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Review on Functional and Rheological Attributes of Kafirin for Utilization in Gluten Free Baking Industry

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Abstract

In the developing countries, the problems related to food safety, huge population and escalating prices of animal proteins give rise to the search of some new exceptional protein sources. In this milieu, agricultural by-products are becoming popular as novel protein source. Sorghum is one of the most significant crops in the world and about 50% of the world's population uses sorghum due to its higher protein content and supply of energy. Further, it has protein content ranges from 13-17% (w/w) which is to some extent higher as compared to other cereals and it has no harm for the patients suffering from gluten intolerance. So, this review is documented on utilization of sorghum protein kafirin in baking industry on the basis of different functional and rheological parameters.

Keywords

Sorghum, Kafirin, Celiac Disease, Functional, Rheological

1. Introduction

Sorghum (Sorghum bicolor) is a significant member of cereal family with more than 35% cultivation directly for human uptake (in semi-arid tropics of Africa and Asia is utilized mainly as a food crop), the rest for industrial products, as alcohol and fodder. Generally, it is more frugal to grow, withstand drought and other environmental strains [10]. Sorghum kernel, just like other cereals, contains three anatomical parts: pericarp, endosperm and germ. Proximate composition of sorghums is affected by environment and genetics; fiber is concentrated in pericarp, whereas protein, fat and ash are abundant in the germ portion. The endosperm is comprised of starch as a major portion along with small amounts of protein, fat and fiber [23]. Seed coat or testa is present beneath the pericarp while certain types of sorghum contain phenols and tannins as their rich component. The endosperm comprises of the aleurone layer, peripheral, and floury areas and it depends upon arrangement of protein and starch. Germ comprises scutellum and the embryonic axis [58].

Protein profile and type differs because of genetics, soil fertility, water availability, environmental conditions and temperature throughout grain maturation. Protein content in sorghum is normally 11-13% but greater values have also been cited [79]. The main fractions of protein present in sorghum are prolamines after glutelins. A characteristic of sorghum protein lacks gluten and cake bread cannot be prepared from sorghum flour.

Sorghum protein is a rich source of essential amino acids necessary for human consumption. Sorghum has the lowest lysine score on the basis of amino acid content but high in total essential amino acids as compared to other cereals. According to finding of [19] the total content of essential amino acids like cysteine and tyrosine was 38.8 (g/16g nitrogen) in sorghum and 33.4 (g/16 g nitrogen) in wheat. The in vivo and in vitro study showed no toxicity of proteins and peptides present in sorghum [20]. Amount of protein in sorghum is very high than that of other cereals because it is more nutritious and contains an important amino acid alanine, which is higher in quantity than that of wheat, corn and rice [12]. The protein content in sorghum ranged from 10.8-13.1% [52].

2. Celiac Disease

Celiac disease (CD) is a multisystemic auto-immune disorder that occurs in genetically susceptible individuals as a response to gluten protein fractions found in wheat, rye, and barley. Samuel Gee, M.D. first described celiac disease's classical features in 1887 as a failure to thrive, diarrhea and lassitude, but it was not until 1953 that Willem Karel Dicke identified wheat, rye, and barley as the cause. Originally, considered to be a uncommon childhood disease, recent improvements in serological testing and public screenings have shown the occurrence of celiac disease in the common population to be 1 in 133 [47].

2.1. Treatment

The only known cure for celiac disease is a lifelong diet free from the protein fractions glutenin, gliadin and hordein found in cereals of the family Triticum and related grains such as barley, triticale and rye [20] (Figure 1). Grains, legumes and pseudocereals are considered gluten-free and safe for celiacs include amaranth, arrowroot, all types of pulses, buckwheat, corn, mesquite, millet, quinoa, rice, sorghum, soy and teff [20]; [31]; [47]. Upon first diagnosis, celiac patients typically suffer from iron, folate and calcium deficiencies due to malabsorption in the small bowel. Secondary lactose intolerance is common in celiac patients due to decreased lactase production from damaged villi in the small intestine. Deficiency of fat soluble vitamins (ADEK) is also common; while after treatment with a gluten-free diet damage to the small intestine heals and serological values return to normal [6].

Figure 1. *Lifelong diet for celiac patients.*

2.2. Gluten-free Bread

Bread is a staple food in many homes; however upon diagnosis with celiac disease traditional wheat based breads are no longer an option for consumers. One of many challenges to individuals with celiac disease is the increased cost of gluten-free foods. On average, gluten-free food is approximately 245% more expensive as compared to their gluten based products [41]; [70]. Further studies showed that 56.5% of participants reported it was difficult to find gluten-free food outside the home, while 75.3% of participants reported that quality of gluten-free products was a significant concern (Leffler *et al.,* 2008). Quality and availability of gluten-free foods to adolescents in schools cause adolescent noncompliance to gluten free diet [75]. Thus appealing gluten-free foods must be available in schools or else for celiacs will be unable to maintain a gluten-free diet [48].

Developing gluten-free breads from whole grain and pseudo cereal flours like buckwheat, flax, quinoa, brown rice, teff, legumes, sorghum and nuts would be an ideal option for improving the nutritional quality and the dietary fiber content of gluten-free breads [59]. Sorghum is an ancient grain and major crop in the United States, which should be significantly less expensive than many of the other ancient grains. It is increasing in availability with some major companies considering its utiliziation in products designed for gluten-free consumers. Sorghum is an ideal grain to be used in gluten-free bread production, as it has no harm for consumption of the celiac patients [53].

Though it has not been widely used for food production in the United States; sorghum has been a staple food in Africa, China, India and other areas because of its robust production under drought conditions and it is free of aflatoxins. White, tan-plant food-grade sorghums have a mild flavor and are ideal for gluten-free breads [72]. Sorghum flour is also commonly suggested as an ingredient in gluten-free cookbooks for individuals who prefer to prepare products at home. Lack of a supply chain in the United States limits its use in commercial gluten- free products. This is being solved with new hybrids that are white with sweet bland flavor. Other sorghums are very high in antioxidants with a wide array of unique levels of flavonoids and rare 3-deoxyanthocyanins. Gluten-free breads made with sorghum require higher water content creating a batter-like system, unlike wheat-based doughs.

3. Sorghum Proteins

Protein is found throughout the caryopsis with approximately 80% in the endosperm, 16% in the germ and 3% in the pericarp for sorghum. Of all the proteins, about half are alcohol soluble proteins known as prolamin. The protein fraction of sorghum was given its name due to it higher amount of proline and glutamine. Kafirin is known as the prolamine of the sorghum. Kafirin is higher in glutamic acid and aspartic acid when compared to zein. Kafirins are hydrophobic proteins located primarily in tightly packed protein bodies found in both the hard and soft endosperm [28].

Four types of kafirins have been identified in grain sorghum. These are α, β and δ- kafirins. Their classification is based on molecular weight, solubility and structure. The α-kafirin is the most prevalent form and is found in the innermost regions of the protein body. B-kafirin and γ-kafirin contain high levels of cysteine that form disulfide bonds. While covalently bonded; these proteins form the dense web of protein material that makes up the majority of the outermost layer of the protein body [79].

In sorghum, the other proteins are in the form of glutelins, albumins, and globulins, which make up enzymes, cell material, and provide assistance in seed structure and plant development. Like most cereal grains, sorghum's limiting amino acid is lysine. Lysine is only present at approximately 45% of the FAO/WHO recommended levels [39].

During the grain development, the kafirins are deposited mainly in the endosperm [66] make protein bodies which bound starch granules [16]. Watterson [78] with his collaborators working on three sorghum varieties with varying hardness and percent vitreousness found that the α-kafirins constituted the highest proportion of the kafirin proteins in sorghum endosperm accounting for 66% to 84% of the total kafirin fraction in the sorghum endosperm. This fraction contained 1 mol cysteine. The β-kafirins accounted for 7-13% of the total kafirins in the sorghum endosperm tissue and contained about 5.8 mol cysteine.

The γ-kafirins accounted for 9-21% of the total kafirin fraction of the sorghum endosperm. Shull [67] while working on purified kafirins, found that γ-kafirins contained about 7 mol cysteine. Due to presence of disulphide bond in the native state of γ-kafirins they were insoluble but soluble in water as reduced subunits form. The δ-kafirins have not yet been well-known at the protein level though it has been suggested that they have lower methionine contents than δ-zeins in maize [22].

The SDS-PAGE was used to analyze the protein profile of the kafirin under reducing conditions [60]. According to Watterson [78] in the vitreous endosperms the α-kafirins are almost 81-83% while in the opaque endosperms it is almost 65-72% of the overall amount of proteins while γ-kafirin has 8-13% and 18-22% in opaque endosperms of the total kafirin fraction.

4. Kafirin Protein and Functional Properties

Wheat is a distinctive cereal grain which contains proteins having ability to form a viscoelastic network in the presence of water and energy input. Viscoelastic materials in the response of applied force show elastic or viscous property. Wheat has ability to form gluten networks which is responsible for the formation of moldable, handleable dough and good quality baked products. Due to ability of wheat gluten to form a good quality viscoelastic dough with higher consumer acceptability, it is tough to find a source which could have the same viscoelastic properties but with no wheat allergies. Presently, very few proteins from the non-wheat sources have been identified to have a good degree of viscoelastic properties. Proteins of the carob germ and isolated maize proteins have both been found to have gluten-like properties [40]; [68].

The physical and chemical properties of sorghum protein kafirin are similar to the main storage protein found in maize such as zein. Because of the similarities between kafirin with zein, it's possible to obtain comparable functional characteristics. Due to the parallels in between kafirin and zein, the characteristics of zein can be used as a model to know about kafirin's functionality [79]. Within these types of tests extracted zein had been combined with starch inside a percentage associated with 10% zein and 90% maize starch to stimulate both prevalent fractions associated with whole wheat flour (protein as well as starch). Whenever combined with water over zein's glass transition temperature (Tg), zein-starch composite flours created wheat-like visco-elastic characteristics. It had been discovered that the visco-elasticity associated with zein had been determined by the Tg (Lawton, 1992). Having properties similar to zein, kafirin can also provide the same visco-elastic activity.

Whenever rheology is tested with farinograph, the dough at 35°C instead of 25 or 30°C creates probably the most desirable visco-elastic dough. Lawton, additionally discovered that not only in whole wheat dough, but also the development associated with viscoelastic zein-starch dough had been determined by the actual improvement of the network associated with proteins fibers. It had been additionally figured that below zein's Tg absolutely no proteins fibers were created and for that reason the viscoelastic dough wasn't formed. To follow the Lawton's study which described the actual conditions essential to create visco-elastic dough through zein as well as starch, Mejia [43] determined the necessary secondary structures associated with zein proteins required to form visco-elastic dough.

The evaluation of the actual functionality associated with kafirin in order to zein had been initially done by [49]. Kafirin could form resins more like zein whenever plasticized along with oleic acid at a level of 50% kafirin (w/w). Even though kafirins with this test could form comparable resins as zein, but the actual proteins were not able to create visco-elastic doughs as defined by (Lawton, 1992). The scientists concluded that commercial zein have just α-zeins based on SDS-PAGE. Kafirins nevertheless, had been found to have both α-kafirin as well as γ-kafirin fractions in the laboratory extracted protein isolate. The scientists further discovered that small fraction of γ-protein is present in each zein and kafirins and is more hydrophobic in nature as compared to α-kafirin and as a result it does not allow proper hydration and plastisization [25].

The very first bread created from visco-elastic zein-starch dough was prepared by [62]. For this purpose, adjustments needed to be made from the initial dough formula as referred by (Lawton, 1992) as well as utilized by other people [43]; [49]. Composition of flour for that zein-starch dough as well as breads had been 20% zein and 80% maize starch. Hydroxypropyl methylcellulose (HPMC) had been additionally included like a functional component. Other elements incorporated were water, yeast like a leavening agent, salt as well as sugar. The dough had been mixed at 40 °C rather than 35 °C [21]. The alterations had been made because of the failure associated with Lawton (1992) zein-starch dough formula in order to keep sufficient gas to create acceptable breads under the conditions of these experiments. HPMC is a surface active hydrocolloid which not just aided along with gas retention, but additionally permitted zein fiber formation from the experiment [43].

Evaluating lab extracted kafirins with the industrial extracted zein proteins in terms of functionality ought to be carried out with extreme caution as the both products were not made in the same way. Preferably, kafirins and zein ought to be isolated in a similar method to provide knowledge about variations within their functionality. The commercially available zein can be used as a model in determining the actual causative elements associated with each zein and kafirin functionality, to ensure the reproducibility of functional proteins isolates produced at laboratory scale. Understanding this study, Schober et al., 2008 discovered exactly how various extraction methods influenced zein as well as kafirin functionality. Relatively α-zein isolated within the laboratory had been discovered to possess a few qualities of commercial zein. Nevertheless, lab extracted kafirins didn't possess the same properties.

To make kafirin functional as commercially available zein, a complete knowledge of viscoelasticity of commercial zeins is required. It's obvious from the previous study that all isolated zein did not match the results in terms of functionality. The commercially isolated zein from maize gluten has been subjected to different procedure like exposures to organic solvents, alkalilne pH and high temperatures [36]. These treatments could possibly alter the proteins which have the role in functionality of zein. Through knowing the reason as well as process behind the functionality associated with remote zein, the procedure for the production of viscoelastic kafirin starch dough can also be achieved. This may be essential for gluten-free market where sorghum proteins might be isolated through flour milling residues or even dried grains from the bio-ethanol industry [77].

The proteins which were extracted and modified for conjugating polysaccharides, probably were a combination of albumins, globulins and γ-kafirins and considered to be water soluble [12]. Even though absolutely no proteins yields had been documented by [7]; kafirin made up about 68-73% from the overall proteins present in sorghum flour [32], therefore just a part of the entire protein within sorghum might have been removed. Nevertheless, the extraction and modification associated with kafirins or even zeins is definitely a fascinating idea and kafirins or even zeins conjugated in order to polysaccharides or some other proteins might have the obvious change in the functionality which could lead to enhanced utilization of sorghum and corn proteins.

Kafirin with oleic acid has ability to form viscoelastic dough like in zein. Each kafirin-resin and resins through industrial zein possess comparable extensional rheological characteristics to whole wheat gluten dough along with higher extensional viscosity and obvious strain hardening. Regardless of the variations within molecular weight and hydrophobicity in between kafirin and zein when compared with whole wheat gluten, the resins associated with kafirin and zein were found to possess comparable extensional rheological properties in sorghum and maize required for the development of baked leavened breads. Functional properties of the protein play significant role in textural attributes of the final product [11].

Use of quality protein isolates furnishes food with rich amino acid profile. Plant proteins, being less expensive than animal source, are used to supplement foods with desirable functional attributes [61]. Textural and functional properties of protein isolates vary with the processing conditions used for extraction and product manufacturing [33].

However, thorough study of protein functionality is necessary for food applications. An important parameter that determines the packaging requirement of a product is the bulk density [65]. Bulk density denotes the conduct of a product in dry mixes and it differs with the excellence of particles. Water and oil absorption properties depend on protein structure and relative proportion of polar and non-polar side chains for hydration and fat binding [45].

Protein structure has both hydrophilic and hydrophobic properties and thereby interacts with water and oil simultaneously in the food system [17]. Water binding ability of proteins not always positively correlates to solubility [2]. Water absorption capacity of bran protein concentrates decreases with the increase in temperature during bran processing whereas oil absorption exhibits an inverse response [54]. Lipoxigenase reduction is not only necessary to check the rancidity but also to keep the gelling property of isolated proteins; residual enzyme affects gelling ability and results in a coarse textured gel [38].

Foaming capacity of protein depends upon its molecular flexibility that is attributed to globular protein structure and surface tension [4]. Foaming properties of bran protein concentrates are analogous with that of casein [15].

Emulsion properties of protein may vary with hydrophobicity, molar mass, conformational stability and physicochemical factors like pH, ionic strength and temperature [37]. Surface hydrophobicity correlates to surface tension and emulsifying properties [50]. Dry heat protein concentrates exhibits 54% emulsion activity whilst more than 50% stability [54]. Emulsion properties of spray dried protein isolates stay better than those obtained by freeze drying as type of drying method influences protein functionality [35]; [71]. Due to the presence of hydrophobic and hydrophilic groups of protein, the formation of emulsions occurs.

5. Rheological Characteristics

Rheology is the branch of science dealing with the deformation and flow of materials [24]. Information of the rheological and mechanical properties of different food systems is important in the scheme of flow processes for quality control, in forecasting storage, stability measurements and in understanding and manipulating texture [56]. Rheological arrangement and measurements have become vital tools in analytical laboratories for characterization of essential materials and end products, to handle process conditions and foresees product performance and consumer acceptance [34]. Rheological properties of dough change during every stage of bread making process. When the dough is mixed in a high speed mixer it is converted into an elastic and coherent mass due to high stress conditions prevailing at this speed [69].

Rheological behavior is associated directly with textural qualities such as mouthfeel, taste and shelf stability [24]. In order to reduce problems arising from large quantities of non-specific use of flours and for the manufacturing of specific end product, full understanding of the protein compositions of flour and the rheological properties of dough is required. Rheological properties of dough, such as farinographic and extensographic properties, reflect the processing adaptability of flours [51].

6. Effect of Kafirin on Dough Rheology

Dough rheological behavior is mainly affected due to protein quantity and quality of flour. The rheological characteristics are the source of information regarding dough handling behavior. Consequently, dough properties play a key role in quality of finished products. Dough rheological properties are influenced by the structure of aggregates and their tendency to interact with each other. Quality and quantity of the proteins affect the water absorption capacity of the dough. Farinograph and mixograph are the important equipments used for measuring the rheological properties of dough during mixing. In the present findings, it is noticeable that protein isolate blends have lower water absorption which produces dough low in moisture which in turn produces dry and stiff breads of poor quality.

Bugusu [13] strengthened the rheological characteristics and breads producing high quality of the sorghum-wheat composite flour dough by supplementing it with zein above its Tg. These researchers concluded that kafirin and zein with almost similar chemical properties have the same reactivities and kafirin if present in the dough can be precipitated in the dough development process just like zein. The purpose of this particular process has been to find out whether kafirin offers viscoelastic features within dough-like mechanism and to evaluate its dough developing qualities along with zein and whole wheat gluten.

Variation in water absorption depends upon protein quality and damage starch contents. Similarly protein isolates fortified blends have less development time as compare to wheat flour [29]. Dough stability increases with the storage of flour samples, might be attributed to the difference in the protein contents and quality [64]. Differences in farinographic characteristics may be due to variations in protein quality and quantity [27].

The mixograph and amylograph quantify the physical properties of dough by recording the resistance of dough to mix. Protein isolates blends owing to differences in their protein quality and quantity exhibited significant variation in the mixing time [26]. Butt [14] reported that peak height in amylograph and mixograph of different wheat varieties ranged from 43-65%. Rasool [57] reported that incorporation of non-wheat plant materials results in decreased peak height of composite flours. The variations in results could be attributed in utilization of local varieties, environmental factors like temperature fluctuations, soil characteristics and the other contributory features.

7. Food Applications of Sorghum

Soghum had been used as an ingredient for the production of food in late 1970's when Nigerians produced a fermented cereal porridge named ogi; lately, it was used by Sudanese to make fermented weaning food known as Nasha. Before 2000, it was mainly being used for the production of porridges, tortillas, roti/chappati (mostly used in India and Pakistan as a staple diet), ready to eat breakfast cereals and cookies. Recently, several studies have found its role to form a gluten free food products and it is capturing its market as a cheapest source of food for gluten free food industry. Table 1 shows the use of sorghum in production of different food products.

Table 1. *Type of food prepared from Sorghum.*

	Food product name	Reference
A Nigerian fermented cereal porridge	OGI	[9];[3]; [1]
Bakery	Cookies	[8]
A traditional Sudanese fermented sorghum weaning food	Nasha	[30]
Breakfast food	Thin porridges	[63]
Cooked	Tortillas	[73]
Snacks	Ready-to-eat breakfast cereals	[76]
Leavened product	Chapatti/roti (India)	[46]
Extruded food	Pre-cooked pasta	[18]
Gluten-free foods	Sorghum breads and pasta	[5]
Setting agents	Pasta, crackers, pizza crust, and leavened bread	[44]
Chinese food	Noodles from decorticated sorghum flour	[42]
Functional food	Ready-to-eat sorghum-cowpea African porridge	[74]
Baking food	Sorghum biscuits	[55]

8. Conclusions

Presently, sorghum is mostly consumed as animal feed due to some unacceptable attributes but the processing treatments like fermentation, germination and other heat treatments could help in improving the protein digestibility. This review provides an insight about the use of kafirin in the production of baking products. Sorghum not only has a plenty of protein but also it is gluten free. Further, the functional and rheological attributes of kafirin makes it a valuable ingredient for the production of a food which is rich in protein and gluten free. Further research is needed to increase the utilization of sorghum protein at industrial scale to provide a cheaper but nutritious diet.

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