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Effects of processing method and solute interactions on pepsin digestibility of cooked proso millet flour

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ABSTRACT

Previous studies have reported a substantial decline in in vitro digestibility of proso millet protein upon cooking. In this study, several processing techniques and cooking solutions were tested with the objective of preventing the loss in pepsin digestibility. Proso millet flour was subjected to the following processing techniques: high pressure processing (200 and 600 MPa for 5 and 20 min); germination (96 h); fermentation (48 h); roasting (dry heating); autoclaving (121 °C, 3 h), and treatment with transglutaminase (160 mg/g protein, 37 °C, 2 h). To study the interaction of millet proteins with solutes, millet flour was heated with sucrose (3–7 M); NaCl (2–6 M); and $CaCl_2$ (0.5–3 M). All processing treatments failed to prevent the loss in pepsin digestibility except germination and treatment with transglutaminase, which resulted in 23 and 39% increases in digestibility upon cooking, respectively, when compared with unprocessed cooked flours. Heating in concentrated solutions of sucrose and NaCl were effective in preventing the loss in pepsin digestibility, an effect that was attributed to a reduction in water activity (a_w). $CaCl_2$ was also successful in preventing the loss in digestibility but its action was similar to chaotrops like urea. Thus, a combination of enzymatic modification and cooking of millet flour with either naturally low a_w substances or edible sources of chaotropic ions may be useful in processing of proso millet for development of novel foods without loss in digestibility. However, more research is required to determine optimum processing conditions.

1. Introduction

In order to promote proso millet (*Panicum miliaceum*) as human food, our previous work focused on quality of proso millet storage proteins, panicins. Unexpectedly, we found a drastic decline in digestibility of panicins upon cooking due to formation of hydrophobic aggregates (Gulati et al., 2017). This discovery could prove to be a stymie in the promotion of proso millet as human food. Hence, it is important to explore strategies that could prevent the observed decline in digestibility of proso millet flour.

Potential processing methods that would not result in poor protein digestibility could be focused on either inhibiting the formation of the hydrophobic aggregates, or breaking of the hydrophobic aggregates after they are already formed. The formation of the hydrophobic aggregates may be inhibited either by modifying the structure of proteins prior to cooking or by creating an unfavorable environment for their formation during cooking. Hydrophobic aggregates may be broken after being formed by external stress like high pressure.

Several processing techniques [e.g., roasting, autoclaving, high pressure processing (HPP), fermentation, germination] are known to improve protein digestibility, functionality, and flavor of cereals by modifying protein structure (Hugo, Rooney, & Taylor, 2003; Poutanen, Flander, & Katina, 2009; Tiwari & Awasthi, 2014). HPP and autoclaving have been useful in either breaking protein aggregates or making them more soluble by disrupting the ionic and hydrophobic forces that are essential for the tertiary and quaternary structure of proteins (Galazka, Dickinson, & Ledward, 2000). Germination and fermentation, on the other hand, result in proteolysis and denaturation of storage proteins by intrinsic or microbial enzymes, respectively, which may change protein digestibility (Ganzle, Loponen, & Gobbetti, 2008; Ghumman, Kaur, & Singh, 2016; Hammes et al., 2005; Li, Oh, Lee, Baik, & Chung, 2017; Szewińska, Simińska, & Bielawski, 2016). Among fermented cereal products, sourdoughs formed by lactic acid bacteria and yeast fermentation of cereal flour slurries are widely recognized for imparting changes in cereal starches and proteins that result in a product with improved structure and stability (Poutanen et al., 2009).

Enzymes can also modify protein structure by crosslinking. Transglutaminase (TGase) is an enzyme approved for food processing

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that catalyzes the formation of an iso-peptide bond between the ε-amine group of lysine and the γ-amide group of glutamine residues (Motoki & Seguro, 1998). TGase has been used either to crosslink proteins (Nonaka, Sakamoto, Kawajiri, Soeda, & Motoki, 1992) or create internal covalent crosslinks (Renzetti, Behr, Vogel, & Arendt, 2008). Hassan, Osman, and Babiker (2007) observed that TGase treatment of pearl millet prolamins were able to resist heat-induced aggregation. In our study, we hypothesized that TGase could crosslink panicins and prevent hydrophobic aggregation upon cooking and avert the observed loss in digestibility.

Protein aggregation can also be inhibited in the presence of co-solutes (e.g., salts and sugars). These compounds can create an unfavorable environment for aggregate formations by either reducing the water activity or interacting with hydrophobic amino acids and preventing them from associating (Ohtake, Kita, & Arakawa, 2011).

Thus, the objective of this research was to identify techniques that would result in high digestibility of proso millet protein after processing. The above processing strategies to either break hydrophobic aggregates after they are formed or to prevent the formation of the aggregates altogether were tested.

2. Materials and methods

2.1. Samples

Commercially available dehulled proso millet grains were obtained from Clean Dirt Farms (Sterling, CO, USA) and milled using a cyclone sample mill (UDY, Fort Collins, CO, USA) fitted with a screen size of 1 mm. The milled flour had a mean particle size of 374 μm (American Society of Agricultural and Biological Engineers, 1992). The flour was kept under refrigerated conditions until further analysis. The flour was analyzed for moisture, fat, and ash content using approved methods (AACC International, 1999a, 1999b, 1999c, 1999d, 1999e). Protein content was analyzed using a nitrogen analyzer (FP 528, Leco, St. Joseph, MI, USA) with a protein factor of 6.25. Total starch content was analyzed using a total starch assay kit (K-TSTA, Megazyme, Bray, Ireland) following the KOH format. Whole proso millet grains (variety: sunrise) used in the study were grown at Scottsbluff, Nebraska, USA.

2.2. Preliminary heating in water

Four hundred milligrams of proso millet flour were dispersed in 10 mL of water and heated from 25 °C to 100 °C in 15 °C increments on a magnetic stir plate with constant stirring. The temperature was recorded constantly during heating and once the temperature was reached the flasks were cooled immediately over ice and the time required to reach the respective temperature was recorded. In vitro pepsin digestibility was measured on cooled samples.

2.3. In vitro pepsin digestibility

Pepsin digestibility was measured using the residue method developed by Mertz et al. (1984) with modifications as reported by Gulati et al. (2017). This method measures changes in solubility of cereal proteins, which are normally largely insoluble, after hydrolysis with pepsin, and matches closely with in vivo digestibility of cereal proteins in humans. If samples were to be cooked, 400 mg of flour or treated sample was dispersed in 10 mL water and heated at 100 °C for 20 min after accounting for time required to reach the temperature (see Section 2.2). Uncooked samples (400 mg) were dispersed in 10 mL of water and not subjected to cooking. To the cooked or uncooked samples, 60 mL of phosphate buffer (pH:2) containing pepsin (1.5 mg/mL) were added and digested for 2 h at 37 °C with intermittent mixing. After 2 h, the reaction was stopped by adding 4 mL of 2 N NaOH and contents of the flask were centrifuged and pellet washed twice with deionized water. After digestion, the pellet was oven dried at 40 °C overnight and used to measure pepsin digestibility according to the following equation: Pepsin digestibility (%) = $[(N_i - N_f)/N_i] \times 100\%$, where N_i was the total concentration of N in the sample before digestion and N_f was the concentration of N in the recovered pellet after digestion.

2.4. Processing of millet flour and grains

Different processing techniques that may either break the heat-induced hydrophobic aggregates formed in millet proteins or prevent their formation by protein structure modification were tested. The processing techniques tested in this research included both high temperature processing (i.e., autoclaving) and non-thermal processing (i.e., HPP, fermentation, germination). Depending on processing technique, heating of the sample to test for changes in pepsin digestibility upon cooking was performed before, during, or after the processing step was completed as described in this section. Unprocessed millet flour was used as a control for all processing treatments.

2.4.1. Autoclaving

Four hundred milligrams of proso millet flour was dispersed in 35 mL of water in a flask, covered, and autoclaved at 120 °C for 3 h. The samples were cooled to room temperature and analyzed immediately for pepsin digestibility.

2.4.2. Sourdough fermentation

Sourdough bread was made according to a modification of the 'non-conventional flour' sourdough bread procedure described previously using a spontaneous or natural microflora culture approach (Coda, Rizzello, & Gobbetti, 2010). De-hulled millet flour (100 g) was mixed with water (100 g), covered, and allowed to stand at room temperature for 24 h, whereupon fresh flour (100 g) and water (100 g) were added and the mixture was allowed to stand for another 24 h. One hundred grams of this slurry was retained and fresh flour (100 g) and water (100 g) were added and fermented for another 24 h. This step was repeated twice more before the sourdough starter was ready to be used. To prepare the bread dough 75 g of sourdough starter was mixed at speed 2 in a Kitchen Aid stand mixer (Benton Harbor, MI) for 5 min with 150 g millet flour, 75 g water, 3 g salt, 22 g sugar, and 10 g oil. The high load of sourdough starter was added because we considered that adding a higher load of sourdough would lead to the greatest changes in pepsin digestibility. Batters were deposited directly into 20 × 10 cm greased loaf pans, covered, and proofed for 4 h at room temperature before baking at 190 °C for 25 min. After cooling, the bread was sliced, frozen, and freeze-dried before measuring pepsin digestibility without further cooking.

2.4.3. High pressure processing

Millet flour slurry in water (1:10 w/v) was vacuum sealed (Impulse sealer, Wu-Hsing Electronics, Ltd., Taiwain) in a bag and then placed in a new bag and vacuum sealed again at 100 mbar (Model C200, Multivac Inc., Kansas City, MO). The double bagged samples were further placed in another vacuum pouch and sealed to avoid any contamination or leakage (VacMaster VP215, Ovaerland Park, KS). Some samples were heated (100 °C, 20 min) prior to vacuum sealing and others were not. The triple bagged samples were then subjected to either 200 MPa or 600 MPa pressure for 5 or 20 min at 20 °C in a complete 2 × 2 factorial design. Pressure treatments were carried out using a 2.0 L Stansted ISO Lab high pressure processing unit (FPG 9400:922, Stansted Fluid Power Ltd., Essex, UK). The pressure-transmitting fluid used was a mixture of ethylene glycol and water. The temperature increase due to the adiabatic heating effect during processing was approximately 3 °C per 100 MPa. After pressure treatment, the millet flour slurry was centrifuged at 4000 × *g*/10 °C for 15 min and the pellet was freeze dried. The dried material was then subjected to pepsin digestibility assays directly or after cooking in water. Thus, HPP treatment was compared on flours cooked before, after, and both before and after HPP treatment.