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Sorghum extrusion process combined with biofortified sweet potato contributed for high iron bioavailability in *Wistar* rats

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ABSTRACT

This study aimed to evaluate the effect of sorghum and sweet potato on the bioavailability of iron, gene expression of proteins involved in iron metabolism and the plasma antioxidant capacity in animals fed with whole sorghum grains processed by dry heat or extrusion, combined or not with sweet potato flour with high content of carotenoids. Five experimental groups were tested (n = 7): dry heat sorghum flour (DS); extruded sorghum flour (ES); whole sorghum flour + sweet potato flour (DS + SP); extruded sorghum flour + sweet potato flour (ES + SP) and positive control (FS). The evaluations included: hemoglobin gain, hemoglobin regeneration efficiency, gene expression of divalente metal transporter 1 (DMT-1), duodenal citochroma B (DcytB), ferroportin, hephaestin, transferrin and ferritin and total plasma antioxidant capacity (TAC). The ES + SP group showed higher ($p < 0.05$) expression of DcytB, ferroportin and hephaestin when compared to the control group. The DS group showed high ($p < 0.05$) expression of DMT-1 and the ES showed high mRNA expression of transferrin and ferritin. The changes in the sorghum physicochemical properties from extrusion process reduced the iron and phytate content, and increased the gene expression of proteins involved in iron metabolism, improving iron bioavailability. The combination of sweet potato and sorghum flour (dry or extruded) improved the iron capture and total antioxidant capacity, probably due to the presence of β-carotene and antioxidant compounds.
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1. Introduction

Iron deficiency anemia is a nutrition deficiency found in developing countries, affecting around 42% of the world population (WHO, 2015). New public health programs have been developed to control micronutrient deficiencies, such as biofortification food (Bouis et al., 2011; La Frano et al., 2014).

Sorghum (*Sorghum bicolor* L.) is the fifth cereal with the highest productivity in the world and its nutritional composition suggests that it is a source of vitamins and minerals as well as bioactive compounds (phenolic compounds and tannins) which can reduce iron bioavailability (Cardoso et al., 2015a; Petry et al., 2010; Moraes et al., 2012; Hart et al., 2015). Sorghum genotypes are an important source of iron for many people (Ashok Kumar et al., 2013) despite its low iron bioavailability. Thus, Embrapa Milho e Sorgo (Brazilian Agricultural Research Corporation) selected within a database of 100 genotypes, one with high iron, zinc, antioxidant activity, total phenolics and tannins contents (Cardoso et al., 2015a).

To minimize the effect of phytochemicals in the sorghum and enhance the iron absorption, it has been used on diets with pro-vitamin A carotenoid (Layrisse et al., 1998). Embrapa has been producing Beauregard sweet potato (*Ipomoea batatas*) with high content of carotenoids, which in association with sorghum can increase the iron bioavailability. Vitamin A can act in iron mobilization of organic tissue stocks, favoring the availability of this

Abbreviations used: ANOVA, analysis of variance; DcytB, duodenal citochroma B; DMT-1, divalente metal transporter 1; DS, dry heat sorghum flour; DS + SP, whole sorghum flour + sweet potato flour; ES, extruded sorghum flour; ES + SP, extruded sorghum flour + sweet potato flour; FER, food efficiency ratio; FS, positive control; HRE, hemoglobin regeneration efficiency; RBV HRE, biological hemoglobin regeneration efficiency; TAC, total plasma antioxidant capacity.

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mineral for hematopoiesis and hemoglobin synthesis (Semba and Bloem, 2002).

The iron homeostasis is controlled by transcriptional mechanisms that regulate gene expression of proteins involved in this mineral metabolism (Tako et al., 2013). The duodenal cytochrome b (Dcytb), divalent metal carrier protein (DMT-1), ferroportin and duodenal hephaestin are proteins that regulate iron absorption, while transferrin and ferritin are proteins synthesized in the liver and act in the transport and storage of iron, respectively (Tako et al., 2013; Grotto, 2008).

There are no studies about the iron bioavailability interference of the extrusion process combined with high carotenoid content crops. Thus, it is important to assess the effect *in vivo* of sorghum flour combinations (dry heat or extruded) with biofortified sweet potato. We aimed to evaluate the effect of the heat treatment and extrusion process of sorghum flour and its combination with high carotenoid content crop on the iron bioavailability, gene expression of proteins involved in iron metabolism, and their antioxidant effect.

2. Materials and methods

2.1. Sorghum and sweet potato

Dry or extruded whole sorghum (*Sorghum bicolor* L.) of SC 319 genotype (origin: Uganda; brown pericarp with proanthocyanidins and 3-deoxidanthocyanidins − 3DXAs) was used. The seeds were grown at Embrapa Milho e Sorgo (Nova Porteirinha, Minas Gerais, Brazil) between June and October 2011. The experimental plots were composed of two rows of 3 m long with spacing of half a meter between rows. The fertilization at planting consisted of the application of 300 kg/ha of formulated 08-28-16 (NPK). After 25 days of planting, fertilization with 50 kg/ha N was performed. Sweet potato (*Ipomoea batatas*), registered in National Register of Cultivars under the name Beauregard (number 26934) with high concentration of carotenoids were supplied by Embrapa Meio Norte (Terezina, Piauí, Brazil). The sweet potato was planted at a spacing of 1 m between furrows and 30 cm between plants. The vines were planted to between 25 and 35 cm or at least 5 gems, buried at the top of the windrow, leaving 1/3 out. The harvest took place between 120 and 150 days after growth. The productivity may range from 20 to 40 tonnes per ha, may exceed that value in cases of more fertile soil. After the harvest, the foods were stored in insulated boxes and then packed in polyethylene bags and stored at −18 ± 1 °C.

2.2. Staple food crop flours preparation

The SC 319 sorghum grains were subjected to two processing methods, dry heat/milling (DS); and extrusion/milling (ES), as described below:

DS - The whole grains were subjected to dry heat (121 °C; 25 min) in an oven with forced air circulation (Marconi®, MA 093, São Paulo, Brazil). Subsequently, the grains were ground in a mill (850 μm screen) (Marconi®, MA 090, São Paulo, Brazil).

ES - The extrusion process of sorghum flour was conducted at Embrapa Agroindústria de Alimentos Rio de Janeiro, Brazil. The whole grains previously milled (850 μm) were processed in a co-rotating twin-screw model Evolum HT 25 (Clextral, Firminy, France) (temperature between 30 and 150 °C) (Vargas-Solórzano et al., 2014). To compensate moisture differences in the samples and provide a final moisture content of 12%, distilled water was injected between the first and second feeding zones using a plunger metering pump model J-X 8/1 (AILIPU Pump Co. Ltd., China). The samples were collected over 15–20 min. Subsequently, the extrudate was ground in a mill (850 μm) (Marconi®, MA 090, São Paulo, Brazil) and stored in polyethylene bags, at −18 ± 1 °C, until analysis.

The sweet potatoes were peeled and sliced on multiprocessor (Philips Walita, Amsterdam, Netherlands) and dried in an air oven for 6 h at 60 °C (Dias et al., 2015). The sweet potato was ground in a 090 CFT mill (Marconi, São Paulo, Brazil) at 3000 rpm, sieved (600 mesh screen). All samples were stored in a freezer (−18 ± 1 °C).

2.3. Chemical composition

The determination of iron content of food and the iron content of the diet were performed according to Gomes (1996). Briefly, 1.0 g of the samples was oxidized with 10 mL of nitric acid for 8 h at room temperature. Later, the samples were heated in the digester block with exhaust to approximately 120 °C for 16 h. The iron and zinc concentrations were determined by coupled plasma atomic emission spectrometry (model Optima 3300 DV, Perkin Elmer, MA, USA), with an inducible plasma argon source. Analysis was performed under the following conditions: power of 1300 W, plasma argon flow rate of 15 L min^{-1}, auxiliary argon flow rate of 0.7 L min^{-1}, nebulizer argon flow rate of 0.5 L min^{-1}, rate of sample introduction of 1.5 mL min^{-1}. Calibration curves were used to prepare standard solutions of iron concentration, according to Cardoso et al. (2015b). The analyses were performed in triplicate.

The phytate content was quantified according to the method proposed by Latta and Eskin (1980), with modifications by Ellis and Morris (1986). For the extraction of phytates, 0.1 g in triplicate was weighed and 15 mL of HCl 2.4% were added, remaining under horizontal stirring for 12 h at 250 rpm. The extract was vacuum-filtered on büchnner funnel and purified using ion exchange column, stationary phase consisting of AG® 1-X4 Resin (Bio-Rad Laboratories, São Paulo, SP, Brazil). The column was preconditioned with NaCl 2 M and the extract obtained from the previous steps was applied to it. The inorganic phosphors were eluted with NaCl 0.05 M followed by elution of the retained phytates with NaCl 2 M. Phytate was determined colorimetrically, based on the pink coloration of Wade's reagent, formed from the reaction between ferric ion and Sulfosalicylic acid, which exhibits maximum absorbance at 500 nm. An analytical curve of phytic acid (myo-inositol hexaphosphate) (Sigma-Aldrich®, St. Louis, MO, USA, code P8810) at concentrations of 10–100 μg mL^{-1} was created using the Linear regression equation ($y = -0.0035x + 0.4818$; $R^2 = 0.9965$) to express the phytate content in milligram of phytic acid per gram of sample.

The concentration of pro-vitamin A carotenoid (α and β-carotene) in pumpkin and sweet potato was determined by Rodriguez et al. (1976). Five grams of sample were ground in 60 mL of chilled acetone for approximately 2 min and the material was vacuum filtered on a Buchner funnel using filter paper. The filtrate was transferred to a separator funnel, in which 50 mL of cooled petroleum ether were added to transfer the pigment to the acetone ether. Each fraction was washed three times with distilled water to remove all acetone. The concentration of material was performed by evaporation of the petroleum ether extract using a rotary evaporator at 35 °C. The pigments were dissolved again in a known amount of petroleum ether and stored in amber glass vials at 18 °C. For analysis, an aliquot (2 mL) of the extract stored in petroleum ether was evaporated under nitrogen flow and then recovered in the same amount of methanol and filtered through a filter unit with 0.45 μM porosity. The analyses of carotenoids were performed in triplicate by high performance liquid chromatography (HPLC) using the chromatographic conditions developed by Pinheiro-Sant'Ana et al. (1998) as follows: HPLC-DAD system (diode array detector); chromatographic column Phenomenex Gemini RP-18,

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