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Molecular features of fermented and sprouted sorghum flours relate to their suitability as components of enriched gluten-free pasta

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ABSTRACT

Enrichment with other components may improve the textural and nutritional characteristics of rice-based pasta, often used in gluten-free diets. In this work, formulations based on enrichment with sorghum flour were tested. Sorghum flour was used in the absence of treatments, after fermentation by lactic acid bacteria, or was prepared from sprouted grains. Both fermentation and sprouting affected sorghum proteins, but with different mechanisms. Different proteins were affected by fermentation and sprouting, that also had a different impact on the thiol/disulfide balance. Sprouting, but not fermentation, resulted in significant breakdown of sorghum starch. As a result of these modifications, it was not possible to prepare acceptable rice-based pasta upon enrichment with sprouted sorghum flour. Conversely, fermented sorghum flour gave pasta with improved cooking properties with respect to both rice-only pasta and to pasta enriched with untreated sorghum flour.

1. Introduction

Grains and pseudo-grains that do not contain gluten proteins, such as sorghum, teff, amaranth, millet, quinoa, and buckwheat are attracting the interest of consumers outside their country of origin. In the Western world, foods prepared from these “alternative grains” are regarded as niche products associated with specific or generic health benefits, and are seen as fashionable because of their association with traditional ethnic foods (Jones, 2013) and with very ancient food habits (Guyot, 2010). Some of these crops are used after a biotechnological pre-treatment of grains or flours – usually fermentation or sprouting – in order to improve flavor, structure, and stability of baked goods (Guyot, 2010; Hugo, Rooney, & Taylor, 2003). The use of fermentation has significant potential for improving the nutritional quality and the health effects of foods and food ingredients (Katina et al., 2005).

Sorghum is one of the main staples for the world's poorest and most food-insecure people, especially in the most arid and marginal sub-tropical areas. Cereal-based tropical fermented foods do not have market significance in industrialized countries (Guyot, 2010), in spite of their nutritional value, also because of processing issues. From a nutritional standpoint, sorghum represents an excellent source of proteins and of antioxidant compounds (Taylor, Belton, Beta, & Duodu, 2014). Also, the release of sugars from sorghum starch is slower than in other cereals, which is of interest for diabetic or obese people (Taylor & Emmambux, 2010).

The overall digestibility of sorghum is low because starch and proteins are associated in compact complexes, and a pre-fermentation step is often used in the preparation of various foods and beverages from sorghum (Elkhalifa & El Tinay, 1995; Elkhalifa et al., 2006; Hassan & El Tinay, 1995). Various approaches – including malting, fermentation, intrinsic amylases and proteases, and of enzymes of microbial origin – have been investigated as for increasing starch and protein digestibility in sorghum (Correia, Nunes, Barros, & Delgadillo, 2010).

There is also an increasing interest in using sorghum as a gluten-free ingredient, either alone or in combination with other non-gluten cereal flours (Asif, Rooney, Acosta-Sanchez, Mack, & Riaz,

Abbreviations used: RF, rice flour; UF, unfermented sorghum flour; FF-4h, sorghum flour after 4 h of fermentation; FF-8h, sorghum flour after 8 h of fermentation; SF, sprouted sorghum flour; RP, brown rice pasta; UP, unfermented sorghum-enriched pasta; FP, fermented sorghum-enriched pasta; SP, sprouted sorghum-enriched pasta; DTT, dithiothreitol; DTNB, 5,5′-dithiobis-(2-nitrobenzoate); BU, Brabender Units; CU, Ceralpha Units.

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2010; Taylor, Schober, & Bean, 2006). However, formation of a gluten-like protein network is strongly impaired when using untreated sorghum flour as the predominant component of the mixture (Marti & Pagani, 2013). The major protein fractions in sorghum are globulins and prolamins, that typically are present as compact aggregates in protein bodies surrounding the starch granules. This peculiar structure makes a pre-treatment of sorghum flour almost necessary to facilitate transformability into either the common foods consumed in the Countries of origin, or in foods closer in their appearance to those consumed in the Western world.

In view of using modified sorghum flour for producing gluten-free pasta containing nutritionally significant amounts of sorghum, this study addresses: *i)* the nature and extent of starch and protein modification occurring during either sorghum germination or fermentation, and *ii)* the effects of these molecular changes on parameters that may have practical relevance for processing purposes and on some markers of pasta quality.

2. Materials and methods

2.1. Sorghum flours

A tannin-free white sorghum cultivar (Tabat) was obtained from the Food Research Center, Shambat, Sudan. Sorghum was fermented in Sudan as described by Elkhalifa et al. (2006). Sorghum (1 kg) was mixed with 2 L of water in a round earthenware container, and 300 g of a previously fermented sourdough (300 g) was added to the mixture. At given fermentation times (4 or 8 h at 35–37 °C) the samples were placed in a hot air oven (Heraeus UT 5042, Germany), dried at 56 °C for 16 h, and ground into flour (FF) in a hammer mill to pass through a 0.4 mm screen. The same milling procedure was used to prepare flour from un-fermented sorghum (UF).

Sorghum grain was sprouted in the dark for three days as described by Elkhalifa and Bernhardt (2010). After a given sprouting time the samples were dried in a hot air oven and ground into flour as above. Flours (SF) were stored in polyethylene bags at 4 °C.

2.2. Pasta samples

Sorghum-enriched pasta samples were prepared by adding 15 g of each of the various sorghum flours to a mixture made of 60 g of parboiled brown rice flour (Riso Viazzo s.r.l., Crova, Italy) and 25 g of pre-gelatinized rice flour (Molino Favero, Padova, Italy; amylose content 0.265 g (g total starch)$^{-1}$)for 100 g of the final product. A rice-only pasta sample was prepared from a 3/1 mixture of parboiled brown rice flour and pre-gelatinized rice flour. Brown rice was used to improve the fiber content of the products. A total of four pasta samples were therefore available: a control, rice-only pasta (RP), and rice-based pasta containing 15% of untreated sorghum flour (UP), of sprouted sorghum flour (SP), or of fermented sorghum flour (FP). Thus, 100 g of all the sorghum-containing samples contained 25 g of pregelatinized rice flour, 60 g of parboiled brown rice flour, and 15 g of sorghum-derived flour.

Pasta was prepared in the pilot plant at DeFENS. Flour mixtures and water were blended to a final moisture content of 40%, and formed by into macaroni shape (7 mm external diameter) in a lab scale extruder (20 kg h^{-1}; MAC 30, Italpast, Parma, Italy), keeping the extrusion temperature at 50 °C. Pasta was dried using a low-temperature drying cycle (50 °C for 14 h). All pasta samples were stored at room temperature, and ground – when required – to a 0.5 mm particle size with a laboratory mill (IKA Universalmühle M20, Staufen, Germany) prior to analysis. The mill was fitted with a water-cooled jacket to avoid overheating.

2.3. Chemical analysis

Analyses were performed according to AOAC (2005) for moisture (934.01), protein (960.52), ash (942.05). Total carbohydrates were calculated by difference. All analytical data are from triplicate determinations on two sets of materials. Total titratable acidity was determined on 10 g of sample homogenized with 90 mL of distilled water and expressed as the volume of 0.1 mol L^{-1} NaOH to get a pH of 8.5. The pH value was determined by a Crison GPL22 pH meter (Crison Instruments, Alella, Barcelona, Spain).

2.4. Protein solubility and thiol accessibility

Protein solubility in native and denaturing conditions was determined by suspending 0.5 g of a finely ground sample in 10 mL of 0.05 mol L^{-1} sodium phosphate buffer, pH 7.0, containing 0.1 mol L^{-1} NaCl, and 8 mol L^{-1} urea or 8 mol L^{-1} urea and 0.01 mol L^{-1} dithiothreitol (DTT) when indicated. Suspensions were stirred for 30 and 60 min at 25 °C. After centrifugation (10,000 × g for 20 min, 20 °C) the amount of protein in the supernatant was determined by a dye-binding method (Bradford, 1976) using bovine serum albumin as a standard. Results are expressed as mg proteins (g sample)$^{-1}$.

Accessible –SH groups were measured by suspending 0.5 g of finely ground sample in 10 mL of 0.05 mol L^{-1} sodium phosphate buffer, pH 6.8, containing 0.1 mol L^{-1} NaCl and 0.2 mmol L^{-1} 5,5′-dithiobis(2-nitrobenzoate) (DTNB). After 15 min at room temperature, insoluble material was removed by centrifugation at 12,000 × g for 10 min at 15 °C, and the absorbance at 412 nm of the supernatant was read against a DTNB blank (Barbiroli et al., 2013; Cabrera-Chavez et al., 2012). Total accessible thiols were measured according to the same protocol, but adding 8 mol L^{-1} urea to the DTNB-containing buffer.

2.5. SDS-PAGE

The protein profile in the various samples under the extraction conditions mentioned above was analyzed by SDS-PAGE in a 12% gel after denaturation in the presence of 2-mercaptoethanol using a MiniProtean Apparatus (BioRad, Richmond, VA) as described in previous studies (Barbiroli et al., 2013; Cabrera-Chavez et al., 2012). Gels were stained with a silver staining kit (BioRad, Richmond, VA, USA). Sample volumes were adjusted to load 0.01 mg of protein per lane. Low molecular weight markers (Amersham Biosciences, Amersham, UK) were used for calibration.

2.6. Starch pasting properties

Alpha-amylase activity was determined at least in duplicate according to AACC standard n. 303, by using the Megazyme Amylase Assay Procedure (Megazyme International Ireland Ltd., Bray Business Park, Bray, Co. Wicklow, Ireland). Pasting properties were measured in triplicate in a Brabender Micro-Visco-AmyloGraph (Brabender, Duisburg, Germany) (Marti, Seetharaman, & Pagani, 2010), on samples ground to particles smaller than 0.5 mm.

2.7. Pasta color analysis

A reflectance color meter (CR 210, Minolta Co., Osaka, Japan) was used to measure the lightness and saturation of the color intensity by utilizing the CIE-LAB uniform color space procedure. CIE-LAB-System color values L*, a*, and b*, that measure lightness, redness–greenness, and yellowness–blueness, respectively, were