitution increased. Only the F25 sample did not differ significantly from the control sample.

The elasticity parameters showed a similar behaviour to the cohesion, with lower values observed in the sample with high hardness.

Table 7: Textural parameters of the gluten-free control, rice-based, and fonio-enriched breads.

Samples	Hardness (N)	Springiness	Choesiveness	Resilience
F25	11.99 ± 1.72d	0.99 ±0.01 a	0.56 ± 0.01 b	0.30± 0.01b
F50	18.29 ± 2.39c	0.90 ± 1.53 a	0.44 ±0.004 c	0.22± 0.010 c
F75	51.32 ± 5.72 b	0.95± 0.023 b	0.40 ± 0.026 d	0.17± 0.011 d
F100	61.11 ± 3.31 a	0.96± 0.021 b	0.42 ± 0.022 cd	0.17± 0.013 d
C	10.95± 0.75 d	0.98± 0.008 a	0.56± 0.010 b	0.30 ± 0.011 b
R75	9.21± 2.03 d	0.98 ± 0.012 a	0.60± 0.028 a	0.32± 0.020 a
R100	8.83 ± 0.71 d	0.97 ± 0.002 ab	0.57 ± 0.019 ab	0.29 ± 0.018 b

Mean values ± standard deviation of textural properties. The values with the same following letter do not differ significantly from each other ($p<0.05$).

5.Conclusion

The results of the study indicate that a bread with a good leavening, structure and texture can be obtained with the addition of less than 25% of fonio. According to the Rapid Visco Analyser results, the water absorption and viscosity increased with an increase in fonio substitution. Furthermore, increasing the fonio addition resulted in decreased dough stability time. There were significant differences in the colour of both the crust and the crumb.

Based on the results obtained and the characteristics of the cereal flour, the use of less than 25% fonio can maintain the same rheological and textural properties as traditional gluten-free bread recipes. Moreover, these innovative products can be introduced to the expanding market for gluten-free and functional items, high quality proteins, starch fractions and granules, and non-food allergy products. The data obtained in this study demonstrated that the use of less exploited but high-quality food sources locally available in West Africa can contribute to food security and improve the nutritional and organoleptic profile of bakery products.

Further studies could investigate the correlation between the rheological properties of fonio-enriched bread and their sensory profile. This could be achieved through conventional descriptive analysis and consumer liking scores, matching the values of the bakery products. It would be of interest to evaluate in further studies the relationship between rheological properties of fonio-enriched breads and the sensory profile by conventional descriptive analysis and consumer liking scores matching values of the bakery products.

References

1. AACC (2005). *Approved methods of analysis* (10 ed.). St. Paul, MN, USA: AACC International.

2. AACC International Method 22-08.01, ICC Standard No. 161, RACI Method 05-05, American Society of Brewing Chemists Barley-12.

3. Armstrong, M.J., Hegade V.S., Robinson G. (2012). Advances in coelic disease. *Current Opinion in Gastroenterology*, 28, 104-112.

4. Aronsson, C.A., Lee H.S., Liu E., et al. (2015). Age at gluten introduction and risk of celiac disease. *Pediatrics*, 135, 239-245.

5. Ayo, J. A., & Nkama, I. (2004). Effect of acha (*Digitaria exilis*) grain flour on the physico-chemical and sensory properties of bread. *International Journal of Food Properties*, *7*(3), 561-569.

6. Balet, S., Guelpa, A., Fox, G., Manley, M. (2019). Rapid Visco Analyser (RVA) as a tool for measuring starch-related physicochemical properties in cereals: a review,12(3), 2344-2360.

7. Bernardo, D., Peña A.S. (2012). Developing strategies to improve quality of life of patients with gluten intolerance in patients with and without celiac disease. *European Journal of Internal Medicine*, 23, 6-8.

8. Bollaín, C., Angioloni, A., Collar, C. (2006). Relationships between dough and bread viscoelastic properties in enzyme supplemented wheat samples. *Journal of Food Engineering*, 77 (3), 665-671.

9. Chen, M.H., Christine, J., Bergman, M, McClung, A.M., Everette J.D., Rodante E. Tabien J. (2017). Resistant starch: Variation among high amylose rice varieties and its relationship with apparent amylose content, pasting properties and cooking methods. *Food Chemistry*, 234, 180-189

10. Chiotellis, E., Campbell, G.M. (2003). Proving of bread dough II: Measurement of gas production and retention. *Food and Bioproducts Processing*, 81, 207-216.

11. Conte, P., Del Caro, A., Balestra, F., Piga, A., & Fadda, C. (2018). Bee pollen as a functional ingredient in gluten-free bread: A physical- chemical, technological and sensory approach. *Food Science and Technology International*, 90, 1-7.

12. Diop, B. M., Gueye, M. C., Agbangba, C. E., Cisse, N., Deu, M., Diack, O., Fofana, A., Kane, N. A., Ndir, K. N., Ndoye, I., Ngom, A., Leclerc, C., Piquet, M., Vigouroux, Y., Zekraoui, L., Billot, C., & Barnaud, A. (2018). Fonio (*Digitaria exilis* (Kippist) Stapf): A Socially Embedded Cereal for Food and Nutrition Security in Senegal. *Ethnobiology Letters*,*9*(2), 150-165.

13. European Commission. (2018). Fonio: EU novel food approval. *Official Journal of the European Union*, *61*, L323.

14. Fliedel, G., Outtara, M., Grabulos, J., Dramé, D., and Cruz, J. (2004). Effet du blanchiment mécanique sur la qualité technologique, culinare et nutritionelle du fonio, céréale d'Afrique de l'Ouest. In : Brouwer Inge D., Traoré Alfred S. and Trèche, S., (ed.) Voies alimenatires d'amélioration des situations nutritionnelles en Afrique de L'Ouest : Le 140ngl des 140ngland140gies alimentaires et des nutritionnistes : actes du 2e Atelier international, Ouagadougou, 23-28 nov. Ouagadougou *: Presses universitaires de Ouagadougou*, Burkina Faso, pp. 599-614.

15. Fritz, R.D., Chen Y. (2017). Kernel-based gluten contamination of gluten-free oatmeal complicates gluten assessment as it causes binary-like test outcomes. *International Journal of Food Science & Technology*, 52, 359-365.

16. Gao, Y., Janes M. E., Chaiya B., Brennan M.A., Brennan C.S., Prinyawiwatkul W. (2018). Gluten-free bakery and pasta products: Prevalence and quality improvement. *Journal of Food Science & Technology*, 53 ,19-23.

17. Heckenberg, S., Verheyen, C., Jekle, M., Becker, T. (2017). Effect of mechanically modified wheat flour on dough fermentation properties and bread quality. *European Food Research and Technology*, 243, 287-296.

18. Igyor, M. A. (2005). Substitution of wheat flour with "acha" (*Digitaria exilis*) for bread making.*Botswana Journal of Technology*, *14*(2), 51-57.

19. Jideani, V. and Alamu, R. and Jideani, A. (2007). Preliminary study into the production of non-wheat bread from acha (*Digitaria exilis*). *Nutrition & Food Science, 37,* 434-441.

20. Jideani, V. A., Salami, R.A., Jideani, I.A. (2008). Effect of Irish potato starch, yeast and sprouted soybean flour on the quality of acha bread. *British Food Journal,* 110 (3), 271-282

21. Lasekan, D.O. (1994). Chemical composition of acha (*Digitaria exilis*) flour. *Journal of Food Science & Agriculture*, 14:177–179.

22. Leon, E., Piston, F., Aouni. R., Shewry, P.R., Rosell, C.M., Martin, A. Barro, F. (2010). Pasting properties of transgenic lines of a commercial bread wheat expressing combinations of HMW glutenin subunit genes. *Journal of Cereal Science*, 51, 344–349.

23. Liang, X., King, J.M. (2003). Pasting and crystalline property differences of commercial and isolated rice starch with added amino acids. *Journal of Food Science,* 68,832–838.

24. Ludvingsson, J.F., Leffler D.A., Bai J.C. (2012). The Oslo definition for coeliac disease and related terms. *Gut,* 62, 43-52.

25. Marti, A., Cardone, G., Nicolodi, A., Quaglia, L., Pagani, M.A. (2017). Sprouted wheat as an alternative to conventional flour improvers in bread- making. *Food Science &Technology*, 80, 230-236.

26. Nunes, M. H.B., Ryan, L.A.M., Arendt, E.K. (2009). Effect of low lactose dairy powder addition on the properties of gluten-free batters and bread quality. *European Food Research and Technology*, 229:31-41.

27. Olapade, A.A. and Oluwole, O.B. (2013). Bread making potential of composite flour of wheat-Acha (*Digitaria exilis* staph) enriched with cowpea (*Vigna unguiculata* L.walp) flour. *Nigerian Food Journa*l, 3, 6-12.

28. Onyango, C., Mutungi, C., Unbehend, G. and Lindhauer, M.G. (2011). Modification of Gluten-Free Sorghum Batter and Bread Using Maize, Potato, Cassava or Rice Starch. *LWT-Food Science & Technology*, 44, 681-686.

29. Porteres, R. (1976). African Cereals: EfeuJilJe, Fonio. Black Fonio, Teff. Brac:hiaria. Pa.t/W[UIIl. Pel/Ilise/IIII/. and African Rice. In: Origins of African Plant Domestication, eds. J.R. Harlan, J.MJ. deWel. & A.S.L. Siernler. Moulton. The Hauge. Pp. 409-452.

30. Rubio–Tapia, A., Kyle, R.A., Kaplan, E.L., Johnson, R.D., Page, W., Erdtmann, F., Brantner, T.L., Kim, R., Phelps, T.K., Lahr, B.D., Zinsmeister, A.R., Joseph Melton, L.J., Murray A.J. (2009). Increased Prevalence and Mortality in Undiagnosed Celiac Disease. *Gastroenterology,* 137 (1),88– 93.

31. Sandhu, K.S., Singh, N., Singh, Malhi, N. (2007). Some properties of corn grains and their flours I: physicochemical, functional and chapatti making properties of flours. *Food Chemistry,* 101, 938–946.

32. Sapone, A., Bai J.C., Ciacci C., Dolinsek J., Green P.H. (2012). Spectrum of gluten-related disorders: consensus on new nomenclature and classification. *BMC Medicine*, 10, 13.

33. Sollid L.M. (2002). Coeliac disease: dissecting a complex inflammatory disorder. *Nature Review Immunology*, 2, 647-655.

34. Temple, V.J, Bassa J.D. (1991). Proximate chemical composition of acha (*D. exilis*) grain. *Journal of the Science of Food and Agriculture,* 56, 561–563.

35. Vietmeyer, N.E., Borlaugh, N.E., Axtell, J., Burton, G.W., Harlan, J.R. and Rachie, K.O. (1996). (Ed) Fonio (Acha). Lost crop in Africa Chap 3., *BOSTID publication*, 58-75.

36. Villanueva, M., Abebe, W., Collar, C., Ronda, F. (2021). Tef Eragrostis tef (Zucc.) Trotter variety determines viscoelastic and thermal properties of gluten-free dough and bread quality. *LWT- Food Science & Technology,* 135,110065.

37. Xu, X., Xu, Y., Whang, N., Zhung, Y. (2018). Effects of superfine grinding of bran on the properties of dough and qualities of streamed bread. *Journal of Cereal Science*, 81, 76-82.

CHAPTER 5: STUDY III

EFFECT OF DIFFERENT HYDROCOLLOIDS ON RHEOLOGICAL AND STRUCTURAL CHARACTERISTICS ON GLUTEN-FREE RUSKS

Abstract: In recent years, several studies have investigated the use of hydrocolloids in gluten-free bakery products, but there is a lack of research on consumer products such as rusks. The aim of this investigation was to analyse the influence of different hydrocolloid additions, at two different percentages (1-2%), on the formulation of gluten-free rusks.

The rheological properties of the doughs were analysed. The colour and textural characteristics of the resulting breads were evaluated and the resulting rusks were analysed by texture and image analysis.

The rheological properties of the doughs indicate an elastic behaviour. High values of G' and G" were recorded for samples with 2% hydrocolloid addition. The gluten-free (GF) breads with psyllium fibre (1-2%) showed a darker colour. The textural analysis showed that the H1 and X1 samples exhibited the highest hardness values, whereas H2 demonstrated the lowest value. Consistent values were recorded for the textural parameters of the rusks.

These findings provide informative indications that the final product was influenced by the addition of different hydrocolloids and their ratios.

Keywords: Rusk, hydrocolloids, gluten-free rusk, coeliac disease, water adjustment, water hydration capacity

1. Introduction

In recent years, there has been a significant increase in the percentage of people suffering from celiac disease and other gluten-related disorders, due to improved diagnostic techniques and environmental factors that can reduce gluten tolerance in the diet (Cardo et al., 2021).

It is estimated that about one per cent of the population is affected by the disease. The incidence varies with age and location, and is more common in women (Cardo et al., 2021). Celiac disease is a common, chronic, immune-mediated enteropathy of the small intestine that is promoted by exposure to dietary gluten in genetically predisposed individuals (Arslain, et al., 2021).

When gluten is ingested by people with this disease or other gluten-related disorders, the prolamin fraction of grain causes an immune response. This, over time, causes the flattening of villi and microvilli, rendering them unable to consume nutrients (Capriles and Arêas, 2014). The only therapeutic approach to managing celiac disease is lifelong, strict adherence to a gluten-free diet that avoids grains such as wheat, barley, rye, kamut and triticale (Gao et al, 2018).

Maintaining a regular gluten-free diet is a challenging task due to several dietary restrictions. Gluten is found in various foods like flour, bread, pasta, and snacks but may also be concealed in several industrially processed foods, serving as thickening, stabilizing, or flavoring agents (Lindfors et al., 2019). Health and social problems that arise cannot be overlooked. Celiacs are penalised in social, school and work activities, impacting their quality of life greatly. (Bascuñán et al., 2017).

There are many uncertainties about the nutritional adequacy of gluten-free diets, which often contain more carbohydrates and lipids than their gluten-containing counterparts and must be monitored for potential nutritional deficiencies (Hallet et al., 2002).

Gluten is a complex mixture of proteins known as prolamins and glutenins (Wieser, 1996), plays a fundamental role in the production of bakery products (Zhou et al., 2014) and has unique properties that favour its use in many products, such as resistance to stretching, extensibility, mixing tolerance and gas retention (Moreni et al., 2014). For this reason, gluten-free products have technological defects due to insufficient formation of gluten network and affect the quality of final products (El Khoury et al., 2018). The gliadin fraction increases the viscosity of the system food, while the glutenin fraction gives it elastic properties (Delcour et al., 2012).

The production of high-quality gluten-free products with properties similar to those of their gluten-containing counterparts is one of the technological challenges for cereal researchers and bakers (Hager et al., 2013). In addition, the gluten-free products also need to have similar shelf life and sensory properties as wheat products (El Khoury et al., 2018).

The easiest way to improve the structure of gluten-free products is to incorporate ingredients that mimic the action of gluten. Several studies have been carried out to replace gluten, such as non-gluten proteins or hydrocolloids, enzymes and emulsifiers (Sciarini et al., 2012). The hydrocolloids or gums are high molecular weight hydrophilic biopolymers used as functional ingredients in the food industry (Kohajdova & Karovičová, 2009). These additives are widely used in gluten-free products such as bread because they are a good source of soluble dietary fibre (Angioloni et al., 2011), indispensable structural ingredients for increasing volume, improving texture and shelf life (Lazaridou et al., 2007; Anton & Artfield,2008; Capriles & Arêas., 2014). They also improve sensory properties and overall consumer acceptance (Capriles & Arêas., 2014). The sources of hydrocolloids are very different and they can be classified according to their origin: microbial synthetics such as a xanthan gum, seed gums such as guar gum and psyllium, chemical cellulose derivatives such as a hydroxypropyl methyl cellulose, etc. (Houben et al., 2012). Each hydrocolloid has certain specific properties, so when implemented in bakery products, they can improve the kneading, rising and baking process. (Ferrero,2017). The ability to form a gel is highly dependent on the type and concentration.

Many authors have carried out research in recent years focusing on the hydrocolloids suitable for gluten-free bakery products such as bread, biscuits, crackers and cakes, but there is a lack of studies on a product such as rusks. Therefore, the scope of the research was to investigate the effect of adding different hydrocolloids to a gluten-free rusk formulation.

Rusk is a bakery product widely consumed in all countries (Yaseen, 2000), this product is made from slices of bread which are dried and toasted. The toasting process evaporates a lot of water compared to bread and for the same weight, the slice provides a greater energy input.

They are highly valued for their readiness for consumption, convenience and long shelf-life (Gupta et al., 2011; Kaur, et al., 2017).

The few authors who have carried out research on this topic have done so in relation to rusks containing gluten: Yaseen (2000) and Gupta et al. (2011) have been working on the improvement of the nutritional value of rusks through the use of barley flour. In addition, Lazaridou et al. (2019) have conducted research on rusks with barley using hydrothermal treatment. The only study on gluten-free rusks was carried out by Allbban et al. (2020), who produced a rice-based rusk rich in selenium and iron. Zeppa et al. (2011) made a structural evaluation of the rusks available on the market. For this reason, hydrocolloids should be considered as a candidate to improve the rheological properties of bread in gluten-free rusk product.

The aim of this study was to evaluate the effect of different hydrocolloids on the rheological properties, dough quality and textural characteristics of gluten-free rusks, characterised by the absence of a gluten network.

2. Materials and Methods

2.1. Materials and rusk formulations

Rice flour and maize starch were supplied by Chimab Campodarsego (PD Italy). Gluten free formulations also contained compressed yeast, sunflower oil, salt and sugar were purchased from local supermarket. These ingredients have been used throughout in the present study for all rusk formulations.

Additionally, in this study five commercially available hydrocolloids were used: guar gum and psyllium fiber (Chimab Campodarsego, PD Italy), hydroxypropyl methylcellulose (Bio Line S.r.l), tara (Aglumix®01, Silvateam Food Ingredients S.r.l., Bergamo, Italy). and xanthan gum (Chimab, Campodarsego, Italy).

All gluten-free formulations contained 80% of maize starch, 20% of rice flour, 3% of sugar, 1,8% salt, 5% sunflower oil, 2% yeast. Hydrocolloids were added at 1 and 2 % w/w (maize starch and rice flour basis).

Amount of water added in the different samples were predicted by an equation according Horstmann et al., (2018). To obtain the right amount of water to be added depending on the hydrocolloid used and its percentage of addition, the water holding capacity (WHC) of the different hydrocolloids and flour/ starches used was calculated. All gluten-free formulations studied in this work and their symbols are summarized in Table 1.

Table 1. Gluten-free rusk formulations.

Hydrocolloids	Level of hydrocolloids	Amount of water added (%)	Symbol of formulations
Guar	1	83.06	G1
Guar	2	100.00	G2
Hydroxypropylmethylcellulose	1	69.55	H1
Hydroxypropylmethylcellulose	2	73.00	H2
Tara	1	82.91	T1
Tara	2	99.66	T2
Psyllium	1	83.95	P1
Psyllium	2	101.74	P2
Xanthan gum	1	73.35	X1
Xanthan gum	2	80.53	X2

2.2. Preliminary trials to define the water content in gluten-free batters

The water content of the control gluten-free rusk recipe containing 80 % maize starch, 20% rice flour, and 2% guar gum, was determined by introducing different water content at different percentages (90, 100 %, 110%, 120%, and 130%) based on starch and flour mixture. The breads obtained from these different amounts of water were then assessed based on the specific volume, crumb structure and crumb texture. the optimal water content of 100% is determined in control formulation (Guar 2%). In breadmaking, the water content is one of the most important parameters (Sahin et al., 2019).

The correct water content in gluten-free dough was obtained using two equations such as Horstman et al., (2018). The moisture content of rice flour, maize starch and hydrocolloids was calculated to know the quantity needed to estimate the water holding capacity (WHC). AACC method 56-30.01 with some modification was used to determine the WHC of maize starch, rice flour, guar gum, hpmc, tara, xanthan gum and psyllium. Briefly samples (0.5 g) were mixed with 25 mL of distilled water using an ultra-Turrax equipped with dispersion element (Ultra turrax T25 basic IKA Werke) for 15 s. Subsequently the suspensions were shaken for 60 min at 200 rpm using a platform shaker at room temperature (Universal table shaker 709, ITALY), followed by centrifugation at 3500 rpm for 10 min at 18°C.

WHC was calculated as ml of water retained per gram of solid, using equation:

WHC [mL water/ g ingredient] = (W2-W1)/W0

Where:

W1= weight of tube plus the sediment

W2= weight of tube plus the sample

W0= weight of sample

Water content [%] = (((a/ 100 * c1) + (b /100*c2)) *d)/e

Where:

a= WHC of starch/flour

b=WHC of Hydrocolloid

c1= % of starch / flour used in the formulation based on dry ingredients

c2=% of hydrocolloid used in the formulation based on dry ingredients

d= % (based on starch) - optimal amount of water added to the base formulation

e= ml - combined WHC of the base formulation

2.3. Rusk preparation

Breads were prepared according to the method described by Conte at al. (2018) and roasted according to the method described by Lazaridou et al. (2019) with some modifications.

All gluten-free doughs were prepared by suspending sugar, salt and yeast in aliquots of warm water (26 °C) before adding the pre-mixed dry ingredients. The sunflower oil and the dissolved ingredients were then mixed in a mixer (KitchenAid, Artisan, 5KSM150, USA) equipped with a flat beater at speed 1 for 5 min and at speed 2 for 8 min.

The dough is kept in non-stick aluminium baking trays (20x6x7cm), purchased from Siccardi (TO). 300g of dough was put into each box, which was then covered and left for fermentation. Proofing was carried out in a climatic chamber at 26 °C and 90 % relative humidity (RH) until the dough had doubled in volume from its initial state.

The bread was baked in an electric oven (Europa Molina di Malo, VI, Italy) at 200 °C for 60 min and left to cool at room temperature for 120 min.

The breads were mechanically sliced (GRAEF GmbH & Co. KG Donnerfeld 6 D-59757 Amsberg) to a thickness of 1 cm. The slices were toasted at 130 °C until the final product had a moisture content of 7% for each formulation.

2.4. Dough rheology properties

The rheological properties of gluten-free dough formulations with different hydrocolloids were investigated using a Physica MCR 102 rotational rheometer (Anton Paar GmbH, inc., Graz, Austria) with a parallel plate geometry (25 mm diameter and 2 mm gap). The temperature was controlled at 20 °C with an accuracy of ±0.1 °C. After loading, the dough sample was allowed to rest for 2 min. Excess dough was trimmed off. A thin layer of vaseline oil was applied to the edge of the exposed sample just before the measurement to avoid moisture loss during the resting period. The test performed on the dough samples was a strain sweep test in the range of 0.001 to 100 s-1 at a constant frequency of 10 Hz. Based on these results, a target strain of 0.01% (within the linear range) was selected for measurement. Frequency sweeps were performed over the range 0.1 - 10 Hz at 0.01% strain. Each test was performed on different dough samples, at least in triplicate.

2.5. Bread measurements

2.5.1. Specific volume

Bread volume of the samples was measured according to the AACC 10-05.01 method of rapeseed displacement (AACC, 2005) and the specific (volume mL/g) was calculated as bread volume/bread weight.

2.5.2. Colour determinations

The crumb and crust colour of fresh bread was measured with a tristimulus colorimeter (DP-301, Konica Minolta Sensing, Osaka, Japan) equipped with a CR-300 measuring head (Konica Minolta Sensing, Osaka, Japan) using a D65 illuminant and a CIE 10° standard observer angle and calibrated against a white tile supplied with the instrument. Colour measurements were expressed as Hunter values for L* (lightness), a* (redness) and b* (yellowness). Colour measurements were taken on the day of firing. Three measurements were taken, one in the middle and two in the distal part of the bread. For each sample, the crumb colour was measured at three points on three central slices of five breads.

2.5.3. Bread Texture

The mechanical properties of breads were determined using a texture analyser (TA-XT2 Texture Analyser, Stable Microsystems, Surrey, UK). The evaluation was carried out using Texture Profile Analysis on 2 cm wide slices.

The slice was compressed with a 25 mm cylindrical aluminium probe at a constant speed of 1 mm/s with a 30 s gap between compressions. Bread texture parameters were taken as the force required to compress the bread sample by 50%. All the parameters mentioned above were measured on at least three slices taken from two breads.

2.6. Rusk Texture

The breaking strength (or fracture toughness) of rusks has been measured using a three-point bending test, equipped with a three-point bending ring (HDP/3PB).

A force is applied to the centre of the sample (which is also the centre of the supports) and the breaking stress is determined.

To obtain a value for the three-point failure force, the peak force (hardness) applied was recorded in Newtons.

Three numerically independent parameters were obtained from this test: Hardness (N), Brittleness/flexibility (distance in mm) and Toughness (gradient in N/mm).

All the parameters mentioned above were measured on 9 rusks and determined after roasting for 10 min.

2.7. Image Analysis

Crumb grain characteristics were defined using Image J (NIH, USA), a digital image analysis system. Images were acquired using an Epson Perfection V500 Photo scanner (Epson, Suwa, Japan). A 30x30 mm square was taken from the centre of each image (n = 3). The parameters used to characterise the crumb grain are the number of cells, the ratio of cells to total area, the mean size of the cells and the % of the area of the cells in the sections (Conte et al., 2018). These parameters were generated according to dimensional categories from 0 to infinity (mm^2).

2.8. Statistical analysis

Statistical analyses were performed using STATISTICA software, version 6.0 (Statsoft, Inc., Tulsa, OK, USA). All experimental data of dough, bread and rusk parameters were analysed by one-way ANOVA according to the generalised linear model to investigate the effects of different hydrocolloids at different addition ratios. Differences between means were identified using the least square difference (LSD) test with a significance level of α= 0.05\.

3. Results and Discussion

3.1. Water holding capacity

The water holding capacity (WHC) is defined as the quantity of water (in grams) bound per gram of hydrocolloids in an aqueous dispersion (Horstman et al., 2018). The WHC of ingredients used in food formulation influences their functional and sensory properties.

Different hydrocolloids have different WHC values expressed in g/g sample weight.

Among the various hydrocolloids, psyllium exhibited the highest value of 50.92, while guar gum and tara exhibited values of 48.45 and 48.04, respectively. The HPMC had the lowest value recorded at 11.16, followed by Xanthan gum at 21.65.

Horstman et al. (2018) conducted an experiment and found similar values of WHC for samples of HPMC and xanthan gum at 10.39 and 18.72 respectively. However, they found a different value for guar gum at 21.05.

3.2. Dough rheological properties

The results of dough rheological properties were reported in table 2. In all batter samples the component elastic of the material (G'), was higher than the viscous component of the material (G"), indicated that the dough samples had an elastic-like behaviour. This behaviour is also demonstrated by the values of Loss Tangent (tanδ) which for all samples is <1.

Regarding elastic modulus G’, the samples H1 and T2 have no statistically significant differences compared to the G2. The highest values, compared with G2, were recorded in the samples of X2, X1, P2, H2, P1. The lowest values of G’, respect to the G2 were recorded in the G1 and T1 samples.

Higher values of G' and G" are recorded in all 2% concentration samples than in 1 % concentration samples. Compared to the G2, there are significant differences between the samples. The highest values of G” was recorded in X2 sample, followed by H2, X1, P2, T2, e H1.

The lowest value of G”, compared to the control, were recorded in P1, G1, T1 samples.

Tan δ values were found to be in the range of 0.16-0.49 suggesting that the addition of different hydrocolloids shows different effects from each other.

Table 2 Rheological properties of gluten-free dough with different hydrocolloids and at different concentration (1-2 %).

Samples	**Storage Modulus (G')**	**Loss Modulus (G")**	**Loss Factor (tanδ)**
G1	943 ± 92 ^{g}	344 ± 61^{g}	0.33 ± 0.03 ^{b}
H1	1484 ± 9 ^{f}	561 ± 36 ^{e}	0.38 ± 0.03 ^{b}
T1	820 ± 89^{g}	375 ± 32 ^{fg}	0.46 ± 0.03 ^{a}
P1	2156 ± 150 ^{e}	338 ± 24 ^{g}	0.16 ± 0.001 ^{e}
X1	7227± 163 ^{b}	1341 ± 152 ^{c}	0.19 ± 0.02 ^{de}
G2	1634± 43 ^{f}	529 ± 22 ^{ef}	0.32 ± 0.01 ^{c}
H2	3271 ± 142 ^{d}	1587 ± 65 ^{b}	0.49 ± 0.02 ^{a}
T2	1408 ± 234 ^{f}	671± 73 ^{de}	0.48 ± 0.03 ^{a}
P2	3847 ± 132 ^{c}	768± 127 ^{d}	0.20 ± 0.03 ^{d}
X2	9116± 332 ^{a}	1883± 188 ^{a}	0.21 ± 0.02 ^{d}

Mean values ± standard deviation. Within columns, values with the same letter do not differ significantly from each other according to LSD test ($p<0.05$).

3.3. Crust and crumb colour of bread

The crust and crumb colour of the breads are shown in Table 3. The use of different hydrocolloids significantly affected the colour of both the crumb and the crust of the experimental gluten-free breads. In terms of crust colour characteristics, the gluten-free bread with psyllium fibre (1% and 2%) showed a darker colour due to the natural colour of the powder. The tara sample, compared to the G2, had no significant differences for L* and similar but statistically different values for yellowness (b) and greenness (-a*). Xanthan (1 - 2%) and HPMC at 2% addition showed the highest brightness L*.

The samples G2, T1 and T2 showed a negative value of a*. The highest value of a* of crust was found in hpmc samples at both percentages of gum, followed by samples with xanthan, psyllium, guar and tara. For the b* coordinate, the yellow samples are H1, H2 and X2. With regard to the colour of the crumb, the sample with the highest L* value was hpmc with 1% addition, followed by hpmc with 2% addition. There were no statistically significant differences in L* between the G2, G1, T (1-2%) and X1. The X2 and P1 samples showed a lower L*.

All samples except P2 showed negative a* values, while for the b* coordinate the recorded values are always positive. The difference in L*, a* and b* values is related to the different nature of the hydrocolloids but also to the chemical and molecular bonds established with the ingredients used.

Table 3 Crust and crumb colour attributes of gluten-free bread added with different hydrocolloids at two level of concentration.

Crust Colour			
Sample	**L**	**A**	**B**
G1	70.12 ± 1.94 [c]	0.94 ± 2.35 [de]	19.62 ± 2.38 [cd]
H1	74.42 ± 2.38 [b]	5.54 ± 1.47 [a]	28.25 ± 3.94 [a]
T1	68.51 ± 2.35 [d]	-0.21 ± 0.60 [fg]	16.30 ± 2.18 [e]
P1	59.61± 2.17 [e]	3.64 ± 0.71 [c]	21.25 ± 1.30 [bc]
X1	75.64 ± 1.82 [ab]	0.35 ± 1.34 [ef]	19.19 ± 3.45 [d]
G2	67.70 ± 2.12 [d]	-0.48 ± 0.73 [g]	15.21 ± 2.22 [e]
H2	76.57 ± 2.64 [a]	4.60 ± 1.58 [b]	27.27 ± 3.64 [a]
T2	67.82 ± 2.30 [d]	-1.51 ± 0.30 [h]	11.84 ± 0.91 [f]
P2	57.26 ± 1.57 [f]	3.87 ± 0.70 [bc]	15.51 ±2.11 [e]
X2	76.10 ± 2.38 [a]	1.36 ± 1.39 [d]	22.54 ± 3.99 [b]
Crumb Colour			
G1	67.37 ± 0.52 [c]	-1.79 ± 0.08 [de]	4.34 ± 0.26 [d]
H1	81.04 ± 0.57 [a]	-1.90 ± 0.05 [e]	6.63 ± 0.06 [a]
T1	66.20 ± 0.93 [c]	-1.72 ± 0.09 [d]	3.85 ± 0.06 [ef]
P1	56.77 ± 1.24 [e]	-0.41± 0.09 [b]	3.70 ± 0.04 [fg]
X1	66.09 ± 1.24 [c]	-2.33 ± 0.11 [g]	5.76 ± 0.36 [b]
G2	66.21 ± 1.04 [c]	-1.78 ± 0.05 [de]	3.57 ± 0.29 [fg]
H2	78.24 ± 0.17 [b]	-1.33 ± 0.11 [c]	4.16 ± 0.24 [de]
T2	65.71 ± 1.28 [c]	-1.74 ± 0.06 [de]	3.39 ± 0.07 [g]
P2	57.91 ± 1.88 [e]	0.76 ± 0.18 [a]	4.25 ± 0.07 [de]
X2	63.93 ± 0.23 [d]	-2.15 ± 0.12 [f]	5.29 ± 0.54 [c]

Mean values ± standard deviation. Within columns, values with the same letter do not differ significantly from each other according to LSD test ($p<0.05$).

3.4. Textural properties of bread

The mechanical behaviour of the sample was measured on the day of baking to assess the quality of the bread in terms of hardness, springiness, cohesiveness, chewiness and elasticity. Mir et al. 2016, reported that hydrocolloids could improve the texture of gluten-free bread. The hardness of the bread crumb is considered to be a very important quality of the bread. The effect of adding different hydrocolloids at two concentration levels (1-2%) on the textural properties of bread is shown by the data in Table 4.

The highest hardness values of 14.93 (N) and 13.48 (N) were recorded for H1 and X1 samples respectively, followed by X2 (10.04 N), with respect to the G2.

The samples G1 (7.73 N), T1 (7.53 N), T2 (6.63 N) and P1 (6.67 N) do not show statistically significant differences and have a lower hardness compared to the G2.

Sample H2 (4.46 N) had the lowest hardness of all samples, followed by P2 (6.16 N).

Springiness is related to the aeration and elasticity of bread, and high values are desirable (Torres et al., 2013). In general, springiness decreased with increasing concentration of hydrocolloids added, except for formulations containing HPMC.

The highest springiness value was recorded in the samples of P1, compared to the G1, but also compared to all the samples. The samples of T1 (0.99), G1 (0.98) and X1 (0.98) did not show any statistical differences and compared to the sample G2 they have a higher springiness.

There are no statistical differences between the sample G2, H1, H2, T2, P2 and X2, all these samples have lower springiness values.

Cohesiveness is a measure of the extent to which the structure of the food can be deformed before it breaks. When the cohesiveness values were compared with the G2, statistically significant differences were found between all the samples. The highest value with respect to G2 is recorded in the H2 sample, followed by G1, T2, P2 and H1. The lowest value was recorded in X2, P1, T1 and X1. In terms of resilience, the sample with the highest value is G1. The lowest value is recorded in sample H1.

Table 4. Textural parameters of gluten free bread added with different hydrocolloids at 2 level of concentration.

Sample	Hardness (N)	Springiness	Cohesiveness	Chewiness (N)	Resilience
G1	7.73± 0.26 [cd]	0.98 ± 0.006 [a]	0.70 ± 0.008 [ab]	5.29 ± 0.21 [cd]	0.43 ± 0.006 [a]
H1	14.93 ± 3.28 [a]	0.95 ± 0.020 [ab]	0.65 ± 0.03 [cde]	9.32 ± 2.37 [a]	0.33 ± 0.02 [e]
T1	7.53 ± 3.10 [cd]	0.99 ± 0.010 [a]	0.61± 0.02 [fg]	4.55 ± 1.87 [de]	0.36 ± 0.02 [de]
P1	6.67± 0.38 [cd]	0.99 ± 0.004 [a]	0.63 ± 0.03 [ef]	4.18 ± 0.42 [de]	0.39 ± 0.04 [c]
X1	13.48 ± 0.48 [a]	0.98 ± 0.006 [a]	0.60 ± 0.01 [g]	7.88 ± 0.37 [b]	0.34 ± 0.02 [e]
G2	9999999	0.98 ± 0.005 [a]	0.65 ± 0.01 [de]	5.28 ± 0.34 [cd]	0.38 ± 0.008 [cd]
H2	4.46 ± 0.31 [e]	0.85 ± 0.02 [c]	0.72 ± 0.03 [a]	2.72 ± 0.18 [f]	0.34 ± 0.02 [e]
T2	6.63 ± 0.49 [cd]	0.99 ± 0.009 [a]	0.68 ± 0.02 [bc]	4.44 ± 0.38 [de]	0.40 ± 0.02 [bc]
P2	6.16 ± 1.55 [de]	0.92 ± 0.16 [b]	0.67 ± 0.05 [bcd]	3.81 ± 1.19 [ef]	0.42 ± 0.05 [ab]
X2	10.04 ± 0.46 [b]	0.98 ± 0.003 [ab]	0.64 ± 0.02 [def]	6.27 ± 0.30 [c]	0.35 ± 0.02 [e]

Mean values ± standard deviation. Within columns, values with the same letter do not differ significantly from each other according to LSD test ($p<0.05$).

3.5. Rusk texture

The hardness value of the rusks is influenced by the hydrocolloids. The parameters of the texture are shown in Table 5.

The samples with the highest hardness value are those with 1 % xanthan (46.79 N), followed by those with guar (1 and 2 %) (45.18 and 43.55 N). The sample with the lowest hardness value is Hpmc at 2% addition, followed by Tara at 1% and 2% concentration. The most brittle/flexible samples are H1 and H2 and the least brittle/flexible samples are T1 and X1. The G2 smple shows no statistically significant differences with the sample containing Tara and Xanthan 2%. In terms of toughness, the highest value of the sample was recorded in X1, followed by T1 and G2. The lowest toughness value was recorded in the sample with Hpmc at 1% concentration, followed by 2% Hpmc. The G2 sample shows no statistically significant differences with the sample containing 2% Xanthan.

Table 5. Gluten-free rusk texture added with different hydrocolloids at different use ratio (1-2%).

Samples	Hardness N	Brittleness/Flexibility Mm	Toughness N/mm
G1	45.18 ± 2.61 [ab]	2.15 ± 0.50 [cd]	21.66 ± 4.58 [bcd]
H1	26.93 ±3.19 [f]	3.26 ± 1.02 [a]	8.93 ± 3.06 [f]
T1	36.11± 6.53 [de]	1.43 ± 0.51 [e]	27.39 ± 9.91 [ab]
P1	39.23 ± 0.90 [cd]	2.27 ± 0.67 [bc]	18.59 ± 5,48 [de]
X1	46.79 ± 4.03 [a]	1.66 ± 0.48 [de]	29.61 ± 7.16 [a]
G2	43.55± 3.20 [ab]	1.87 ± 0.48 [cde]	24.87 ± 8.77 [abc]
H2	34.28 ± 4.34 [e]	2.85 ± 0.74 [ab]	12.51 ± 2.73 [ef]
T2	37.22 ± 3.69 [cde]	1.97 ± 0.71 [cde]	20.64 ± 6.61 [cd]
P2	39.06 ± 2.51 [cd]	2.16 ± 0.18 [cd]	18.01± 1.45 [de]
X2	41.29 ± 8.39 [bc]	1.75 ± 0.59 [cde]	25.58 ± 9.48 [abc]

Mean values ± standard deviation. Within columns, values with the same letter do not differ significantly from each other according to LSD test ($p<0.05$).

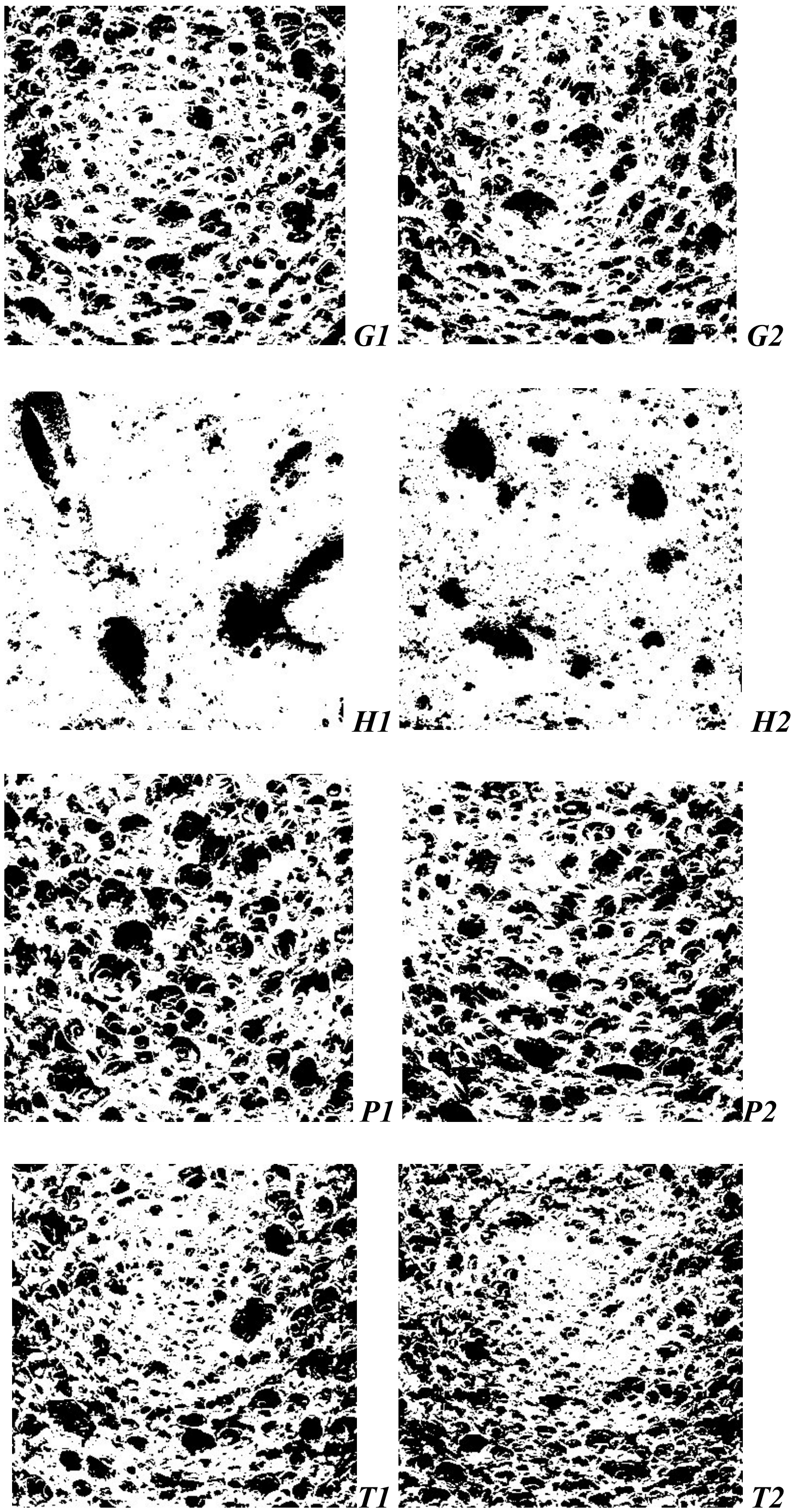
3.6. Image Analysis

The analysis carried out on the crumb characteristics, as shown in Table 6, shows a significant decrease in all the parameters studied for the H1 samples compared to the other samples analysed, except for the average size of the alveoli. However, the data obtained do not accurately reflect the visually observed properties of samples made with HPMC. These samples often have a limited number of very large alveoli, hiding the remaining areas where the number of alveoli was high and of a smaller size, as required by the characteristics of rusks. This may be due to some problems of dough development during leavening and that could be the subject of future evaluations on possible further studies for the optimization of the use of hydroxypropylmethylcellulose.

According to the percentage of alveoli present on the surface of the rusks under the conditions of this study, the hydrocolloids G and P showed significantly higher values, independent of the concentration of use, while for T and X the results were different in relation to the increase in the percentage of use.**Table 6.** Parameters of crumb image analysis of gluten free rusk

Slice	Count	Total area	Average size	Area %
G1	960.67 ± 72.53 [a]	234.57 ± 8.86 [a]	0.25 ± 0.03 [ab]	25.96 ± 0.97 [a]
H1	351.33 ± 291.14 [b]	83.04 ± 10.35 [c]	0.77 ± 1.04 [a]	9.23 ± 1.15 [c]
T1	968.33 ± 33.23 [a]	231.42 ± 28.10 [a]	0.24 ± 0.03 [ab]	25.71 ± 3.12 [a]
P1	876.00 ± 18.19 [a]	267.00 ± 10.15 [a]	0.31 ± 0.01 [ab]	29.67 ± 1.13 [a]
X1	772.00 ± 69.54 [a]	236.68 ± 11.40 [a]	0.31 ± 0.01 [ab]	26.30 ± 1.27 [a]
G2	964.25 ± 18.15 [a]	256.08 ± 15.71 [a]	0.27 ± 0.01 [ab]	28.43 ± 1.79 [a]
H2	763.00 ± 408.72 [a]	106.10 ± 23.23 [c]	0.17 ± 0.09 [b]	11.79 ± 2.58 [c]
T2	1034.00 ± 81.19 [a]	180.20 ± 44.19 [b]	0.17 ± 0.03 [b]	20.00 ± 4.92 [b]
P2	823.67 ± 52.50 [a]	236.94 ± 21.98 [a]	0.29 ± 0.01 [ab]	26.33 ± 2.44 [a]
X2	748.00 ± 186.68 [a]	164.29 ± 26.76 [b]	0.22 ± 0.02 [b]	18.25 ± 2.97 [b]

Mean values ± standard deviation. Within columns, values with the same letter do not differ significantly from each other according to LSD test ($p<0.05$).


G1
G2
H1
H2
P1
P2
T1
T2

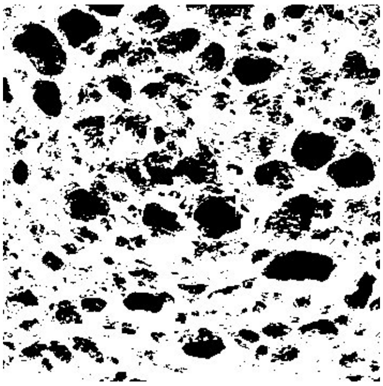
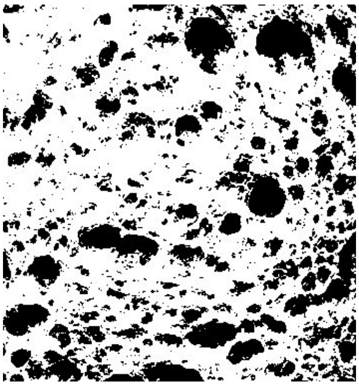
 X1  X2

Fig.2 Effect of different hydrocolloids at different use ratio on the rusks processed by image j

4. Conclusions

In this work the behaviour and the rheological aspect of the doughs with gluten free ingredients integrated with five different hydrocolloids at two concentration, 1% and 2% and the interactions between these ingredients and the dough have been evaluated. In addition, the texture, colour and crumb grain structure of rusk and any influences arising from the hydrocolloids on the appearance were evaluated.

The data demonstrate that final product changed with different hydrocolloids added and their ratios.

These results may be used to improve knowledge regarding the use of hydrocolloids on a product such as rusks and their changes of texture parameters. However, with the percentages of additives used, no friable product has been obtained.

Further research needs to be done to examine other additional percentages of hydrocolloids and consumer acceptance of this product.

References

1. Allbban, A. M., & Mouminah, H. H. (2020). Supplementation of Gluten-Free Rice Flour with Yeast and Date Powder to Produce Rusk Rich in Selenium and Iron. *Life Science Journal*, 17(9).

2. Angioloni, A., Collar, C. (2011). Physicochemical and nutritional properties of reduced-caloric density high-fibre breads. *Food Science & Technology,* 44, 747-758.

3. Anton, A., Artield, S., (2008). Hydrocolloids in gluten- free breads: a review. *International Journal of Food Science and Nutrition,* 59 (1), 11-23.

4. Arslain, K., Gustafson, C. R., Baishya, P., & Rose, D. J. (2021). Determinants of gluten-free diet adoption among individuals without celiac disease or non-celiac gluten sensitivity. *Appetite*, 156, 104958.

5. Bascuñán, K. A., Vespa, M. C., & Araya, M. (2017). Celiac disease: understanding the gluten-free diet. *European Journal of Nutrition*, 56(2), 449-459.

6. Cardo, A., Churruca, I., Lasa, A., Navarro, V., Vázquez-Polo, M., Perez-Junkera, G., & Larretxi, I. (2021). Nutritional imbalances in adult celiac patients following a gluten-free diet. In *Nutrients* (Vol. 13, Issue 8). MDPI.

7. Capriles, V. D., & Arêas, J.A.G. (2014). Novel approaches in gluten-free breadmaking: Interface between food science, nutrition, and health. Comprehensive Reviws in *Food Science and Food Safety*, 13 (5), 871-890.

8. Conte, P., Del Caro, A., Balestra, F., Piga, A., & Fadda, C. (2018) Bee pollen as a functional ingredient in gluten-free bread: A physical- chemical, technological and sensory approach. *Food Science and Technology International*, 90, 1-7.

9. Delcour, J.A.; Joye, I.J.; Pareyt, B.; Wilderjans, E.; Brijs, K.; Lagrain, B. (2012) Wheat gluten functionality as quality determinant in cereal-based food products. Annu. Rev. *Food Science & Technology*, 3.

10. El Khoury, D., Balfour-Ducharme, S., & Joye, I. J. (2018). A review on the gluten-free diet: Technological and nutritional challenges. *Nutrients*, 10(10), 1410.

11. Ferrero, C. (2017). Hydrocolloids in wheat breadmaking: A concise review. *Food Hydrocolloids,* 68,15-22.

12. Gao, Y., Janes M. E., Chaiya B., Brennan M.A., Brennan C.S., Prinyawiwatkul W. (2018). Gluten-free bakery and pasta products: Prevalence and quality improvement. *Journal of Food Science & Technology*, 53 ,19-23.

13. Gupta, M., Bawa, A. S., Semwal, A. D. (2011). Effect of barley flour blending on functional, baking and organoleptic characteristics of high-fiber rusks. *Journal of Food Processing and Preservation*, 35, 46-63.

14. Hager, A.S., Arendt, E. K. (2013). Influence of hydroxypropyl methylcellulose (HPMC), Xanthan gum and their combination on loaf specific volume, crumb hardness and crumb grain characteristics of gluten-free breads on rice, maize, teff and buckwheat. *Food Hydrocolloids,* 32, 195-203.

15. Hallert, C., Grant, C., Grehn, S., Granno, C., Hulten, S., Midhagen, G., Strom, M., Svensson, H., Valdimarsson, T. (2002) Evidence of poor vitamin status in coeliac patients on a gluten-free diet for 10 years. *Alimentary Pharmacol Ther Journal,* 16(7):1333–1339.

16. Horstmann, S.W., Axel, C., & Arendt, E.K. (2018). Water absorption as a prediction tool for the application of hydrocolloids in potato starch- based bread. *Food Hydrocolloids,* 81,129-138.

17. Houben, A., Hochstotter, A., Backer, T. (2012). Possibilities to increase the quality in gluten-free bread production: an overview. *European of Food Research Technology*, 235(2), 195-208.

18. Kaur, R., Kaur, M., Purewal, S.S. (2018). Effect of incorporation of flaxseed to wheat rusks: Antioxidant, nutritional, sensory characteristics, and in vitro DNA damage protection activity. *Journal of Food Processing and Preservation,* 42, 13585.

19. Kohajdova, Z., Karovičová, J. (2009). Application of hydrocolloids as baking improvers: a review. *Chemical Papers*, 63(1),26-38.

20. Lazaridou, A., Duta, D., Papagergeorgiou M., Belc, N., Biliaderis, C. (2007). Effects of hydrocolloids on dough rheology and bread quality parameters in gluten-free formulations. *Journal of Food Engineering*, 79 (3), 1033-47.

21. Lazaridou, A., Marinopoulou, a., Biliaderis, C.G. (2019). Impact of flour size and hydrothermal treatment on dough rheology and quality of barley rusks. *Food Hydrocolloids*, 87, 561-569.

22. Lindfors, K.; Ciacci, C.; Kurppa, K.; Lundin, K.E.A.; Makharia, G.K.; Mearin, M.L.; Murray, J.A.; Verdu, E.F.; Kaukinen, K. (2019). Coeliac disease. *Nature Reviews Disease Primers,* 5, 3.

23. Moreni, M.L., Comino, I., Sousa, C. (2014). Alternative Grains as Potential Raw Materials for Gluten-Free Food development in the Diet of Celiac and Gluten-Sensitive Patients. *Austin Journal of Nutrition and Food Sciences*, 2(3):1016.

24. Sahin, W. A., Wiertz, J., & Arendt K.E. (2020). Evaluation of the method to determine the water addition level in gluten-free systems. *Journal of Cereal Science,* 93, 102971.

25. Sciarini, L.S., Ribotta, P.D., León, A.E., Pérez, G.T. (*2012).* Incorporation of several additives into gluten free breads: effect on dough properties and bread quality. *Journal of Food Engineering*, 111: 590–597.

26. Wieser, H. (1996). Relation between gliadin structure and coelic toxicity. *Acta Paediatrica*, 85, 3-9.

27. Witczak, M., Ziobro, R., Juszczak, L., & Korus, J. (2016). Starch and starch derivatives in gluten-free systems–A review. *Journal of Cereal Science*, 67, 46-57.

28. Yaseen, A. A. E, (2000). Formulating a new high fiber rusk for production on large scale. *Nahrung* 44 (2), 110-113.

29. Zeppa, G., Bertolino, M., Rolle, G.C. (2011). Valutazione strutturale di fette biscottate presenti in commercio. *Industrie Alimentari.*

30. Zhou, W., Therdthai, N., Hui, Y.H. (2014). Introduction to baking and bakery products. In: *Bakery Products Science and Thecnology*,1-16.

CHAPTER 6: GENERAL CONCLUSION

Fonio flour showed good performance when techno-functional, pasting, rheological and gelling properties were compared with other gluten-free sources.

Fonio has the potential to be used in food products, especially in preparations such as porridge, where better gelling properties are appreciated. Therefore, it is suggested that fonio is a promising starch source to compete with other commercially important flours, such as rice, in industrial applications.

The results of the second study indicate that a bread with good leavening, structure and texture can be obtained with the addition of less than 25% fonio.

Based on the results obtained and the characteristics of the cereal flour, the use of less than 25% fonio can maintain the same rheological and textural properties as traditional gluten-free bread recipes. Furthermore, these innovative products can be introduced to the expanding market for gluten-free and functional products, high quality proteins, starch fractions and granules, and non-food allergy products. The data obtained in this study demonstrated that the use of less exploited but high-quality food sources locally available in West Africa can contribute to food security and improve the nutritional and organoleptic profile of bakery products.

Further studies could investigate the relationship between the rheological properties of fonio-enriched breads and their sensory profile. This could be achieved through conventional descriptive analysis and consumer liking scores, in line with the values of the bakery products.

For people with special needs, such as those with coeliac disease, it is essential to diversify raw sources, but also to develop conventional gluten-free products that increasingly resemble gluten substitutes. It is for this reason that the third study focused on the development of a product such as a gluten-free rusk with different hydrocolloids.

The data show that the final product changes with the addition of different hydrocolloids and their proportions. These results can be used to improve knowledge of the use of hydrocolloids on a product such as rusks and their changes in textural parameters. However, no friable product was obtained with the proportions of additives used.

Further research is needed to investigate other additional hydrocolloid percentages and consumer acceptance of this product.

Nutritional, Physicochemical, and Sensory Evaluation of Gluten Free Bread Supplemented with *Digitaria Exilis* Stapf Flour

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The aim of this study is to explore if the Fonio (F) is suitable for the productions of gluten-free (GF) breads. Different percentages of F flour (25-50-75-100%) were substituted in a GF bread formulation and compared to the conventional GF breads formulations. Physicochemical, technological, (sensorial) and nutritional characteristics of gluten free products have been assessed on flours, doughs and GF breads produced.

MAIN OBJECTIVES:
1) The determination of the viscometric and rheological properties of flours and doughs at different percentages of F flour.
2) Textural properties of the breads produced substituting different percentages of F to the conventional GF formulations.

1. Samples

Table 1: Dough and bread formulations.

SAMPLE CODE	RICE FLOUR (%)	CORN STARCH (%)	FONIO FLOUR (%)	WATER LEVEL (%)
C	50	50	0	90
F25	25	50	25	88.3
F50	0	50	50	88.5
F75	0	25	75	97
F100	0	0	100	110
R75	75	25	0	98
R100	100	0	0	110

Abbreviations: C, control; F100,fonio 100%; F75,fonio 75%; F50,fonio 50%; F25,fonio 25%; R75, rice 75%; R100,rice 100%.

GF control (C) was prepared using a conventional GF formula based on 50% corn starch, 50% rice flour (R), 90% water, 1.8% NaCl, 1.8% yeast, 5% sunflower oil, 1.5% guar gum, and 1.5% psyllium fiber.

Fonio-enriched GF breads were prepared by replacing the rice flour and the corn starch with increasing percentages of F, as shown in Table 1.

In addition, to compare the behavior of F-enriched GF breads with other widespread GF formulations, two other R based formulations were prepared.

Leavening properties

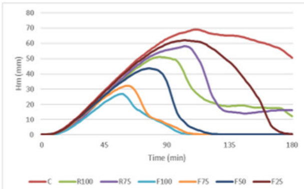

Fig 2 : *Dough development curves of the GF control, rice-based, and fonio-enriched doughs.*

Table 2 : Gaseous release parameters of the experimental GF doughs.

SAMPLES	$CO_{2\,TOT}$ (mL)	$CO_{2\,REL}$ (mL)	$CO_{2\,RET}$ (mL)
C	1348 c	68 e	1280 a
F25	1414 a	132 d	1282 a
F50	1299 d	152 c	1147 d
F75	1348 c	199 b	1149 d
F100	1385 b	226 a	1159 d
R75	1370 bc	130 d	1239 b
R100	1378 b	164 c	1214 c

Mean with the same following letter do not differ significantly from each other ($p < 0.05$). Abbreviations: $CO_{2\,TOT}$: total gas production; $CO_{2\,REL}$: gas released by the dough; $CO_{2\,RET}$: gas reteined by the dough.

Increasing amounts of fonio flours in the GF formulations proportionally reduced the maximum development of the dough (Hm) compared to C, leading to final breads with an unfavorable weight/volume ratio.

Although the total gas production of almost all the F-enriched GF doughs was similar or even higher than that observed for both control and R-based samples, the final volume of CO_2 retained by the fortified doughs was similar to that of the control only in the case of F25 sample, indicating that F supplementation weakened the dough structure.

2.Flour and dough characterization

Flours Pasting profiles

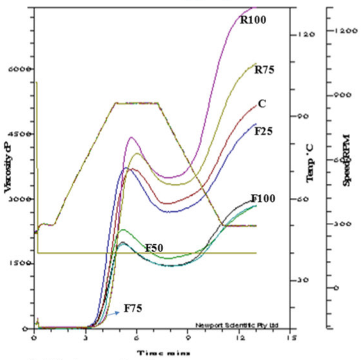

Fig 1: *Pasting properties of flours*

All the F-samples showed viscometric profiles lower than those observed in both control and rice-based samples, especially during the cooking cycle. In particular, at increasing levels of fonio substitution corresponded lower values of peak viscosity. On the contrary, during the cooling cycle, the lowest values of final viscosity were observed for all the F-samples, indicating a low tendency to starch retrogradation.

3. Fresh Bread

Tab 3: *Texture parameters of the GF control, rice-based, and fonio-enriched doughs.*

SAMPLES	HARDNESS (N)	SPRINGINESS	COHESIVENESS	RESILIENCE
C	10.95 d	0.98 a	0.56 b	0.29 b
F25	11.99 d	0.99 a	0.56 b	0.29 b
F50	18.29 c	0.98 a	0.44 c	0.21 c
F75	51.31 b	0.95 b	0.40 d	0.16 d
F100	61.10 a	0.95 b	0.41 cd	0.16 d
R75	9.21 d	0.97 a	0.59 a	0.32 a
R100	8.83 d	0.97 a	0.57 ab	0.29 b

Mean with the same following letter do not differ significantly from each other ($p < 0.05$).

TPA analysis evidenced that all the F-enriched GF breads, except for sample F25, showed higher values of hardness and lower values of cohesiveness and resilience than those observed in both C and R-based samples. On the contrary, the incorporation of F flour did not change the springiness of the resulting breads, at least at levels of substitution up to 50%.

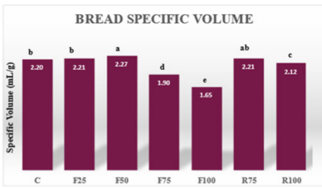

Fig 3 : *Specific Volume.* Mean with the same following letter do not differ significantly from each other ($p < 0.05$)

Fig 4 : *Crumb images of the experimental GF bread samples.*

With respect to both control and R100 sample, the incorporation of 50% of F flour increased the specific volume of the GF breads. On the contrary, at the highest levels of F substitution (75% and 100%) the fortified breads showed a significant reduction of the specific volume values.

CONCLUSIONS: The incorporation of increasing percentages of F flour in a conventional GF formulation significantly worsened the technological properties of both doughs and breads when the level of substitution used were higher than 25%.

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Techno-Functional and Gelling Properties of Acha (Fonio) (*Digitaria exilis stapf*) Flour: A Study of Its Potential as a New Gluten-Free Starch Source in Industrial Applications

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Abstract: Fonio (*Digitaria exilis Stapf*) is an ancient African cereal that represents a rich source of carbohydrate, fat, fiber, vitamins, minerals, and sulfur-containing amino acids. Processing and utilization of fonio require adequate knowledge of its structural, chemical, and nutritional characteristics. The present work evaluates the structural, techno-functional, and gelling properties of fonio and compares them to other major gluten-free cereals (rice, maize, sorghum, and millet). Fonio flour presented significantly higher water absorption index and swelling power, while it scored a lower water solubility index than the reference flours. The pasting viscosity profile of fonio was similar to that of rice, with equivalent peak viscosity but a breakdown viscosity 24% lower than rice, indicative of higher stability and resistance to shearing and heating. Rheological properties demonstrated that fonio generates gels with remarkably strong structures. At 15% concentration, fonio gel withstood stress 579% higher than those observed in the reference flours without breaking its structure. Fonio flour presented the highest gelatinization enthalpy (11.45 J/g) and a narrow gelatinization temperature range (9.96 °C), indicative of a better-packed starch structure than the other analyzed flours. The texture of the gels made with fonio showed higher firmness over the evaluated period. These combined results suggest that fonio is a suitable ingredient for gel-like food formulations.

Keywords: fonio; gelation; gel texture; gel viscosity; rheological properties; thermal properties

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1. Introduction

Over the last decade, the market for gluten-free (GF) products has grown considerably as a consequence of better diagnostic methods for identifying an increasing number of people suffering from celiac disease and other gluten-related disorders, and people who have eliminated gluten from their diet because they perceive it as a healthy improvement [1]. The nutritional composition of gluten-free products can be the most important cause of macro and micronutrient deficiencies in people with celiac disease [2]. For this reason, there are many concerns about the nutritional inadequacy of the gluten-free diet, often characterized by an excess of calories and a reduced intake of fiber, minerals, and complex carbohydrates [3,4]. It is very important for gluten-free diets to be balanced and diverse, making it necessary for more gluten-free sources to be studied and applied in the development of novel food products with improved sensorial quality and nutritional value.

Digitaria exilis Stapf, also known as fonio or acha, is a naturally gluten-free African cereal suitable for use in the diet of celiac patients [5]. Despite its low agronomic yield potential, fonio is gaining importance as a crop and food ingredient due to its superior nutritional characteristics compared with other cereals, the increased market interest in traditional food, and its suitability to be grown in tough conditions, such as in arid soil.

Fonio flour has been mixed with other ingredients to improve the nutritional and textural quality of different food products such as malts, beverages, sourdough, bread, puddings, crackers, breakfast cereals, and biscuits [6,7].

Fonio has a high content of calcium and iron, compared to the other cereals indicated in the food composition table of Mali [8]. Potassium and magnesium appear to be the major mineral elements in fonio grains [6]. The protein content of fonio is like that found in white rice [9,10], although it has a higher sulfur amino acid content (methionine and cystine [11]. Fonio has a high pentosan content, which gives it the capacity to absorb water to produce a very viscous solution, an attribute known for good baking operation [12]. Fonio has also been linked to health benefits such as the prevention and treatment of constipation, cardiovascular diseases, and hypertension [13]. The presence of polyphenols in fonio leads to antioxidant and free radical scavenging activities [6]. It is believed that fonio may have nutraceutical properties with a role in preventing and managing prediabetes and type 2 diabetes [5]. Sartelet et al. [14] have demonstrated the presence of apigenin and luteolin in fonio manifest strong anti-thyroid peroxidase (TPO) activities. Given these characteristics, fonio flour has the potential to be used as an ingredient to improve gluten-free nutritional profiles without compromising the taste and quality of products.

Despite the nutritional interest and health benefits of fonio flour, there is a lack of research on fonio compared to other major cereal grains [6]. An in-depth study into the techno-functional and gelling properties of fonio has not been covered in the available literature so far. The aim of this work is to evaluate the techno-functional, pasting, and thermal properties of fonio flour and the rheological and textural characteristics of the gels made from it. Other major gluten-free cereals, such as maize and rice (the two main gluten-free ingredients used in Europe), millet and sorghum (two warm-season grains that belong to the same family (*Poaceae*) and sub-family (*Panicoideae*) as fonio), were included in the study as reference flours.

2. Materials and Methods

2.1. Material

Commercial fonio (*Digitaria exilis stapf*) flour was obtained from Obà Food (Roma, Italy). Maize (*Zea Mais* L.) flour was purchased from ADPAN (Asturias, Spain). Indica rice (*Oryza sativa* L.) flour was kindly provided by Herba Ricemills S.L.U. (Valencia, Spain). Sorghum (*Sorgum bicolor*) and millet (*Panicum miliaceum*) flours were kindly supplied by Salutef (Palencia, Spain).

2.2. Flour Proximate Composition

The flours' composition was determined following the 44–19 (moisture), 08–01 (ash), 30–25 (fat), and 46–11 (protein) AACC methods [15]. Carbohydrates were determined by difference to 100%.

2.3. Techno-Functional Properties

Foaming capacity (FC) and foam stability (FS) were determined as described by Abebe et al. [16]. The flour sample (2 g) was mixed with 40 mL of distilled water at 30 °C in a 100 mL measuring test tube. To produce foam, the suspension was shaken manually for 5 min. The volume of foam was measured after 0 min (V_0) and 60 min (V_{60}). FC was established directly from V_0, and FS was calculated as (V_{60}/V_0) 100.

Water absorption capacity (WAC), water absorption index (WAI), water solubility index (WSI), and swelling power (SP) were determined evaluating dispersions of 2 g of flour sample in 20 mL of distilled water using 50 mL centrifuge tubes, following the methods indicated by Abebe, Collar, and Ronda [16]. The WAC was determined by the centrifugation method. The dispersions were kept at room temperature for 30 min, then vortexed for 30 s and finally left to rest for 10 min. Immediately after, the tubes were centrifuged for 25 min at 3000× *g*. The supernatant was removed, and the sediment was weighed. Results were expressed as g H_2O retained/g of flour dry matter.

WAI, WSI, and SP were determined after cooking the flour dispersions for 15 min in a 90 °C water bath. The gels formed were cooled at room temperature for 1 h and centrifuged at 3000× *g* for 10 min. The supernatant was placed into an evaporating capsule to determine the soluble solid content (WSI (g of soluble solids/100 g flour dry matter) and SP (g of sediment/g insoluble solids flour dry matter)). The sediment was used to determine WAI (g of sediment/g of dry flour matter).

2.4. Least Gelation Concentration

The least gelation concentration (LGC) of the studied flours was determined with slight modifications of the method indicated by Joshi et al. [17]. Glass test tubes containing 2 mL of distilled water and the corresponding amount of flour to achieve a concentration of 2, 4, 6, 9, 10, 11, 12, 14, 16, 18, and 20 g/100 mL were kept for 1 h in a boiling water bath to form the gel. The tubes were cooled down by placing them under running water and stored at 4 °C for 2 h. LGC was determined as the minimum concentration where the gel did not drop or slip when the glass tube was inverted.

2.5. Pasting Properties

Pasting properties of flour samples were determined using a Kinexus Pro+ rheometer (Malvern Instruments Ltd., Malvern, UK) equipped with a starch cell and controlled by rSpace software. Each flour sample (3.0 g, dry basis) was transferred to the evaluation canister and mixed with 25 mL of distilled water. The sample was equilibrated at 50 °C for 60 s, heated to 95 °C at 6 °C/min, maintained at 95 °C for 300 s, cooled to 50 °C at 6 °C/min, and maintained at 50 °C for 120 s. The paddle speed rate was set at 160 rpm during the whole analysis. Parameters calculated from the pasting profile were pasting temperature (PT), peak viscosity (PV), trough viscosity (TV), breakdown viscosity (BV), final viscosity (FV), and setback viscosity (SV). Each sample was analyzed at least in duplicate.

2.6. Rheological Properties of Flour Gels

The gels of fonio, millet, sorghum, maize, and rice were analyzed by a dynamic oscillatory test, performed using a Kinexus Pro+ rheometer (Malvern Instruments Ltd., Malvern, UK) equipped with a parallel plate geometry (40 mm diameter) of serrated surface at a gap of 1 mm. Gel samples were obtained following the method described for the determination of flour pasting properties (Section 2.5). Dispersions of flour in water at different concentrations (6-8-10-12-15 g/100 g) were used to prepare the gels. Once the gel was prepared, it was placed in the plates and was left to rest for 5 min to allow relaxation. The temperature was stabilized at 25 °C. Strain sweeps were performed from 0.1 to 1000% at a constant frequency of 1 Hz to establish the maximum stress (τ_{max}) in the Linear Viscoelastic Region (LVR) and the stress at the cross point ($G' = G''$) [18]. The limit of the LVR (τ_{max}) was identified as the sharp decrease of G' modulus, which coincided with the sudden increase of tan(δ). Frequency sweeps were made from 1 to 10 Hz at a constant strain of 1% of the LVR. The values G_1', G_1'', and $\tan(\delta)_1$ were obtained from fitting the frequency sweeps experimental data to the power-law model as described by Villanueva, De Lamo, Harasym, and Ronda (2018) [19]; these variables represent the elastic and viscous moduli and the loss tangent, respectively, at a constant strain of 1%. The exponents obtained from the fitting (a, b, and c) quantify the dependence of the elastic and viscous moduli and the loss tangent to the oscillation frequency. The gels and the rheological tests were carried out in duplicate.

2.7. Thermal Properties

Thermal transitions (gelatinization, retrogradation, and amylose-lipid complex dissociation) were determined by differential scanning calorimetry (DSC) (DSC-822e, Mettler Toledo, SAE). Samples (~6 mg) were prepared at a 30:70 (*w*/*w*) flour:water ratio in 40 μL aluminum pans. The scan made for the evaluation went from 0 to 115 °C at a heating rate of 5 °C/min, using an empty pan as reference. Zinc and indium were employed to calibrate the calorimeter. The enthalpy change (ΔH, J/g dry basis) and the gelatinization temperatures [onset (To), peak (Tp), and endset (Te)] were recorded. Endothermic transitions of the retrograded starches were determined with a second scan made after 7 days of sample storage at 4 °C, following the same procedure described for the gelatinization evaluation. Each experimental sample was measured at least in duplicate.

2.8. Texture of Fresh Gels and Its Evolution with Time

The texture of gels was measured with a TA-XT2 Texture Analyser (Stable Microsystems, Surrey, UK) provided with the software "Texture expert exceed" version 2.63. The gels were prepared in 50 mL centrifuge tubes by heating 28 g of flour dispersion (at a concentration of 15 g/100 g) in a boiling water bath for 30 min, stirring the dispersion every 2–3 min until the boiling temperature was achieved. The tubes were cooled at room temperature and stored for 2, 6, 10, 24, 48, 96, and 192 h at 4 °C. Gels were left at room temperature for 15 min before the analysis of the texture. Measurements were done on gel cylinders of 2.7 cm diameter and 2 cm height. A "Texture Profile Analysis" (TPA) double compression test was performed using a 75 mm diameter aluminum probe (SMSP/75) to suppress 50% depth, at 1 mm/s speed test, with a 30 s delay between compressions. Firmness (N), springiness, cohesiveness, and gumminess (N) were calculated from the TPA graphs.

2.9. Statistical Analysis

One-way ANOVA with parametric tests was used for the statistical analysis using Statgraphics Centurion XVIII software (Bitstream, Cambridge, MN, USA). The Levene test was used to check the homogeneity of variances. The normality of the studentized residuals was evaluated with the Kolmogorov–Smirnov test. Fisher's Least Significant Difference test at 95% confidence intervals ($p < 0.05$) was used to establish significant differences among means.

3. Results and Discussion

3.1. Flour Proximate Composition

The composition of fonio and the other studied gluten-free flours are presented in Table 1. It can be noted that the composition of fonio flour was very similar to rice flour. Both flours were the richest in carbohydrates and the poorest in proteins, along with maize. Fonio showed the lowest content of fat and ash among the studied flours. Analyzing the literature available for the fonio flour, large variations of the chemical and nutritional composition were found. Ballagoun [20] reported the following ranges in the main fonio flour components: 5.1–11% proteins, 1.3–5.2% fat, 1–6% ash. These differences can be attributed to genetic factors, geographical situation, environmental influences, agronomic characteristics, and analytical methods used in the determination.

Table 1. Proximal composition of fonio compared to the other gluten-free flours. Except for the moisture content, all presented values are referred to dry basis.

Flour	Moisture (%)	Ash (%)	Fat (%)	Proteins (%)	Carbohydrates (%)
Fonio	12.90 ± 0.01 e	0.45 ± 0.02 a	1.1 ± 0.2 a	6.6 ± 0.3 a	91.9 ± 0.9 c
Millet	10.47 ± 0.01 a	1.73 ± 0.09 e	4.2 ± 0.8 c	12.9 ± 0.5 d	81.2 ± 1.4 a
Sorghum	11.77 ± 0.01 b	1.35 ± 0.07 d	3.3 ± 0.6 b	10.0 ± 0.4 c	85.3 ± 1.1 b
Maize	11.96 ± 0.01 c	1.14 ± 0.06 c	4.4 ± 0.9 c	7.5 ± 0.3 b	87.0 ± 1.3 b
Rice	12.66 ± 0.01 d	0.57 ± 0.03 b	1.3 ± 0.2 a	7.8 ± 0.3 b	90.4 ± 0.6 c

Data are the mean ($n = 2$) ± standard deviation. Values with a letter in common in the same column are not significantly different ($p > 0.05$).

3.2. Functional Characteristics

Functional characteristics are summarized in Table 2. The studied flours have shown different values of foaming capacity (FC) and foaming stability (FS). The foaming capacity of flours is mainly related to proteins, which form a continuous, cohesive film around the air bubbles in the foam [21]. Fonio did not exhibit any foaming capacity, while millet and sorghum flours exhibited the highest FC and FS, probably due to their higher protein content. It has been reported that protein–carbohydrate interactions in fonio may contribute to the low solubility of fonio proteins [6], resulting in lower availability to interact with water and generate foam. No FS was measured in fonio, given that no foam was formed. The remaining GF flours showed FS values above 50%, suggesting that the native proteins soluble in water are very surface active in these flours [21].

Table 2. Functional characteristics and pasting properties of fonio flour compared to other gluten-free flours.

	Fonio	Millet	Sorghum	Maize	Rice
			Functional properties		
FC (mL)	nd	3.33 ± 0.34 b	4.99 ± 0.35 c	1.57 ± 0.17 a	1.74 ± 0.01 a
FS (%)	nd	52 ± 9 a	82 ± 2 c	76 ± 8 b	50 ± 1 a
WAC (g/g)	1.12 ± 0.02 c	0.97 ± 0.01 a	1.09 ± 0.01 b	1.34 ± 0.02 d	0.98 ± 0.01 a
WHC (g/g)	1.8 ± 0.2 bc	1.8 ± 0.1 abc	1.85 ± 0.07 c	2.4 ± 0.1 d	1.65 ± 0.09 a
WAI (g/g)	7.7 ± 0.2 d	6.20 ± 0.07 b	6.9 ± 0.3 c	6.35 ± 0.06 b	5.77 ± 0.03 a
WSI (g/100 g)	0.7 ± 0.1 a	2.2 ± 0.1 cb	2.2 ± 0.1 c	4.7 ± 0.1 d	1.74 ± 0.03 bc
SP (g/g)	7.7 ± 0.2 d	6.16 ± 0.07 b	6.9 ± 0.3 c	6.26 ± 0.06 b	5.74 ± 0.03 a
			Pasting properties		
PT (°C)	82.2 ± 0.1 b	85.3 ± 0.1 d	91.6 ± 0.2 e	80.29 ± 0.02 a	82.72 ± 0.02 c
Pt (s)	562 ± 3 a	620 ± 1 c	682 ± 6 e	572 ± 5 b	633 ± 1 d
PV (Pa-s)	4.68 ± 0.03 d	2.11 ± 0.03 b	2.56 ± 0.03 c	1.580 ± 0.004 a	5.22 ± 0.09 e
FV (Pa-s)	5.15 ± 0.08 c	4.46 ± 0.02 b	5.4 ± 0.4 c	2.92 ± 0.02 a	5.22 ± 0.09 c
TV (Pa-s)	2.39 ± 0.02 d	1.32 ± 0.01 a	2.00 ± 0.02 b	1.270 ± 0.001 a	2.19 ± 0.04 c
BV (Pa-s)	2.29 ± 0.01 d	0.79 ± 0.04 c	0.56 ± 0.05 b	0.310 ± 0.005 a	3.03 ± 0.05 e
SV (Pa-s)	2.76 ± 0.07 b	3.14 ± 0.02 bc	3.4 ± 0.5 c	1.65 ± 0.02 a	3.03 ± 0.05 bc

FC: foaming capacity, FS: foaming stability, WHC: Water holding capacity (g H_2O/g flour dry matter, dm), WAI: Water absorption index (g sediment/g flour dm), WSI: Water solubility index (g soluble solids/100 g flour dm), WAC: Water absorption capacity (g H_2O/g flour dm), SP: swelling power (g/g insoluble flour matter). PT: Pasting Temperature, Pt: peak time, PV: peak viscosity, FV: final viscosity, TV: Trough Viscosity, BV: Breakdown viscosity, SV: Setback viscosity. Data are mean ± standard deviation. Values with a letter in common in the same row are not significantly different ($p > 0.05$).

Flour hydration properties varied significantly among GF flours, which could be explained by the different compositions of the flours, mainly protein, fiber, and starch [21]. In order to obtain good quality from alternative materials (such as novel gluten-free sources), it is necessary to know their hydration properties to balance formulations and adequate technological production processes to counteract changes in the rheological properties caused by the substitution of gluten [22]. It has been documented that hydration is a critical factor in many manufacturing processes of cereal-based products such as pasta, couscous, and bread [22]. The WHC, which quantifies the ability of a matrix to absorb and retain water without the influence of external forces, and the WAC, which depends on the source's susceptibility to form hydrogen bonds between starch, influenced by the hydrophilic parts in carbohydrates and proteins [23], followed a similar trend. Fonio showed average values for a GF flour in these two parameters, above millet, rice, and sorghum, which presented the lowest water binding capacities, and below maize flour. Fonio flour exhibited the highest WAI and SP and the lowest WSI among the other analyzed flours. This behavior denotes the ability of fonio starch to absorb the highest amount of water during gelatinization and swelling in excess water and retain it in the formed gel [24].

Swelling power (SP) followed an identical evolution as WAI, given that both parameters associate the weight of the formed gel with the number of insoluble compounds in the flour and the whole amount of flour, respectively. When the fonio flour gelatinizes, better interaction with water is exhibited, which could be due to the very small dimension of the starch granules, ~8 µm [25]. Said small size leads to a high specific surface area, with consequent higher interaction and absorption of water [26]. The very low solubility of fonio in comparison with the other GF flours (WSI value about 3 times lower than that of rice and 7 times lower than maize) is compatible with its very low mineral content; it also means a very low lixiviation and solubility of amylose during starch swelling [27].

3.3. Pasting Properties

The results obtained from viscometric tests are presented in Table 2 and Figure 1. Pasting temperature (PT) expressed the minimum temperature necessary to begin the cooking of the flour, identified as the temperature at which viscosity increases during the heating process [28]. Fonio flour showed an intermediate PT value (82.2 °C), similar to that of rice, higher than maize (80.29 °C), and significantly lower than sorghum (91.6 °C) and millet (85.3 °C). The highest value of PT was registered in sorghum flour, which could indicate the presence of a starch that is highly resistant to swelling and rupturing [29]. Fonio showed the shortest time to reach the peak viscosity (Pt) and a peak viscosity (PV) value significantly higher than millet, sorghum, and, especially, maize flour. The PV of fonio flour was only (slightly) surpassed by rice flour. The PV happens at the equilibrium point between swelling and rupturing of the starch [30]. It is obtained at the maximum swelling of starch granules, and it is linked to the water absorption index of the flour [31], as was shown in Section 3.2. Fonio flour also showed a higher trough viscosity (TV) value than millet, sorghum, and maize, similar to that of rice flour. Fonio showed a breakdown viscosity (BV) value 24% lower than rice flour, denoting higher paste stability and resistance against shearing and heating than one of the most used flours in GF production. Maize, followed by sorghum and millet, showed the lowest BV and the highest stability, although these flours presented significantly lower viscometric profiles (Figure 1), so lower values of BV were expected. The setback viscosity (SV) is an indicator of amylose's tendency to retrograde. In general, higher values of SV indicated a greater tendency of starch to retrograde [32]. The SV value of fonio was similar to that of rice and between those of millet and sorghum (the maximum) and maize (the minimum).

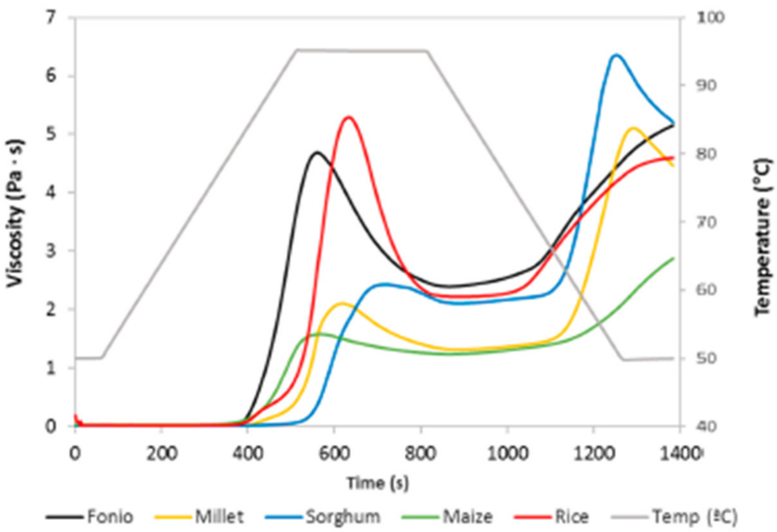

Figure 1. Pasting properties of the gluten-free flours studied.

In millet and sorghum, a second peak of viscosity appeared at the final cooling phase (50 °C) of the pasting curve. It is believed that this behavior could be due to the storage of the flours for a time equal to or greater than two months, given that a second peak during the holding stage was also found by Zhang and Hamaker [33] in sorghum flour when it had been stored for more than two months. The viscometric profile of fresh sorghum flour only showed one peak in the holding period (95 °C).

At the end of the pasting profile, during the temperature holding at 50 °C, the final viscosity (FV) was recorded. This value showed the capacity of the material to form a viscous paste that reflects the retrogradation of amylose [34]. The values registered are maximum for sorghum, fonio, and rice flours, and no significant differences are found among them. The lowest FV value was shown by maize flour.

3.4. Gel Viscoelastic Properties and Their Dependence on Flour Concentration

The viscoelastic properties of the gels were assessed by dynamic oscillatory tests at 25 °C (see Table 3 and Supplementary Figure S1). The samples were produced at different flour concentrations (from 6% to 15%) and analyzed with strain sweeps, allowing the establishment of the end of the linear viscoelastic region (LVR) and the identification of the maximum stress (τ_{max}) that samples could tolerate before the collapse of their structure. The effects of flour type and concentration on the value of maximum stress (τ_{max}) were statistically significant ($p < 0.05$), following an increasing trend with increasing concentration in all the studied GF flours. The strain sweep assays also provided the stress at which the gels passed from a solid-like to a liquid-like behavior (the crossing point of the curves where $G' = G''$ and $\tan(\delta) = 1$) (Table 3). Results indicated that fonio formed gels with a remarkably stronger structure, being the sample that showed the highest values of τ_{max} and cross-over point in all studied concentrations. Differences were particularly marked with higher concentrations; the 15% fonio gel presented a τ_{max} and cross-over point 579% and 1788%, respectively, higher than sorghum (the sample with the lowest results).

The G_1', G_1'', $\tan(\delta)_1$ and a, b and c exponents were generated from fitting the frequency sweeps data in the range of 1–10 Hz to the power-law model. The high value of R^2 (0.955–0.999) indicates to what extent the model adjusted to the studied systems. Elastic and viscous moduli significantly increased when the flour concentration increased. However, the rate of increase varied depending on the type of flour (see Supplementary Figure S1). Except for the lowest studied concentration (6%), fonio gel exhibited markedly higher elastic and viscous moduli than the other GF flours. The marked differences in gel rheological properties of the different gluten-free flours could be attributed to differences in their botanical origin, such as proteins, starch, and lipids contents [35–37].

The viscoelastic moduli of rice gels and the rate of increase of G_1' and G_1'' with concentration were significantly lower than the gels made from the other GF flours, in agreement with previous studies [37].

All gels presented $G_1' > G_1''$, and consequently $\tan(\delta)_1 < 1$, in all the studied concentrations, indicating a solid-like behavior of the gels. This indicates that the gel structure was already formed at a concentration of 6%, regardless of the flour source. Except for the gels made from rice flour, $\tan(\delta)_1$ decreased with the increase in flour concentration (see Table 3 and Supplementary Figure S1). This behavior reveals a strengthening of their structure with concentration. The fonio gels showed the highest decrease in loss tangent with increasing gel concentration, in particular when increasing from 6% to 8%, reaching the lowest value at a concentration of 12% (0.0540). This value was between 50% and 66% lower than those obtained for the gels made with the other GF flours. In the case of rice gels, the loss tangent did not follow a decreasing trend with concentration, but rather the opposite, indicating that in this case, the increase in concentration enhanced the viscosity of the gel rather than its elasticity, resulting in a softer gel structure than that of the more diluted gels [38].

Table 3. Rheological properties of fonio, millet, sorghum, maize, and rice flour gels at different concentrations.

Flour	Concentration (%)	G_1' (Pa)	a	G_1'' (Pa)	B	tan $(\delta)_1$	C	Cross over (Pa)	τ_{max} (Pa)
Fonio	6	45 ± 4 aA	0.121 ± 0.006 eC	10 ± 1 aB	0.370 ± 0.007 dC	0.230 ± 0.007 cD	0.249 ± 0.001 abA	14.5 ± 0.4 aC	8.3 ± 0.1 aC
	8	385 ± 3 bD	−0.134 ± 0.006 aA	30 ± 1 bC	0.267 ± 0.001 cA	0.078 ±0.001 bA	0.401 ± 0.005 bD	574 ± 26 bD	548 ± 18 bD
	10	1242 ± 10 cC	−0.072 ± 0.002 bA	70 ± 1 cC	0.184 ± 0.001 aA	0.056 ± 0.004 aA	0.256 ± 0.003 abD	1347 ± 28 cD	1142 ± 4 cD
	12	1761 ± 66 dE	−0.003 ± 0.003 cA	95 ± 3 dD	0.201 ± 0.002 bC	0.054 ± 0.003 aA	0.204 ± 0.001 aB	1682 ± 102 dD	1387 ± 37 dD
	15	3106 ± 182 eE	0.0150 ± 0.0003 dA	169 ± 11 eBC	0.209 ± 0.005 bC	0.055 ± 0.004 aA	0.193 ± 0.005 aC	2535 ± 4 eD	2151 ± 76 eE
Millet	6	66 ± 5 aBC	0.0320 ± 0.0001 aA	6 ± 1 aA	0.356 ± 0.004 dB	0.099 ± 0.002 cA	0.324 ± 0.003 cC	11.0 ± 0.5 aA	3.9 ± 0.3 aB
	8	167 ± 11 aA	0.064 ± 0.001 eB	17 ± 1 aA	0.290 ± 0.01 cB	0.105 ± 0.003 dB	0.230 ± 0.013 bBC	34 ± 2 aA	12 ± 3 abA
	10	380 ± 36 bB	0.059 ± 0.003 dB	38 ± 3 bA	0.219 ± 0.014 bB	0.101 ± 0.002 cB	0.160 ± 0.010 aB	96 ± 14 bA	29 ± 2 bA
	12	833 ± 31 cC	0.047± 0.003 cB	71 ± 2 cB	0.181 ± 0.009 aB	0.086 ± 0.001 bB	0.133 ± 0.006 aA	204 ± 27 cA	95 ± 4 cA
	15	2213 ± 107 dC	0.0410 ± 0.0003 bB	176 ± 11 dC	0.189 ± 0.005 aB	0.079 ± 0.001 aB	0.148 ± 0.005 aB	526 ± 49 dBC	245 ± 11 dC
Sorghum	6	59 ± 2 aBC	0.043 ± 0.006 aA	6 ± 1 aA	0.363 ± 0.001 dBC	0.101 ± 0.003 aA	0.321 ± 0.006 bB	9.0 ± 0.3 aA	2.0 ± 0.1 aA
	8	202 ± 5 bB	0.079 ± 0.003 dB	27 ± 1 bB	0.240 ± 0.007 cC	0.132 ± 0.001 dC	0.160 ± 0.010 aA	36.6 ± 0.5 bAB	11 ± 2 bA
	10	458 ± 7 cB	0.068 ± 0.001 cBC	56 ± 1 cB	0.185 ± 0.003 bA	0.121 ± 0.003 cC	0.116 ± 0.004 aA	83 ± 1 cA	28.1 ± 0.2 cA
	12	1059 ± 19 dD	0.059 ± 0.002 bC	112 ± 4 dD	0.168 ± 0.001 aA	0.106 ± 0.001 bC	0.109 ± 0.001 aA	194 ± 12 dA	68 ± 6 dA
	15	2671 ± 4 eD	0.056 ± 0.001 bD	280 ± 5 eD	0.179 ± 0.004 bA	0.105 ± 0.002 bC	0.123 ± 0.005 aA	438 ± 6 eA	120.3 ± 0.3 eA
Maize	6	54 ± 1 aB	0.086 ± 0.003 cB	10 ± 1 aB	0.311± 0.001 eA	0.187 ± 0.004 eC	0.225 ± 0.003 aA	13.8 ± 0.7 aB	3.6 ± 0.3 aB
	8	226 ± 6 bC	0.07 ± 0.02 bcB	36 ± 2 bD	0.242 ± 0.001 dC	0.158 ± 0.003 dE	0.170 ± 0.020 aAB	59 ± 3 bBC	34.6 ± 0.4 aB
	10	407 ± 69 cB	0.062 ± 0.007 abBC	57 ± 11 cB	0.220 ± 0.004 cB	0.139 ± 0.003 cD	0.158 ± 0.005 aB	153 ± 2 cB	85 ± 6 bB
	12	673 ± 9 dB	0.051 ± 0.001 abB	812 ± 2 dE	0.209 ± 0.004 bC	0.121 ± 0.002 bD	0.158 ± 0.004 aA	364 ± 1 dC	292 ± 2 cC
	15	1492 ± 6 eB	0.044 ± 0.001 aC	155 ± 1 eB	0.193 ± 0.006 aB	0.104 ± 0.001 aC	0.149 ± 0.006 aB	759 ± 8 eC	454 ± 3 dD
Rice	6	93 ± 1 aD	0.087 ± 0.001 cB	15 ± 1 aC	0.314± 0.001 bA	0.156 ± 0.003 bB	0.227 ± 0.001 bA	36 ± 2 aC	22.7 ± 0.7 aD
	8	174 ± 3 bA	0.0530 ± 0.0001 aB	25 ± 1 bB	0.305 ± 0.001 aD	0.143 ± 0.001 aD	0.252 ± 0.001 dC	118 ± 1 bC	60.4 ± 0.7 bC
	10	196 ± 2 cA	0.072 ± 0.002 bD	32 ± 1 cA	0.321 ± 0.001 cC	0.164 ± 0.003 cE	0.249 ± 0.003 cC	206 ± 2 cC	97.3 ± 0.2 cC
	12	225 ± 4 dA	0.088 ± 0.003 cD	40 ± 1 dA	0.334 ± 0.001 dD	0.180 ± 0.001 dE	0.25 ± 0.02 bC	302 ± 4 dB	154 ± 1 dB
	15	338 ± 5 eA	0.121 ± 0.001 dE	61 ± 1 eA	0.341 ± 0.001 eD	0.181 ± 0.001 dD	0.220 ± 0.002 aD	460 ± 9 eB	217 ± 4 eB

The power-law model was fitted to experimental results from frequency sweeps: $G'(\omega) = G_1'\cdot\omega^a$; $G''(\omega) = G_1''\cdot\omega^b$; $\tan\delta(\omega) = \tan\delta_1\cdot\omega^c$. G_1', G_1'' and $(\tan\delta)_1$: represent the elastic and viscous moduli and the loss tangent at 1% strain. The a, b, and c exponents quantify the dependence degree of dynamic moduli and the loss tangent with the oscillation frequency. τ_{max}: maximum stress that samples can tolerate in the LVR. Data are the mean ± standard deviation. Values with a letter in common in the same column for each flour are not significantly different ($p > 0.05$). Lowercase letters compare the effect of concentration in the same flour, and capital letters compare different flours at the same concentration.

The low values of "a" exponents for all gels indicate that G′ was not dependent on the applied frequency and suggested a stable gel structure [30]. As can be seen in Table 3, the lowest values of "a" were obtained for fonio gels, while the highest ones were presented by rice gels. The dependence of the viscous modulus and loss tangent with frequency, evaluated from the "b" and "c" exponents, respectively, were higher than that of the elastic modulus ("a") and similar among gels of different nature. Both exponents decreased with the concentration of flour in the gel, except for rice flour gels, where b increased with concentration. This impact of concentration on the stability of gels and batters has been reported in previous works [30–36].

The high dependence of storage modulus on the concentration allows gathering information of the gelation efficiency and the structure of the particle network of the gel [39]. Clark et al. [40] estimated the relation between concentration and storage moduli using a power-law equation. Power-law functions between concentration and G_1' and G_1'' were obtained for the dispersions: $G_1' = m * C^n$ and $G_1'' = p * C^q$, where m and p represent the G′ and G″ moduli values at a gel concentration of 1% and a frequency of 1 Hz, and n and q, the exponents, quantify the dependence degree of the viscoelastic moduli to the concentration and reflect the nature of the association behavior in the gel and its network structure [41] (Table 4). The R^2 coefficients ranged from 0.9561 to 0.9997, indicating a good fitting of experimental results to the potential model. In the case of fonio and rice, the evolution of the elastic modulus with concentration would also be compatible with a linear model (see Supplementary Figure S1A). Linear correlations between G′ and concentration have been reported for potato, wheat, corn, and rice starch gels [42]. Therefore, the variation of G_1' with the concentration of millet, sorghum, and maize gels, is probably more related to the protein content than to the starch content of the flours, while in the case of fonio and rice, their naturally low protein content could explain the observed behavior, more similar to that of starch gels. However, to allow comparison among different flours, the potential equation was chosen to model the evolution of all GF gels viscoelastic moduli versus concentration. As can be seen (Table 4), the n and q values of fonio gels were 2.8 and 2.5, respectively. These values were greater in millet, sorghum, and maize flours, which reflects the formation of a more ordered gel matrix. Rice was the only one with significantly lower exponents (n = 1.26 and q = 1.53) than fonio. This denotes a higher increase in fonio gels' viscoelastic moduli with flour concentration and a higher modulation capacity of the gel's viscoelasticity by varying its concentration than rice gels. The exponents n and q of millet and sorghum were notably higher than that of fonio; however, their viscoelastic moduli at low concentrations (quantified by the m and p coefficients) were much lower than those obtained for fonio. The combination of a high consistency at low concentrations (compared to millet, sorghum, and maize) and a high increase in consistency with increasing concentration (compared to rice) makes fonio an interesting ingredient for the production of GF products of gel-like nature.

Table 4. Parameters obtained from fitting to the power-law model the experimental G_1' and G_1'' data in function of the flour concentration in the gels ($G_1' = m \cdot C^n$; $G_1'' = p \cdot C^q$).

Parameter	Fonio	Millet	Sorghum	Maize	Rice
m (Pa)	2 ± 1 d	0.015 ± 0.004 a	0.030 ± 0.005 b	0.20 ± 0.08 c	11 ± 5 e
n	2.8 ± 0.3 b	4.4 ± 0.1 e	4.21 ± 0.06 d	3.3 ± 0.1 c	1.26 ± 0.17 a
R^2	0.979	0.9992	0.9997	0.9971	0.9561
p (Pa)	0.18 ± 0.09 b	0.005 ± 0.002 a	0.006 ± 0.001 a	0.15 ± 0.06 b	1.0 ± 0.2 c
q	2.5 ± 0.2 b	3.9 ± 0.1 c	4.00 ± 0.07 c	2.6 ± 0.2 b	1.53 ± 0.09 a
R^2	0.989	0.9985	0.9995	0.9934	0.9914

The coefficients m and p represent the values of the G_1' and G_1'' moduli at a concentration of 1%; n and q exponents inform about the dependence degree of both moduli on flour concentration. Values with a letter in common in the same row are not significantly different ($p > 0.05$).

3.5. Thermal Properties

Thermal properties of the gluten-free flours were evaluated using Differential Scanning Calorimetry (DSC). Gelatinization, retrogradation, and amylose-lipid complex data obtained from DSC are shown in Table 5. Thermograms of flour samples showed 2 wide endothermic transitions in the first scan; one due to starch gelatinization, which appeared at 73–77 °C, and the second peak at 93–98 °C, due to the dissociation of amylose-lipid complex [43]. The reversibility of this second endotherm indicates the presence of an amorphous amylose-lipid complex within these samples [44]. The different flour samples showed significant differences in gelatinization enthalpy (ΔH_{gel}). Fonio and rice gels presented the highest values (11.5 and 10.5 J/g, respectively), while maize presented the lowest (5.1 J/g). Higher values of ΔH_{gel} are indicative of a better-packed starch structure requiring more energy to fully gelatinize. Significant differences were also found among gelatinization temperatures. Ji et al. [45] discovered that higher crystallites perfection is linked to greater gelatinization temperatures required to melt them. Fonio presented a high value of onset temperature (To), statistically equal to that of rice, surpassed by millet and sorghum. Peak temperature (Tp), where the endothermic transition reaches a maximum, presented the highest value in millet flour (76.98 °C), while the lowest values were recorded in fonio and maize flours, being 73.5 and 70.07 °C, respectively. The width of the gelatinization temperature range (Te-To) was the highest in maize (17.3 °C), followed by rice (11.3 °C), sorghum (10.6 °C), fonio (9.96 °C), and millet (7.45 °C). Lower values of the gelatinization peak width indicate higher starch crystallites homogeneity and a better organized granular structure, requiring a shorter temperature range to fully hydrate. The formation of amylose–amylose linkages and amylose–lipid complexes within the starch granule and a more stable configuration have been associated with higher gelatinization temperatures [46]. The dissociation enthalpy of amylose–lipid inclusion complex obtained in the first run for fonio was equal to those of millet, sorghum, and rice.

A second scan was performed to assess the retrogradation properties of the flours after storage of the gelatinized samples in their corresponding pans at 4 °C for 7 days (Table 5). Two visible peaks were detected from this scan. The first peak, which appeared at a temperature around 50 °C, was linked to the melting of the recrystallized amylopectin (ΔH_{ret}) during the gel staling. All values determined for ΔH_{ret} were lower than their corresponding ΔH_{gel} value, but showed the same trend, with fonio presenting the highest value. The width of this first peak was observed to be higher in fonio flour, while maize presented the lowest value. The second peak registered was related to the reversible amylose–lipid complex dissociation and appeared approximately at the same temperature as it did in the first scan. The enthalpies of the amylose–lipid complex were increased in the second scan with respect to the first scan. Eliasson [44] indicated that the increased values during the second scan are due to better conditions for complex formation after the first heating. This is related to the leaking of amylose out of the granules that occur at temperatures above the gelatinization temperature range.

3.6. Texture of Fresh Gel and Its Evolution with Storage Time

The texture properties of the gels made from the studied flours and their evolution during a period of 192 h of storage at 4 °C, depicted in Figure 2 and Table 6. Fonio gels presented the highest values of firmness in the studied range, in agreement with the information obtained from gel's rheology. As could be expected, the firmness of gels increased with storage time regardless of the type of flour. As can be seen in Figure 2A, except for the fonio gel, all gels reached a constant/asymptotic firmness value after 24–48 h of storage. In the case of fonio, however, a constant increase was obtained during all 192 h, reaching a value of 30.37 N, representing an increase of 109% with respect to the initial firmness. This change over storage periods was mainly related to amylopectin recrystallization [47], hence highly dependent on the starch composition and botanical origin of the sample.

Table 5. Thermal properties of fonio, millet, sorghum, maize, and rice flours.

	First Scan						Second Scan					
Samples	ΔH_{gel} (J/g)	To_{gel} (°C)	Tp_{gel} (°C)	Te_{gel} (°C)	ΔH_{am-lip} (J/g)	Tp_{am-lip} (°C)	ΔH_{ret} (J/g)	To_{ret} (°C)	Tp_{ret} (°C)	Te_{ret} (°C)	ΔH_{am-lip} (J/g)	Tp_{am-lip} (°C)
Fonio	11.5 ± 0.3 e	68.92 ± 0.03 b	73.5 ± 0.1 b	78.88 ± 0.08 b	0.91 ± 0.01 b	97.0 ± 0.2 bc	7.9 ± 0.3 b	32 ± 6 a	50.5 ± 0.3 a	68 ± 4 b	1.3 ± 0.2 a	97.8 ± 0.1 c
Millet	7.0 ± 0.1 c	73.45 ± 0.01 d	76.98 ± 0.02 d	80.9 ± 0.1 d	0.94 ± 0.05 b	93.3 ± 0.1 a	4.5 ± 0.4 a	38 ± 1 a	52.1 ± 0.1 b	63.5 ± 0.3 ab	3.1 ± 0.2 bc	92.6 ± 0.3 a
Sorghum	6.5 ± 0.4 b	69.8 ± 0.1 c	74.7 ± 0.1 c	80.4 ± 0.2 cd	0.99 ± 0.06 b	98.1 ± 0.2 c	5.1 ± 0.6 a	36 ± 2 a	51 ± 1 a	64.2 ± 0.3 ab	3.6 ± 0.7 c	95 ± 1 b
Maize	5.1 ± 0.5 a	61.1 ± 0.1 a	70.07 ± 0.01 a	78.4 ± 0.3 a	0.16 ± 0.03 a	97 ± 1 bc	4.3 ± 0.1 a	38 ± 1 a	50.7 ± 0.8 ab	62.6 ± 0.2 a	2.0 ± 0.2 a	91.6 ± 0.6 a
Rice	10.5 ± 0.2 d	68.9 ± 0.2 b	74.68 ± 0.06 c	80.2 ± 0.2 c	0.98 ± 0.05 b	96.5 ± 0.2 b	5.2 ± 0.6 a	39 ± 1 a	50.4 ± 0.1 a	62.8 ± 0.5 ab	2.3 ± 0.4 ab	98.0 ± 0.1 c

ΔH_{gel}, ΔH_{am-lip}, and ΔH_{ret}: Enthalpy associated to starch gelatinization, dissociation of amylose lipid complex and melting of the recrystallized amylopectin; To_{gel}, To_{ret}: onset temperature of gelatinization and retrogradation peaks; Tp_{gel}, Tp_{ret}, Tp_{am-lip}: Peak Temperature of gelatinization, retrogradation, and amylose-lipid complex dissociation peaks; Te_{gel}, Te_{ret}: endset temperature of gelatinization and retrogradation peaks; First scan: Scan carried out on native (un-gelatinized) sample. Second scan: scan carried out on gelatinized samples after 7 days of storage at 4 °C. Means values with different letters for the same parameter imply significant differences between means at $p < 0.05$.

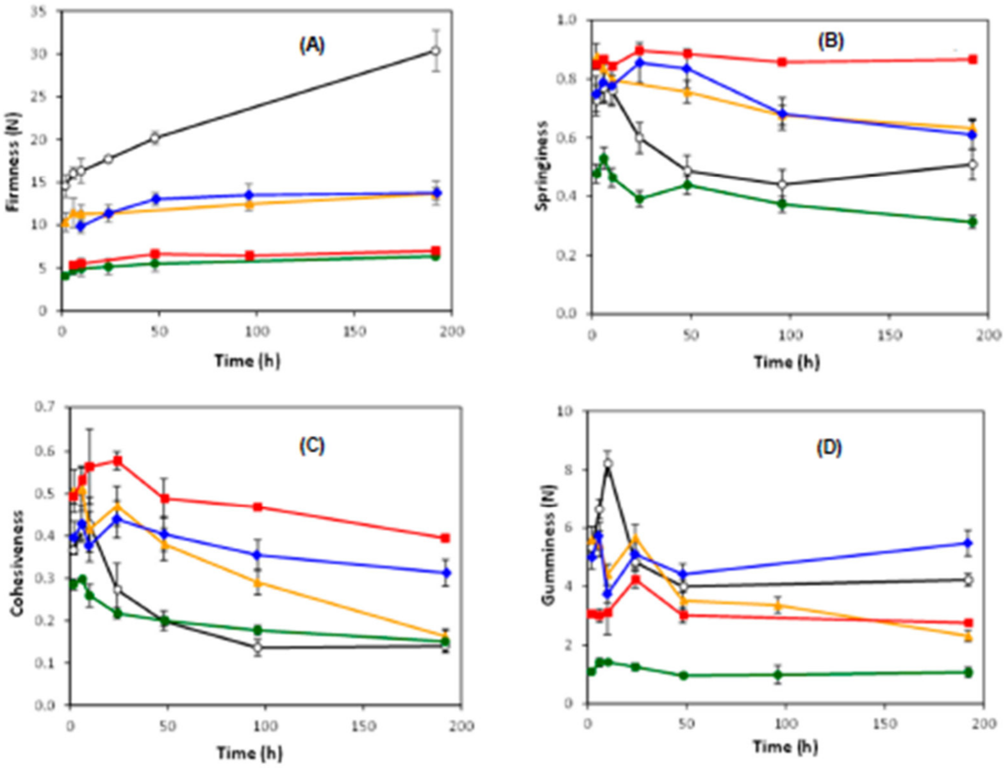

Figure 2. Evolution of firmness (**A**), springiness (**B**) cohesiveness (**C**) and gumminess (**D**) of gels made from fonio (○), millet (▲), sorghum (♦), maize (●) and rice (■) flours at 15% concentration with the storage time at 4 ± 2 °C. The error bars represent the standard deviation.

Springiness is a mechanical textural attribute related to the rapidity and degree of recovery from a deforming force [48]. The initial springiness of fonio gel was lower than those gels made from the remaining flours, except for maize. Gels' springiness decreased significantly ($p < 0.05$) with storage time, except for rice gel which showed a very stable springiness value during the whole studied period. Fonio was the only sample to show some springiness recovery after 100 h, with the value corresponding to 192 h being higher than those determined at 48 and 96 h. In general, the cohesiveness of all gels showed a similar trend to that obtained for springiness, with decreasing values during the studied storage time.

The value of gumminess increased after 6 h and decreased at longer times. The highest value was recorded in fonio and millet gels, but after 192 h, the fonio gel had only lost 21% of this maximum value, while millet lost 59%. This parameter is often used to characterize the necessary energy to disintegrate semisolid foods and is adequately correlated to sensory evaluation by a trained panel [49].

Table 6. Textural parameters of fresh gels (15% flour concentration) and their evolution with storage at 4 °C for 192 h (8 days).

Parameter	Fonio	Millet	Sorghum	Maize	Rice
Firmness (N)					
P_0	15 ± 1 d	11 ± 1 c	12.7 ± 0.4 cd	3.9 ± 0.3 a	6.2 ± 0.1 b
ΔP (%)	109 ± 17 c	27 ± 6 a	9 ± 11 a	65.0 ± 0.6 b	12 ± 4 a
Springiness					
P_0	0.73 ± 0.01 b	0.88 ± 0.01 c	0.75 ± 0.03 b	0.48 ± 0.05 a	0.85 ± 0.01 c
ΔP (%)	−30 ± 10 a	−28 ± 13 a	−7 ± 16 ab	−34 ± 11 a	−2 ± 1 b
Cohesiveness					
P_0	0.37 ±0.01 b	0.51 ±0.04 c	0.40 ± 0.03 b	0.29 ± 0.01 a	0.49 ± 0.02 c
ΔP (%)	−62 ± 4 a	−68 ± 4 a	−21 ± 21 b	−47 ± 9 ab	−20.0 ± 0.7 b
Gumminess (N)					
P_0	5.3 ± 0.6 c	5.60 ± 0.06 c	5.0 ± 0.5 c	1.10 ± 0.03 a	3.08 ± 0.08 b
ΔP (%)	−21 ± 2 ab	−59 ± 8 a	−13 ± 32 b	−13 ± 15 b	−10 ± 2 b
Resilience					
P_0	0.17 ± 0.01 b	0.36 ± 0.05 d	0.21 ± 0.02 bc	0.09 ± 0.01 a	0.23 ± 0.02 c
ΔP (%)	−57 ± 11 a	−75 ± 4 a	−13 ± 33 b	−41 ± 17 ab	−40 ± 5 ab

ΔP = 100·(P_8 − P_0)/P_0, P = textural parameter, P_8 = textural value after 8 d of storage, P_0 = initial textural value. Values with a letter in common in the same row are not significantly different ($p > 0.05$).

4. Conclusions

Fonio flour demonstrated good performance when comparing its properties to other gluten-free sources, showing significant differences in techno-functional, pasting, rheological, and gelling properties. Fonio presented a higher ability to absorb water during gelatinization and swelling in excess water, resulting in the highest determined values of WAI and SP, believed to be influenced by its small starch granule size and high specific surface area. This greater ability to interact with water was also verified in the evaluated gel properties. Fonio had a higher pasting viscosity, as well as high shearing and heating stability and resistance, consistent with a better-packed starch structure than the other analyzed GF flours, as shown by the thermal analysis. Rheological properties revealed that fonio flour generated gels with a remarkably strong structure, particularly at high flour concentrations (12% and 15%). Fonio gels also exhibited notably higher elastic modulus and firmness values than the rest of the GF flours. These combined results lead to the conclusion that fonio has the potential to be used in food, especially in preparations such as porridge, where better gelling properties are appreciated. Therefore, it is suggested that fonio is a promising starch source to compete with other commercially important flours, such as rice, in industrial applications.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/foods11020183/s1, Figure S1: Evolution of the elastic modulus (A), viscous modulus (B), and of the loss tangent (C) of gels.

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References

1. Foschia, M.; Horstmann, S.; Arendt, E.K.; Zannini, E. Nutritional therapy-Facing the gap between coeliac disease and gluten-free food. *Int. J. Food Microbiol.* **2016**, *239*, 113–124. [CrossRef]
2. Alvarez-Jubete, L.; Auty, M.; Arendt, E.K.; Gallagher, E. Baking properties and microstructure of pseudocereal flours in gluten-free bread formulations. *Eur. Food Res. Technol.* **2010**, *230*, 437–445. [CrossRef]
3. Sollid, L.M. Coeliac disease: Dissecting a complex inflammatory disorder. *Nat. Rev. Immunol.* **2002**, *2*, 647–655. [CrossRef] [PubMed]
4. Bernardo, D.; Peña, A.S. Developing strategies to improve quality of life of patients with gluten intolerance in patients with and without celiac disease. *Eur. J. Intern. Med.* **2012**, *23*, 6–8. [CrossRef]
5. Jideani, I.A.; Jideani, V.A. Developments on the cereal grains *Digitaria exilis* (acha) and *Digitaria iburua* (iburu). *J. Food Sci. Technol.* **2011**, *48*, 251–259. [CrossRef]
6. Zhu, F. Fonio grains: Physicochemical properties, nutritional potential, and food applications. *Compr. Rev. Food Sci. Food Saf.* **2020**, *19*, 3365–3389. [CrossRef]
7. Abrouk, M.; Ahmed, H.I.; Cubry, P.; Šimoníková, D.; Cauet, S.; Pailles, Y.; Bettgenhaeuser, J.; Gapa, L.; Scarcelli, N.; Couderc, M.; et al. Fonio millet genome unlocks African orphan crop diversity for agriculture in a changing climate. *Nat. Commun.* **2020**, *11*, 4488. [CrossRef]
8. Barikmo, I.; Outtara, F. Protein, carbohydrate and fibre in cereals from Mali- How to fit the results in a food composition table and database. *J. Food Compos. Anal.* **2004**, *17*, 291–300. [CrossRef]
9. Temple, V.J.; Bassa, J.D. Proximate chemical composition of acha (*D. exilis*) grain. *J. Sci. Food Agric.* **1991**, *56*, 561–563. [CrossRef]
10. Jideani, I.A.; Akingbala, J.O. Some physicochemical properties of acha (*Digitaria exilis*) and iburu (*Digitaria iburua*). *J. Sci. Food Agric.* **1993**, *63*, 369–374. [CrossRef]
11. Ayo, J.A.; Nkama, I. Effect of acha (Digitaria exilis) grain flour on the physico-chemical and sensory properties of bread. *Int. J. Food Prop.* **2004**, *7*, 561–569. [CrossRef]
12. Lasekan, D.O. Chemical composition of acha (*Digitaria exilis*) flour. *J. Food Sci. Agric.* **1994**, *14*, 177–179.
13. Kamran, M.; Saleem, N.; Umer, Z.N. Ready-to-eat (rte) wheat bran breakfast cereal as a high-fiber diet. *J. Food Processing Preserv.* **2008**, *32*, 853–867. [CrossRef]
14. Sartelet, H.; Serghat, S.; Lobstein, A.; Ingenbleek, Y.; Anton, R.; Petitfrare, E.; Aguie-Aguie, G.; Martiny, L.; Haye, B. Flavonoids extracted from fonio millet (*Digitaria exilis*) reveral potential antithyroid properties. *Nutrition* **1996**, *12*, 100–106. [CrossRef]
15. American Association of Cereal Chemists (AACC). *Approved Methods of the AACC Method 44-19*, 10th ed.; AACC: St. Paul, MN, USA, 2000.
16. Abebe, W.; Collar, C.; Ronda, F. Impact of variety type and particle size distribution on starch enzymatic hydrolysis and functional properties of tef flours. *Carbohydr. Polym.* **2015**, *115*, 260–268. [CrossRef]
17. Joshi, D.C.; Sood, S.; Hosahatti, R.; Kant, L.; Pattanayak, A.; Kumar, A.; Stetter, M.G. From zero to hero: The past, present and future of grain amaranth breeding. *Theor. Appl. Genet.* **2018**, *131*, 1807–1823. [CrossRef]
18. Ronda, F.; Pérez-Quirce, S.; Villanueva, M. Rheological Properties of Gluten-Free Bread Doughs: Relationship with Bread Quality. In *Advances in Food Rheology and Applications*; Ahmed, J., Ptaszek, P., Basu, S., Eds.; Elsevier: Cambridge, UK, 2017; Volume 12, pp. 297–334.
19. Villanueva, M.; De Lamo, B.; Harasym, J.; Ronda, F. Microwave radiation and protein addition modulate hydration, pasting and gel rheological characteristics of rice and potato starches. *Carbohydr. Polym.* **2018**, *201*, 374–381. [CrossRef] [PubMed]
20. Ballagoun, V.Y. Sistemes Post-Récolte, Transformation, Qualité du Fonio et Produits Dérivés au Nord du Benin. Ph.D. Thesis, Faculté des Sciences Agronomique, Université d'Abomey-Calavi, Cotonou, Benin, 2013; p. 152.
21. Solaesa, A.; Villanueva, M.; Vela, A.J.; Ronda, F. Protein and lipid enrichment of quinoa (cv. Titicaca) by dry fractionation. Techno-functional, thermal and rheological properties of milling fractions. *Food Hydrocoll.* **2020**, *105*, 105770. [CrossRef]
22. Boucheham, N.; Galet, L.; Patry, S.; Zidoune, M.N. Physicochemical and hydration properties of different cereal and legume gluten-free powders. *Food Sci. Nutr.* **2019**, *7*, 3081–3092. [CrossRef]
23. Gani, A.; Ashwar, B.A.; Akhter, G.; Shah, A.; Wani, I.A.; Masoodi, F.A. Physico-chemical, structural, pasting and thermal properties of starches of fourteen Himalayan rice cultivars. *Int. J. Biol. Macromol.* **2017**, *95*, 1101–1107. [CrossRef] [PubMed]
24. Marson, W.R.; Hoseney, R.C. Factors affecting the viscosity of extrusion cooked wheat starch. *Cereal Chem.* **1986**, *63*, 436–441.

25. Carcea, M.; Acquistucci, R. Isolation and physicochemical characterization of fonio (*Digitaria exilis* Stapf) Starch. *Starch/Stärke* **2006**, *49*, 131–135. [CrossRef]
26. Solaesa, A.; Villanueva, M.; Beltrán, S.; Ronda, F. Characterization of quinoa defatted by supercritical carbon dioxide. Starch enzymatic susceptibility and structural, pasting and thermal properties. *Food Bioprocess Technol.* **2019**, *12*, 1593–1602. [CrossRef]
27. Cornejo-Ramirez, Y.I.; Martinez-Cruz, O.; Lizette Del Toro-Sánchez, C.; Javier Wong-Corral, F.; Borboa-Flores, J.; Javier Cinco-Moroyoqui, J. The structural characteristics of starches and their functional properties. *CyTA-J. Food* **2018**, *16*, 1003–1017. [CrossRef]
28. Singh, S.; Singh, N.; MacRitchie, F. Relationship of polymeric proteins with pasting, gel dynamic-and dough empirical-rheology in different Indian wheat varieties. *Food Hydrocoll.* **2011**, *25*, 19–24. [CrossRef]
29. Kaushal, P.; Kumar, V.; Sharma, H.K. Comparative study of physicochemical, functional, antinutritional and pasting properties of taro (*Colacasia esculenta*), rice (*Oryza sativa*) flour, pigeon pea (*Cajanus cajan*) flour of their blends. *Food Sci. Technol.* **2012**, *48*, 59–68.
30. Acevedo, B.A.; Avanza, M.V.; Cháves, M.A.; Ronda, F. Gelation, thermal and pasting properties of pigeon pea (*Cajanus cajan* L.) dolichos bean (*Dolichos lablab* L.) and jack bean (*Canavalia ensiformis*) flours. *J. Food Eng.* **2013**, *119*, 65–71. [CrossRef]
31. Ballet, S.; Guelpa, A.; Fox, G.; Manley, M. Rapid Visco Analyser (RVA) as a tool for measuring starch- Related Physicochemical Properties in cereal: A Review. *Food Anal. Methods* **2019**, *12*, 2344–2360. [CrossRef]
32. Grace, C.F.; Henry, C.J. The Physicochemical Characterization of Uconventional Starches and Flours Used and Flours Used in Asia. *Foods* **2020**, *9*, 182. [CrossRef]
33. Zhang, G.; Hamaker, B.R. Sorghum (*Sorghum bicolor* L. Moench) Flour Pasting Properties Influenced by Free Fatty acids and Protein. *Cereal Chem.* **2005**, *82*, 534–540. [CrossRef]
34. Shafie, B.; Cheng, S.C.; Gani, A.; Ashwar, B.A. In-vitro digestibility, rheology, structure, and functionality of RS3 from oat starch. *Food Chem.* **2016**, *212*, 749–758.
35. Rao, M.A. Rheology of food gum and starch dispersions. In *Rheology of fluid and Semisolid Foods Principles and Applications*; Rao, M.A., Ed.; Spinger Science + Bussines Media LLC: New York, NY, USA, 2007; pp. 153–214.
36. Ronda, F.; Oliete, B.; Gómez, M.; Caballero, P.; Pando, V. Rheological study of layer cake batters made with soybean protein isolate and different starch sources. *J. Food Eng.* **2011**, *102*, 272–277. [CrossRef]
37. Abebe, W.; Ronda, F. Rheological and textural properties of tef Eragrostis tef (Zucc.) Trotter grain flour gels. *J. Cereal Sci.* **2014**, *60*, 122–130. [CrossRef]
38. Weipert, D. The benefits of basic rheometry in studying dough rheology. *Cereal Chem.* **1990**, *67*, 311–317.
39. Renkema, J.M.S.; van Vliet, T. Concentration dependence of dynamic moduli of heat induced soy protein gels. *Food Hydrocoll.* **2004**, *18*, 483–487. [CrossRef]
40. Clark, A.H.; Ross-Murphy, S.B.; Nishinari, K.; Watase, M. Shear modulus concentration relationships for biopolymer gels. Comparison of independent concentration and cooperative crosslink descriptions. In *Food Polymers, Gels, and Colloids*; Burchard, W., Ross-Murphy, S.B., Eds.; Elsevier: London, UK, 1990; pp. 209–229.
41. Avanza, M.V.; Puppo, M.C. Rheological characterization of amaranth proteins gels. *Food Hydrocoll.* **2005**, *19*, 889–898. [CrossRef]
42. Biiliaderis, C.G.; Juliano, B.O. Thermal and mechanical properties of concentrated rice starch gels of varying composition. *Food Chem.* **1993**, *48*, 243–250. [CrossRef]
43. Solaesa, A.; Villanueva, M.; Munoz, J.M.; Ronda, F. Dry- Heat Treatment vs. Heat_Moisture Treatment assisted by microwave radiation: Techno-functional and rheological modifications of rice flour. *LWT Food Sci. Technol.* **2021**, *141*, 110851110851. [CrossRef]
44. Eliasson, A.C. Interactions between starch and lipids studied by DSC. *Thermochim. Acta* **1994**, *246*, 343–356. [CrossRef]
45. Ji, Y.; Ao, Z.; Han, J.A.; Jane, J.L.; BeMiller, J.N. Waxy maize starch subpopulations whit different gelatinization temperatures. *Carbohydr. Polym.* **2004**, *57*, 177–190. [CrossRef]
46. Lewandowicz, G.; Jankowski, T.; Fornal, J. Effect of microwave radiation on physico-chemical properties and structure of cereal starches. *Carbohydr. Polym.* **2000**, *42*, 193–199. [CrossRef]
47. Jacobson, M.R.; Obanni, M.; BeMiller, J.N. Retrogradiation of starches from different botanical sources. *Cereal Chem.* **1997**, *74*, 511–518. [CrossRef]
48. Di Monaco, R.; Cavella, S.; Masi, P. Predicting sensory cohesiveness, hardness and springiness of solid foods from instrumental measurements. *J. Texture Stud.* **2008**, *39*, 129–149. [CrossRef]
49. Truong, V.D.; Walter, W.M.; Hamann, D.D. Relationship between instrumental and sensory parameters of cooked sweet potato texture. *J. Texture Stud.* **1997**, *28*, 163–185. [CrossRef]

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