Journal of Cereal Science 66 (2015) 46–52

Contents lists available at ScienceDirect

Journal of Cereal Science

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

Effect of different soaking conditions on inhibitory factors and bioaccessibility of iron and zinc in pearl millet

Neha Jha, Rateesh Krishnan, M.S. Meera*

Department of Grain Science and Technology, CSIR – Central Food Technological Research Institute, Mysore 570 020, India

ARTICLE INFO

Article history:
Received 11 November 2014
Received in revised form
23 July 2015
Accepted 19 October 2015
Available online 23 October 2015

Keywords:
Pearl millet
Polyphenols
Phytic acid
Bioaccessibility

ABSTRACT

Pearl millet was decorticated to obtain a bran rich and endosperm rich fraction. The two fractions were soaked in solutions with varying pH. Pearl millet grains were germinated and steamed followed by decortication to obtain two fractions. It was observed that bran rich fractions contained high concentrations of iron, zinc, polyphenols, phytic acid, fibre and flavonoids. Soaking for short duration of 3 h did not result in major mineral losses but decreased the inhibitory factors which depended on the pH. Alkaline soaking decreased flavonoid content by 62.7% in the endosperm rich fraction, while acidic soaking decreased phytic acid content to the maximum in the bran rich fraction. Combination of treatments like germination and heat decreased the phytate content to the maximum in the endosperm rich fraction. Acidic conditions improved zinc bioaccessibility in the bran rich fraction (35%) and iron bioaccessibility (2.5%) in the endosperm rich fraction. Bran rich fraction from germinated grain also had enhanced bioaccessibility of both the minerals but comparatively lesser when compared to soaking under acidic conditions. Soaking the grain components under slightly less than neutral conditions also decreased some of the inhibitory factors and improved the zinc bioaccessibility to some extent in the bran rich fraction.

1. Introduction

Pearl millet (*Pennisetum glaucum* (L) R. Br.) one of the four most important cereals grown in the tropics, is cultivated in more than 29 million ha, with Africa (15.0 million ha) and Asia (11.0 million ha), being the largest producers of the crop. India has the largest area under pearl millet cultivation (9.8 million ha) in the world (International Grains Council, 2011). Pearl millet is a principal source of energy, protein, vitamins and minerals for millions of humans in the regions where it is cultivated. It has no husk, contains 5–7 g/100 g fat, proteins (12 g/100 g), carbohydrates (70 g/100 g), iron (8 mg/100 g), zinc (2 mg/100 g), folic acid (45.5 μg/100 g), and niacin (2.3 μg/100 g) which is better than some of the other cereals like rice, wheat, sorghum, maize (Gopalan et al., 1989).

Pearl millet contains a fibrous pericarp which contains high amounts of inhibitory factors like phytic acid, polyphenols and fibre that form complexes with dietary minerals, such as zinc and iron leading to a marked reduction in their bioaccessibility, thus limiting the nutritional value of the grains (Arora et al., 2003). A study on the effect of domestic processing like germination, fermentation and hand pounding on mineral HCl-extractability in African pearl millet varieties had shown that mineral extractability ranged between 23 and 70% (Abdalla et al., 2010). Among the treatments grain fermentation effectively decreased the inhibitory factors but, simultaneously induced organoleptic changes affecting sensory characteristics (Lestienne et al., 2005). Hama et al. (2011) have shown that minerals and inhibitory factors are not distributed uniformly in pearl millet grains. Hence, it would be appropriate to separate the grain fractions and process them in order to decrease the effect of inhibitory factors and improve mineral bioaccessibility. Very few reports are available wherein grain components like bran and endosperm were either processed separately or combinations of treatments were employed to decrease the inhibitory factors effectively. Hence, in the present study it was aimed to determine the effect of different treatment conditions like soaking and germination followed by thermal treatment on the chelating factors in the grain fractions. The effect of treatments on iron and zinc bioaccessibility was also studied.

* Corresponding author.
E-mail address: msmeera_1996@yahoo.com (M.S. Meera).

http://dx.doi.org/10.1016/j.jcs.2015.10.002
0733-5210/

2. Materials and methods

2.1. Materials

Pearl millet (Bajra) variety CO $(CU)_9$ was obtained from Tamil Nadu Agricultural University (Tamil Nadu, India). Grains were cleaned to remove dust and other foreign materials by aspiration and destoning. Uniform sized grains were obtained by sieving grains through 2.0 mm circular holed sieves.

Pepsin, pancreatin and bile extract (all of porcine origin), amyloglucosidase, ferulic acid and dialysis membranes (MW cut-off 8–12 kDa) were procured from Sigma chemical Co., St. Louis, MO, USA. Phytic acid/total phosphorus kit (K-PHYT) was purchased from Megazyme International Ireland Ltd, Bray, Ireland. Reference mineral standard solutions for atomic absorption spectrometry (AAS) were procured from Merck Specialities Pvt. Ltd, Mumbai, India. All other chemicals used were of analytical grade. Triple-distilled water and acid-washed glasswares were employed for the entire study.

2.1.1. Milling

Pearl millet grains were moistened with 3 mL/100 g water and tempered for 5 min. The tempered grains were decorticated in the emery polisher (Satake, Japan) for 5 min to obtain bran and intact endosperm (decorticated head grains) with minimum broken grains. After decortication the polished grains were passed through an 18 mesh sieve (990 μm opening) to separate out the bran, broken grains and intact endosperm.

2.1.2. Treatments

2.1.2.1. Soaking. The intact endosperm (250 g) and bran (100 g) were soaked separately in distilled water (200 mL pH-6.5), 0.1 N tartaric acid solution (pH 4–4.5) or 0.1 N calcium hydroxide solution (pH-11.5–12) for 3 h to obtain acid or alkali soaked endosperm and bran fractions. Tartaric acid was selected since pearl millet is traditionally soaked in tamarind pods to provide acidic conditions and tartaric acid is the major organic acid (Reichert, 1979). After soaking the solution was drained or filtered using filter papers. The soaked endosperm and bran fractions were subjected to steam in an autoclave at atmospheric pressure and 97 °C for 15 min to inactivate the enzymes that might have been activated during the soaking period, and later air-dried overnight.

2.1.2.2. Germination. Pearl millet grains (500 g) were soaked in distilled water for 8 h, water was drained and grains were germinated for 48 h in a Biological Oxygen Demand incubator (BOD, NSW-152, Narang Scientific works Pvt Ltd, India) maintained at 37 °C. Later, the germinated grains were spread on a wire mesh, steamed in an autoclave (Googly, India) to inactivate enzymes and also to harden the endosperm. The germinated and steamed grains were shade dried for 2 days or until the moisture content of 12 g/100 g was achieved. The germinated steamed grains were decorticated as mentioned above to obtain bran rich and endosperm rich fractions.

The acid, alkali, distilled water treated and germinated endosperm and bran fractions were ground in a cyclone mill and sieved through 60 mesh sieve to obtain 250 μm particle size for further analysis.

2.2. Crude fat and ash content

The crude fat was determined by the Soxhlet method according to AOAC method no. 920.85 (AOAC, 2000). The ash content were determined according to AOAC method no. 923.03 (AOAC, 2000).

2.3. Iron and zinc estimation

Ash solution was prepared by dissolving ash in concentrated HCl and mineral (iron and zinc) contents were determined by Atomic Absorption Spectrometry (AAS) (Shimadzu AAF–6701, Japan) according to AOAC method no. 965.09 (AOAC, 2000).

2.4. In vitro bioaccessibility of iron and zinc

Bioaccessibility of iron and zinc were determined by following the method of Luten et al. (1996). Briefly, all the treated samples were subjected to simulated gastrointestinal digestion by adjusting the pH to 2.0, followed by the addition of pepsin (3 mL of 16% pepsin in 0.2 M HCl) and incubation in a shaker water bath at 37 °C for 2 h. An aliquot of digest (20 mL) was tested for its titratable acidity by adding 5 mL of pancreatin–bile extract mixture (4 g of pancreatin and 25 g bile extract in 1 L of 0.1 M $NaHCO_3$) against 0.2 M NaOH until pH 7.5 was attained.

About 20 mL of the digest was subjected to simulated intestinal digestion in Erlenmeyer flasks containing dialysis tubes (molecular cut-off 8–12 kDa) with 25 mL $NaHCO_3$ (equivalent to moles of NaOH determined by titratable acidity). The flasks along with the dialysis tubes were incubated in a shaker water bath at 37 °C for 30 min (until the pH changes to 5.0) and to that, pancreatin–bile extract mixture was added and shaken for another 2 h (until the pH reached 7.0). The total volume of the dialysate was noted and its iron and zinc contents were AAS as mentioned above.

2.5. Determination of inhibitory factors

2.5.1. Insoluble and soluble dietary fibre

The insoluble and soluble dietary fibre contents were determined by enzymatic-gravimetric method (Asp et al., 1983). The defatted samples were gelatinized with heat stable α-amylase and then enzymatically digested with protease and amyloglucosidase to remove protein and starch. The insoluble dietary fibre was removed by filtering and washing the residue with water and 95% ethanol. The soluble dietary fibre in filtrate was precipitated by adding 95% ethanol. The precipitate was filtered and washed sequentially with 78 and 95% ethanol, dried to determine the fibre content followed by incineration at 525 ± 10 °C to determine the ash.

2.5.2. Polyphenols

For the assay of total polyphenols, 500 mg of each of the defatted sample was refluxed with 5 mL of HCl–methanol–water (1:80:10, v/v/v) solvent system for 3 h followed by centrifugation. An aliquot (0.2 mL) of the extract was treated with 1.5 mL of Folin–Ciocalteu's reagent (1:10) and 1.5 mL of sodium carbonate solution. The contents are allowed to stand for 90 min and the absorbance measured at 725 nm against the reagent blank. Ferulic acid (1 mg/1 mL) was used as the reference standard (Gao et al., 2002).

2.5.3. Flavonoids

The total flavonoids were estimated after extracting 500 mg of the sample using acidified methanol. The extract (0.1 mL) was mixed with 4.9 mL of distilled water. At zero minutes, 0.3 mL of (5% w/v) $NaNO_2$ was added and after 5 min, 0.3 mL of (10% w/v) $AlCl_3$ and at 6 min, 2 mL of 1 M NaOH was added, and immediately the volume was made up to 10 mL with distilled water. The mixture was shaken vigorously and the absorbance read at 510 nm (Zhishen et al., 1999). Results are expressed as mg catechin equivalents (CEQ)/100 g of sample.