Food Chemistry 233 (2017) 1–10

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Physicochemical properties and starch digestibility of whole grain sorghums, millet, quinoa and amaranth flours, as affected by starch and non-starch constituents

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ARTICLE INFO

Article history:
Received 17 November 2016
Received in revised form 16 March 2017
Accepted 4 April 2017
Available online 6 April 2017

Keywords:
Cereals
Pseudocereals
Starch
Whole grain
Physicochemical property

ABSTRACT

Minor grains such as sorghum, millet, quinoa and amaranth can be alternatives to wheat and corn as ingredients for whole grain and gluten-free products. In this study, influences of starch structures and other grain constituents on physicochemical properties and starch digestibility of whole flours made from these grains were investigated. Starches were classified into two groups according to their amylopectin branch chain-length: (i) quinoa, amaranth, wheat (shorter chains); and (ii) sorghum, millet, corn (longer chains). Such amylopectin features and amylose content contributed to the differences in thermal and pasting properties as well as starch digestibility of the flours. Non-starch constituents had additional impacts; proteins delayed starch gelatinization and pasting, especially in sorghum flours, and high levels of soluble fibre retarded starch retrogradation in wheat, quinoa and amaranth flours. Enzymatic hydrolysis of starch was restricted by the presence of associated protein matrix and enzyme inhibitors, but accelerated by endogenous amylolytic enzymes.

1. Introduction

Worldwide consumption of products containing whole grain has grown continuously due to increasing awareness of their health-promoting benefits (Marquart, Jones, Chohen, & Poutanen, 2007; Mintel, 2011; Schaffer-Lequart et al., 2015). Whole grain flour is more nutritious than refined flour because higher contents of dietary fibres, micronutrients and phytochemicals from bran and germ fractions are retained. Currently, common cereals such as wheat and corn are used as ingredients for production of most whole grain products. Gluten protein fraction in wheat, however, cannot be tolerated by persons suffering from celiac disease, and although corn is considered to be safe for celiac patients, it lacks some essential nutrients (Hager, Wolter, Jacob, Zannini, & Arendt, 2012).

Recently, interest in the development for whole grain and gluten-free products has been growing, and the lesser known grains such as sorghum, millet, quinoa and amaranth are postulated as promising alternatives for this purpose (Alvarez-Jubete, Arendt, & Gallagher, 2010; Taylor & Emmambux, 2008). Research has shown that these minor grains are rich in several phytochemicals that exhibit antioxidant and free-radical scavenging activity (Taylor, Belton, Beta, & Duodu, 2014). For centuries, these grains are important staple food crops in Africa, Asia and South America. In these regions, whole flours made from these grains are also used as substrates for producing a wide variety of fermented foods and beverages (Hammes, Brandt, Rosenheim, Seitter, & Vogelmann, 2005; Taylor & Emmambux, 2008). Sorghum and millet are well adapted to drought and harsh climate in the arid areas where other crops grow poorly. Pseudocereals such as quinoa and amaranth are known for their superior protein quality containing a high level of lysine that is limited in cereals. The United Nations recognizes quinoa as the alternative food source in fighting against hunger and food insecurity (FAO, 2013). In modern food processing, these grains can be used either as a basic raw material or as admixtures which improve texture, sensory attributes and nutritional value.

Although these minor grains are viewed as promising ingredients for whole grain-based products, their current utilization in modern food industry is still limited by availability, price, insufficient product development and research efforts. Due to the fact

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http://dx.doi.org/10.1016/j.foodchem.2017.04.019
0308-8146/

that these minor grains differ in several important respects from each other and from the common cereals, a sound fundamental knowledge on their grain characteristics and technological properties is required to promote their industrial use. Starch is present as a major component in most cereal and pseudocereal grains. It plays critical roles on technological properties of cereal flours through its physicochemical transformation and interaction with other ingredients. For example, viscosity of starch is one of the key sensory drivers for cereal beverages and porridges. In extruded cereals and snacks, starch constitutes a continuous amorphous phase, and other grain components such as proteins and fibres impact the continuity of the starch phase. In pasta, noodles and baked goods, starch granules represent the discontinuous phase because they are distributed within a continuous gluten network (Robin & Palzer, 2015). In gluten-free product, starch plays important role in providing structure, texture and stability to final products. In cereal fermentation, the enzymatic conversion of starch plays a key role in determining primary fermentation substrates (Hammes et al., 2005). In addition to starch, technological properties of whole grain flour are also affected by flour preparation, dietary fibres, lipids, micronutrients and phytochemicals.

Improving sensorial and textural properties of whole grain products is a major challenge for the food industry (Robin & Palzer, 2015; Schaffer-Lequart et al., 2015). Increased knowledge of structure-function relationship is essential to overcome this challenge, especially for minor cereals and pseudocereals of which less is known. Previous studies mainly focused on chemical composition and direct use of these grains in food formulation to evaluate changes in nutritional and textural properties of final products (Alvarez-Jubete et al., 2010; Hager et al., 2012). Research that aims to understand roles of starch structures and other grain constituents on physicochemical properties of whole grain flours is scarce. In this study, chemical composition, structural features of isolated starch, thermal and pasting properties and starch digestibility were determined for whole grain flours made from sorghum, millet, amaranth, quinoa, in comparison with those of wheat and corn. The characteristics of whole grain flours are discussed in relation to starch structures, and non-starch constituents.

2. Materials and methods

2.1. Materials

Grains of white and red varieties of sorghum (*Sorghum bicolor*), millet (*Pennisetum glaucum*) and corn (*Zea mays* L.) were obtained from Nestlé Research and Development Center in Abidjan, Ivory Coast. Amaranth (*Amaranthus caudatus*) and quinoa (*Chenopodium quinoa*) grains were purchased from Mokamo Agroexportación, Lima, Peru. According to the supplier, saponin was previously removed from quinoa grains by washing with water. Wheat grains (*Triticum aestivum*) were obtained from Grands Moulins de Cossonay (Cossonay, Switzerland). Grains were manually cleaned from impurities, and broken grains were removed. The grains were stored at 4 °C and used within 30 days of receipt. Whole grain flour (WGF) was prepared freshly on the day of analysis by dry-milling the grains through a 0.5 mm screen using a sample mill (Cyclotec-1093, Tecator, Sweden). Average moisture contents (% of fresh weight) of WGFs were determined using Halogen moisture analyzer (Mettler Toledo HR73, Volketswil, Switzerland) at 160 °C.

Porcine pancreas α-amylase (E.C. 3.2.1.1) (A6255 type I-A approx. 1050 units/mg protein in saline solution, 29 mg proteins/ml), pepsin from gastric porcine mucosa (P7000) and protease type XIV isolated from *Streptomyces griseus* (P5147) were purchased from Sigma-Aldrich Chemie GmbH (Buchs SGSwitzerland). Amyloglucosidase from *Aspergillus niger* (EC 3.2.1.3., 3300 units/ml on soluble starch, stabilised liquid in 50% (v/v) glycerol, 0.02% sodium azide) and isoamylase from *Pseudomonas sp.* (EC 3.2.1.68, 500 U/ml in ammonium sulphate suspension, 0.02% sodium azide) were purchased from Megazyme International Ireland Ltd. (Wicklow, Ireland). Glucose assay kit (Wako Pure Chemical Industries, Ltd, Osaka, Japan) based on enzymatic method (Mutarotase-GOD) was purchased from IGZ Instruments AG (Zürich, Switzerland). All chemicals were reagent grade and obtained from Sigma–Aldrich Chemie GmbH (Buchs SGSwitzerland).

2.2. Grain composition

Total starch, damaged starch and phytic acid contents of WGF were determined using enzymatic kits (K-TSTA 07/11 with KOH procedure, K-SDAM 07/11, K-PHYT 12/12, respectively, Megazyme Ltd.). Protein content was calculated from a sum of total amino acids determined by ion-exchange liquid chromatography following the AACC 07-01 and 07-11 methods. Total fat content was determined using a Soxhlet extraction. Total, soluble and insoluble dietary fibres were determined according to the AOAC 991.43 and AOAC 2011.25 methods. The AOAC 991.43 employs the use of thermo-stable α-amylase at 95–100 °C, pH 8.2. Under this method, some parts of resistant starch and non-digestible oligosaccharides (NDO) are excluded. In the AOAC 2011.25, starch is hydrolyzed by pancreatic α-amylase at 37 °C, pH 6.0 to avoid underestimation of resistant starch, whereas quantification of NDO are performed using HPLC (McCleary, Sloane, Draga, & Lazewska, 2012). Total phenolic content was determined by Folin-Ciocalteau assay using catechin as a standard (Chiremba, Taylor, Rooney, & Beta, 2012). Ash content was determined as a weight loss by high temperature incineration (580 °C). For all assays, determinations were made in duplicate.

2.3. Isolation of starch

Starch was isolated from the grains according to the alkaline-protease wet milling procedure (Song & Jane, 2000). Residual proteins were removed by suspending starch pellet in 0.1 M NaCl solution containing 12% v/v toluene under mechanical stirring for 1 h, and allowed starch to settle. This step was repeated until the toluene layer was clear. The resulting starch pellet was washed three times with deionized water, twice with absolute ethanol and dried in a convection oven at 35 °C for 36 h. Isolated starch was stored in a desiccator at room temperature. Starch purity was >95% dry basis determined using the total starch kit.

2.4. Scanning electron microscopy

Isolated starches and starch granules embedded in cereal endosperms and pseudocereal perisperms were observed under a scanning electron microscope (SEM). For the latter, grains were frozen in liquid nitrogen and fractured along the transverse axis using frozen hammer or blade. Samples were mounted on aluminum stubs using a conductive carbon tape. The samples were sputter-coated with a 5 nm layer of gold using a sputter coater SCD-05 (Leica Microsystems, Wetzlar). The ultrastructure was visualized in the Quanta 200F FEG microscope (FEI company, Eindhoven) operated at 8–10 kV in low vacuum mode using a secondary electron detector.

2.5. Amylose content and amylopectin branch chain-length distribution

Amylose content of isolated starch was determined by the lectin concanavalin A (ConA) method using the assay kit (K-AMYL 07/11,