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Iron in fortified biscuits: A simple method for its quantification, bioaccessibility study and physicochemical quality

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ABSTRACT

Iron deficiency is one of the most important nutritional problems in the world. The aims of this study were to determine the total concentration of iron in order to evaluate its bioaccessibility in biscuits produced with fortified flour, check the importance of its contribution to the iron intake and monitor physicochemical parameters such as moisture, acidity and peroxide value (PV) during 150 days of storage. The simple and cheap method for iron determination was validated and proved to be adequate. Forty one samples of biscuits including salt water, cream cracker, cornstarch, and buttery biscuits were analyzed and their iron content were 5.3–7.8; 5.0–8.6; 2.5–6.8; and 3.7–5.7 mg/100 g, respectively. The in vitro assay results varied from 1.2 to 4.3 mg/100 g and from 0.2 to 2.1 mg/100 g to solubility and dialysis, respectively. There was significant difference in total, soluble and dialyzed iron content among the biscuit types analyzed. The intake of a biscuit portion can contribute from 5 to 32.5% of the recommended daily intake of iron, depending on the type of biscuit consumed. Lipid content varied from 9.8 to 18.0% for the biscuit types analyzed. In the end of storage time moisture levels increased 1.5% for the majority of samples, besides it was observed that most biscuits showed an increase (around 50%) of titratable acidity after 150 days of storage. The highest PV was 27.8 meq/kg of oil fat for salt and water biscuit (in 90 days of storage), 23.3 meq/kg of oil for cream cracker (in 120 days of storage), 22.6 meq/kg of oil for cornstarch (in 120 days of storage) and 14.1 meq/kg of oil for buttery biscuit (in 60 days of storage), indicating lipid oxidation. Samples with the highest iron and moisture content also presented the highest peroxide value, indicating oxidation. The consumption of biscuits plays an important role in providing the daily requirement of iron intake. However, it is necessary to improve the stability and to provide the desired delivery of nutrients without causing damage to the quality of food and health of the consumers.

1. Introduction

Biscuits are consumed by people of all ages. They are produced by mixing flour and/or starch with other ingredients through a kneading process, fermented or otherwise, and they may contain different toppings, fillings, shapes and textures. Their long shelf life allows large scale production and wide distribution. Wheat flour is the main ingredient in biscuit formulations, thus providing a matrix in which the other ingredients are mixed to form the dough (Gutkoski, Bonamigo, Teixeira, & Pedó, 2007).

In 2009, 1206 million tons of biscuit were produced in Brazil, representing the second largest production worldwide. Between 2007 and 2009, the annual consumption per capita of biscuits increased from 6.0 to 6.3 kg (ANIB, 2014). Biscuits can be classified according to their characterizing ingredients or by usage of established names (ANVISA, 1978).

In Brazil, the Ministry of Health through the Resolution No. 344 of December 13, 2002, ordered the fortification of wheat and corn flours with iron (4.2 mg/100 g), in order to reduce the risk of anemia associated with the deficiency of this nutrient, as these flours are widely consumed by the Brazilian population (ANVISA, 2002). In Brazil and other countries including Chile, South Africa, Guatemala, Venezuela and Sri Lanka, different iron sources such as ferrous sulfate, ferrous fumarate, iron-EDTA (sodium iron (Fe^{3+}) ethylenediaminetetraacetic acid (EDTA)), reduced iron, electrolytic iron and iron glycinate chelate (ANVISA, 2002), with different bioavailability, can be used for the fortification of wheat and corn flours. The relative bioavailability can vary from 100 to 5 depending on the iron source used in the fortification — to ferrous sulfate, ferrous fumarate and iron-EDTA this values can vary from 100, 27–200 and 30–390, respectively (Miller, 2002; Quintaes, Barberá, & Cilla, 2015). Iron fortification of foods is the strategy recommended by health institutions as the most efficient way to combat iron deficiency anemia (WHO, 2006).

Soeiro, Boen, Pereira-Filho, and Lima-Pallone (2010) found that the iron concentrations in Brazilian wheat flours presented higher values than those recommended by Resolution No. 344. Fortified wheat flour

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can be used in the preparation of various types of food, including bread, cakes, biscuits and others.

Information about the presence of minerals in diet typically refers to their total concentrations; however, these methods do not measure the fraction that can be bioaccessible to the human body. This fraction is dependent on the elements' forms, on the behavior of organometallic species and complexes in the gastrointestinal tract, and on interactions with the food matrix (Khouzam, Pohl, & Lobinski, 2011). The bioaccessibility can be defined as the amount of nutrient converted to soluble forms in gastrointestinal conditions. It is typically evaluated using a sequential analysis with artificial gastric juice and intestinal juice, and the analysis of soluble fractions (Khouzam et al., 2011).

The term bioavailability refers to the fraction of a compound or bioactive nutrient present in a food that is available to be used in physiological functions or to be stored in the organism. Whereas bioaccessibility has been defined as the fraction of a compound that is released from the food on the gastrointestinal tract and so becomes available to the intestinal absorption (Benito & Miller, 1998; Fernández-Garcia, Carvajal-Lérida, & Perez-Galvez, 2009) and is established by performing in vitro assays.

The in vitro dialysis method is based on the simulation of gastrointestinal digestion of food followed by the determination of the amount of nutrient that crosses a semipermeable membrane that simulates the intestinal wall (Cámara, Amaro, Barbera, & CLemente, 2005; Kiskini et al., 2007; Miller, Schriecker, Ramussen, & Van Campen, 1981; Perales, Barberá, Lagarda, & Farreä, 2006). The in vitro bioaccessibility can also be estimated by employing solubility tests. In this technique the gastrointestinal simulation also occurs, however only the mineral soluble fraction is evaluated (Cámara et al., 2005; Sahuquillo, Barberá, & Farré, 2003).

Besides the nutritional aspect, the range of food fortification programs is related to the physicochemical properties that should be monitored on the vehicle used for the nutrient addition and final product. Huma, Rehman, Awan, Murtaza, and Arshad (2007) found that during 42 days of storage of flour fortified with iron lipid oxidation and rancidity developed, caused by the addition of iron.

Several studies about iron fortification on wheat flour are available on literature, including the mineral's quantification, bioaccessability and bioavailability (Hurrell et al., 2010; Hernández et al., 2006; Moretti, Biebinger, Bruins, Hoeft, & Kraemer, 2014; Quintaes et al., 2015; Vitali, Radić, Cetina-Cižmek, & Vedrina Dragojević, 2011). However, these studies focus on evaluating the different iron forms that can be employed on flour fortification or on the incorporation of vegetal origin flour as a minerals' source on food. The objectives of this study were to evaluate the total iron concentration using a simple and cheap validated method, and its bioaccessibility (via dialysis and solubility methods) in biscuits available for consumption (commercial), to check the contribution of this food product to the supply of iron in the human body and monitor some physicochemical parameters during 150 days of storage.

2. Materials and methods

2.1. Reagents and solutions

Analytical grade nitric acid (Synth, Diadema, Brazil), and hydrogen peroxide (Synth, Diadema, Brazil), were employed. The standards used were: iron standard solution 1000 mg/kg (Qhemis, Jundiaí, Brazil), traceable to SRM 136e (NIST, Gaithersburg, USA), certified reference material (CRM) of wheat flour, 1567a (NIST, Gaithersburg, USA), and glucose solution (Merck, Damstadt, Germany). All solutions were prepared with water purified in the Milli-Q Plus system (Millipore, Billerica, USA). Qualitative filter paper of 9 cm diameter was used for filtering the digested acid solutions.

2.2. Equipments

Iron content analyses were performed with flame atomic absorption spectrometry (FAAS), Perkin Elmer (USA), model AAnalyst-200, with a deuterium lamp for background radiation correction, a hollow cathode lamp, for iron determination (248.3 nm), with air flame (2.5 L/h) and acetylene (10 L/h), at a temperature of approximately 2000 °C.

Residual carbon content analysis was performed by inductively coupled plasma atomic emission spectrometry (ICP-OES), Perkin Elmer (USA) model Optima 2000 DV with axial configuration. The operational parameters were: plasma power: 1300 W; gas flow: 15 L/min; observation height: 15 mm; auxiliary gas flow: 0.2 L/min; view: axial; sample introduction flow: 1.5 mL/min; nebulizer flow: 0.60 mL/min; and atomic emission line (nm): CI = 193.030.

The samples were grounded using a grinder (Model A11 — Ika, Germany) and weighed on an analytical balance (model AP210-0 — Ohaus, Japan). A block digestor (Model M242 — Quimis, Brazil) was used for the mineralization of the samples, and an ultrasonic bath (model 1510 — Branson, Brazil) was used to enhance sample solubilization for the transfer of the digested material. pH meter (Tecnal, Brazil), centrifuge (Excelsa II, Fanen, Brazil), lyophilized (LS, Terroni, Brazil) and metabolic bath (Dusnoff, MA 093, Marcone, Brazil) were used for in vitro assays.

Air oven for sterilization and drying (model 400-3ND — Nova Etica, Brazil) was used for the determination of moisture; Wagner type agitator for laboratory tubes (model MA 160/50/CF — Marconi, Brazil) was used for lipid extraction.

2.3. Samples

The biscuit samples were purchased at supermarkets located in the city of Campinas — SP, Brazil. For the analysis of iron content, 41 samples were evaluated. Cream cracker, salt water, and cornstarch biscuit samples from four different brands, with each divided into three distinct batches, were analyzed. The buttery biscuit samples were composed of two brands, with one brand containing two batches and another composed of three batches. Each batch consisted of five packages, with 100 g of biscuits taken from each package, which were then crushed and homogenized. The main brands sold in the Brazilian market were chosen for the analyses. To the in vitro assay, for bioaccessibility estimative, one sample of each biscuit brand was chosen. All the analyses were performed in triplicates. Moisture, acidity and peroxide value were examined monthly for 150 days, during biscuit storage. The total lipid was measured only at the beginning of the experiment.

2.4. Iron determination and bioacessibility estimative

0.60 g of each sample type was weighed into digestion tubes, 8 ml of nitric acid and 2 ml of hydrogen peroxide (30%) were then added. Small funnels were positioned on the tube to maintain a reflux. The tubes were heated for 2 h at 110 °C. After cooling, 5 ml of water was added to the tubes and subjected to ultrasonic bath for 5 min. The tubes' contents were transferred to 50 ml volumetric flasks, and the volume was completed with water. The samples were filtered in paper filters and stored in a polypropylene vial for determination of the iron content.

The solubility method described by Cámara et al. (2005), with modifications, was applied to evaluate soluble iron in biscuits. Five grams of each biscuit was homogenized with 30 mL of deionized distilled water, and the pH was adjusted to 2.0 with 6 M HCl. In order to develop the pepsin–HCl digestion, 0.65 mL of pepsin solution (1.6 g of pepsin, P-7000, from porcine stomach, (Sigma Chemical Co.St., Louis, USA) in 10 ml of HCl 0.1 M) was added. The mixture was then incubated for 2 h at 37 °C in a shaking water bath. To stop simulation of stomach digestion, the sample was maintained for 10 min in an ice bath. Prior to the simulation of intestinal digestion step, the pH of the gastric digests was raised to 5 by drop-wise addition of 1 M $NaHCO_3$. Then