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BREAD BIOFORTIFIED WITH IRON AND ZINC FROM BEAN FLOUR: CHEMICAL CHARACTERISTICS AND SENSORY ACCEPTANCE

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ABSTRACT

This study aimed to develop bread biofortified with bean flour, partially replacing wheat flour and characterize nutritionally and quantify phytates and tannins, mineral content and availability; sensory acceptance and purchase intent. The addition of bean flour (BF) to formulations was efficient to increase the contents of proteins, lipids and fiber. There was an increase of phytates in bread with F15% and tannins in all treatments. The addition of bean flour (15%) to bread was effective to increase contents of Fe contents in samples F10-15 and Zn contents in samples F5-15. Regarding dialysis, the fortified bread produced higher Fe and Zn availability compared the control bread. The sensory analysis showed, sample F10 was more accepted by judges in terms of flavor, aroma, texture and overall impression, in addition to showing the greatest purchase intent. Therefore, the production of bread fortified with bean flour is promising, since the chemical properties and sensory evaluation were considered satisfactory.

KEYWORDS

Bakery products, Beans, Bioavailability, Sensory analysis and Tannins.

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INTRODUCTION

Fortifying foods with Fe, as well as the distribution of these micronutrient supplements to the target population, has been the most commonly used strategies in most developing countries to fight Fe deficiency. Recent research has shown that the development of plants with higher contents of Fe and other minerals can help improve human diet. The little access of populations to foods rich in micronutrients and the presence in inappropriate proportions of inhibitors and anti-nutrients in the diets, besides the low bioavailability of minerals, are the cause of deficiencies of these elements in human diets.

In 2004, in Brazil, works of the program Harvest Plus Brasil were started aimed at developing varieties of rice, bean, cassava, corn, wheat and sweet potato with higher concentrations of Fe, Zn and beta-carotene. This program is coordinated by the Brazilian Agricultural Research Corporation - EMBRAPA (EMBRAPA).

An essential aspect of food fortification is the election of a food vehicle. To be a potential vehicle of fortification, the food must have low-cost, high consumption within the target population and a constant consumption pattern with low-risk of excess (FAO, 2006)[1].

Bread has been widely used for nutritional enrichment purposes, especially for being one of the main caloric sources of diet in many countries and for being widely consumed by individuals of different social classes (Ranhotra *et al*, 2000[2], Kajishima *et al*, 2003)[3].

Due to population nutritional needs and the good acceptance of bakery products, this work used bread biofortified with bean flour in order to increase the nutritional value and ensure good product acceptance. Nutritional changes of bean, due to genetic improvement, can reveal cultivars containing great nutritional profile, including functional or bioactive compounds. The development of bakery products biofortified with bean flour is recent, however, with great potential for application. Therefore, the purpose of the present work was formulate bread with wheat flour, cassava and bean flour with three different concentrations of this legume flour, determine the contents and availability of Fe, Zn; phytic acid, tannins and investigate the acceptance of the formulated bread.

MATERIAL AND METHODS

Materials

Samples of biofortified bean BRS Pontal and yellow-pulp cassava (*Manihot esculenta*) clone 2003-14-11, designed and planted by the Brazilian Agricultural Research Corporation (EMBRAPA), were kindly provided by the institution to the present work.

Study site

This study was conducted at the laboratories of Food Chemistry, Food Sensory Analysis and Agricultural Products Technology (TPA) of the Center for Agricultural Sciences of the Federal University of Espirito Santo (CCA/UFES) in Alegre, Espirito Santo State, Brazil.

Elaboration of biofortified bread

Bean grains were immersed in distilled water 1:2 (m/v) for 16 h. afterward, the water was discarded and distilled water was added again at 1:3 (m/v) for cooking. Next, the bean was dried in air circulation dryer at 60°C for 24 h. The dry bean was milled in a knife-grinder and stored in polyethylene packaging under freezing until the moment of its use in the formulations (Brigide, 2002)[4].

Cassava was peeled, washed and cut into cubes of about 3cm, which were submitted to cooking at boiling temperature at a ratio of 1:4 (cassava: water) for 30 min. From the standard formulation (F0), wheat flour was partially replaced by bean flour at three percentages (5%, 10% and 15%) (Table No.1).

The ingredients were weighed on a digital scale and the dry ingredients were homogenized for 2 min in a mixer (Arno Planetary, 280W, model BPAI). The other ingredients and 80% of water were added and mixed in the dough. The remaining 20% of water was used to adjust the dough texture. The dough was homogenized until the development of the gluten network. Next, it was kneaded manually for 10 min, modeled and placed in a baking pan for fermentation. Fermentation occurred for 60 min in oven at 30°C. The dough was baked in conventional oven at 180°C for 25 min (Moura, 2008)[5].

Analyses

Centesimal composition

The chemical analyses of contents of water, crude protein (CP), ether extract (EE), dietary fiber (soluble and insoluble), ash and minerals were carried out in accordance with the methodology indicated by AOAC (2005)[6]. The carbohydrates were obtained by difference.

Determining tannins

Tannins were quantified through the methodology described by Price *et al*, (1980)[7] using spectrophotometer readings at 500nm (BEL, Photonics, SP 2000UV). For the construction of standard curve, we used catechin and the results were expressed as mEq of catechin.

Determining phytates

The phytate content was determined by ion-exchange chromatography and spectrophotometry, using the Latta and Eskin method (1980)[8], with modifications by Ellis and Morris (1986)[9].

Content and availability of minerals

The contents of minerals (Fe and Zn) were determined according to Whittaker, Fox and Forbes (1989)[10]. An atomic absorption spectrophotometer was used to quantify the mineral contents (Ca, Fe, Zn, Mg). The simulation of gastric and intestinal digestion was carried out by adding 6% of pepsin solution and pH adjusted to 2.0. The solution remained in water bath at 37°C for 2 h. The titration was carried out with NaOH to pH 7.5 to quantify the volume of $NaHCO_3$ that was added to the solution and proceeded to dialysis. After, the samples were subjected to dialysis with a bile-pancreatin solution in water bath at 37°C for 1 h. The samples were stored at around 0-4°C for further analysis of availability of Fe and Zn by atomic absorption.

Sensory analyses

Acceptance test and purchase intent

For the sensory acceptance test of the formulations, the nine-point hedonic scale was used (Reis and Minim, 2013)[11], ranging between the terms "dislike extremely" (Score 1) and "liked very much" (Score 9).

To the sensory acceptance document containing the hedonic scale, it was also included a test on purchase intent, using a five-point scale, as described by Meilgaard *et al*, (1999)[12], varying between "would definitely not buy" (Score 1) and "would definitely buy" (Score 5).

Statistical analysis

The statistical design used was completely randomized. The results were submitted to the analysis of variance (ANOVA) by the F test and the Tukey test. In all cases, the significance level of 5% was adopted. The data were analyzed using the SAS software (SAS Institute Inc.).

RESULTS AND DISCUSSION

Centesimal Composition

The results obtained for the centesimal composition (Table No.2) showed that the composition of bread biofortified with bean flour differed significantly ($p<0.05$) in terms of protein, lipids and ash contents. Treatment F15 presented the highest levels and the control sample (F0) showed the lowest contents of proteins, lipids, and ash in Table 2 show no significant difference between the samples for the water content. The Brazilian Table of Food Composition (2010) determines 40.7% of moisture for each 100g of traditional bread, similar to the level found in our study.

Andrade *et al*, (2015)[13] found similar results for moisture of bread with partial replacement of wheat flour for roasted cowpea bean flour, and Moura (2011)[14] with linseed addition. However, Rizzello *et al*, (2014)[15] investigated the addition of legume flour, such as beans, chickpeas and lentils to wheat flour and found 29.21 to 30% of moisture. Nevertheless, the formulated breads are within the standard established by the RDC No. 90 of October 18, 2000, which preconizes a maximum of 38 g per 100g for breads made with wheat flour (Brasil, 2000)[16].

Samples of breads added with bean flour did not differ in terms of protein and lipid contents. Bread samples F15 showed greater levels compared to the control bread. However, samples F5 and F10 did not differ from the control. The values found in our study were higher than those found by Andrade *et al*, (2013)[17] in breads with partial replacement of wheat flour by toasted cowpea bean flour, by Cadioli *et al*, (2011)[18] in the formulation of bread with soy protein isolate and Polydextrose and by Skrbic *et al*, (2009)[19] that evaluated bread with barley flour and flakes embedded in bread with wheat flour and whole wheat flour. Mcwatters *et al*, (2004)[20] found that replacing 15% and 30% of raw and extruded cowpea flour increased the contents of proteins and lipids in breads, similar to our research. Replacing 15% of wheat flour for bean flour favored the increase in total protein content, which may not be favorable from the technological point of view, since bean flour is not composed of gluten.

The increased lipid contents provided by bean flour may be favorable to a product by offering better features for consumption for a longer period compared to the control bread. According to Zambrano *et al*, (2002)[21], lipid acts on the walls of gas bubbles, increasing their waterproofing and resistance to moisture exit, preventing or delaying starch downgrading and bread aging, which provides a product with firmer inside giving the feeling of a dry product at ingestion. A similar result was found for Rodrigues (2010)[22] by analyzing the lipid content in breads with different contents of cupuacupeel.

The higher ash content was found in bread samples with 15% FF, differing from the others. The values are similar to those found by Skrbic *et al*, (2009)[19], Andrade *et al*, (2013)[17] and Cadioli *et al*, (2014).

The contents of carbohydrates were obtained by difference and percentages were 34.21 and 43.83% for sample F15 and control bread, respectively. The results were below those observed by Moura (2008, 2011)[5,14] of 50.63 to 65.52% for breads with different linseed contents. In addition, due to the high contents of total fibers, the replacement of a carbohydrate-rich flour for a fiber-rich one, the tendency is to reduce the amount of carbohydrates also present in food. Anton *et al*, (2006)[23] found values of 38.39% for pumpernickel, 42.03% for light pumpernickel, 52.10% for bread and 63.10% for French bread, values similar to those found in our research.

According to the Brasil/Anvisa (1998)[24], to declare that the food is a source of fiber, it must contain at least 3% of fibers and food with high fiber content, at least 6%. Therefore, the formulations may be considered as foods with high fiber content.

According to Giuntini, Lajolo and Mark (2003)[25], products with high fiber content not always exhibit good acceptance by consumers, due mainly to changes in characteristics such as texture, flavor and color.

Breads made with bean flour have a significant difference of insoluble fiber in relation to control bread and for soluble fiber, treatment F5 did not differ from the control bread, however, it did not differ from the others. The values are higher than those found by Skrbic *et al*, (2009)[19], Andrade *et al*, (2013)[17] and Cadioli *et al*, (2014) breads with different wheat flours added.

Insoluble fibers have a great capacity for water retention, which can hold up to ten times its weight in water, making it unavailable for gluten development. These fibers are still able to destabilize gas cells by forming physical barriers on gluten during the dough development, resulting in volume decrease of bread. Soluble fibers, in turn, have retention properties of gas bubbles in the dough, favoring the formation of highly viscous solutions, showing positive effect on baking quality (Wang *et al*, 2003[26], Goesaert *et al*, 2005[27], Autio, 2006)[28].

Analyses of Phytates and Tannins

Foods have in their composition substances known as anti-nutritional factors that interact with some minerals by reducing their availability. Average levels of phytates and tannins are presented in Table No.3. There was no trend for increase of phytate and tannin contents in relation to the amount of bean flour added to breads.

Phytate is an organic acid with chelating characteristics, that is, it binds with minerals such as Ca, Mg, Fe and Zn and interfere with their bioavailability in the diet. Tannins have great capacity to associate with other essential biological polymers such as proteins and carbohydrates. Therefore, an anti-nutritional quality was assigned to these structures, that is, the greater the amount of phytates or tannins, the greater the anti-nutritional effect.

There was a trend to increase the phytate contents in relation to the amount of bean flour added to the breads. Sample F15 showed the highest contents, differing from the control and sample F5.

Moura (2008)[5] analyzed the contents of tannins and phytates at different contents of breads made with linseed (0% to 9%) and found mean values of 0.97mg.g^{-1} for tannins and 2.76mg.g^{-1} for phytates. Osman (2004)[29] evaluated the fermentation effects on typical bread with sorghum in phytic acid that ranged from 3.01 to 3.66mg.g^{-1} and tannins from 0.83 to 0, 068mg.g^{-1}. Fantini *et al*, (2008)[30] found values between 0.11 and 0.15mg.g^{-1} of tannins and values between 0.94 and 1.03mg.g^{-1} of phytates in mixtures of foods, such as beans and tomatoes, beans and rice and beans, rice and tomatoes. These values, probably due to the origin, composition and baking losses, are close to those found in our research. Because they are found naturally in foods and in similar quantities to other foods, anti-nutritional factors in formulated bread possibly do not have detrimental effects for consumers.

Kashlan *et al*, (1990)[31] assessed the phytic acid content in wheat flour and reported that it ranged from 238 to 1063mg.100g^{-1} and that in most consumed bread in Kuwait, the content ranged from 22 to 1045mg.100 g^{-1}, these values are attributed to different extraction rates of wheat flour.

Frontela *et al*, (2011)[32] analyzed the concentration of phytates in uncooked dough, after fermentation and baking, in bread with and without whole-wheat flour. The raw materials had the highest average concentration of phytate (762.8mg 100g^{-1} of wheat flour and 4491mg. 100g^{-1} of whole-wheat flour). The fermentation process significantly reduced the concentration of phytic acid in bakery products, greater than 90%. The cooking process reduced phytate content in relation to fermented masses (from 58.6mg.100g^{-1} of 9.4mg.100g^{-1} white bread, from 69.8mg.100g^{-1} to 19.4mg.100g^{-1}of whole-wheat bread).

Sample F15 presented the highest tannin content, similar to that found by Rodrigues (2010)[22] in breads with different concentrations of cupuacu shell flour (fresh) from 0.84 to 1.08mg.g^{-1}. According to Moura, Canniatti-Brazaca and Souza (2009)[30], tannin values remained at 0.96, 0.95, 0.98 and 1.01mg.g^{-1} in rye bread with 0, 3, 6 and 9% of linseed, respectively.

Analyses of minerals

The minerals showed a statistically significant difference. The data presented in Table No.4 show that sample F15 presented the highest content of Zn in relation to the other breads. According to the Brazilian Table of Food Composition (2010), mineral composition for each 100g of bread is 5.7mg of Fe and 1.3mg of Zn. These data are similar in terms of, Zn found in this research and this difference is attributed to the increase of bean flour in bread formulation.

Regarding the Zn content, significant differences were observed in the samples with increased contents of bean flour in bread. These values are higher than those found by Moura (2008, 2011)[5,14] and Rodrigues (2010)[22], which ranged from 10 to 13.75mg. kg^{-1} in breads formulated with mixed flours. However, our results agree with the contents found by Ronda *et al*, (2015)[33].

Analysis and Dialysis

In addition to quantifying mineral contents, there is need to know how much mineral is available for the human body. Table No.5 shows the percentage of minerals availability.

There was an increase in minerals availability with the increase for bean flour (BF) content in the breads. Probably, this was due to increased mineral contents in blends with bean flours, providing interactions that allowed greater availability of minerals in sample components.

Fe availability in formulated breads showed statistical difference between the samples, except for F5 and F10 samples that did not differ from each other, indicating that bean flour increases Fe availability and the treatment with higher Fe concentration had the greatest uptake.

In terms of Zn uptake, there was no statistical difference between the samples. Sample F15 showed greater availability, differing from the control. Probably, this increased availability was due to the addition of biofortified bean flour.

Frontela *et al*, (2011)[32] evaluated the effect of fermentation and cooking on the phytate content in different baked products (white bread, whole wheat bread and muffin) as well as solubility and mineral dialysis. Both Fe solubility and dialysis increased with fermentation. After baking, minerals dialysis increased in most products, which shows that these processes are favorable for minerals availability.

Sensory Analysis - Acceptance Test and Purchase Intent

The sensory analysis was performed by 74 untrained judges of both sexes, chosen at random, between different age groups. A nine-point hedonic scale was used (1 = extremely disliked, 9 = extremely liked).

According to the data presented (Table No.6), the judges were able to identify significant differences in acceptance of the breads, in terms of bean flour contents, except for sample F15. The acceptance score for the appearance attribute for the control bread is between 8 and 9 (liked very much to extremely liked). SampleF5 showed a score between 6 and 7 (liked slightly to liked moderately), F10 had a score between 7 and 8 (liked moderately to really liked) and sample F15 presented an average hedonic score between 5 and 6 (indifferent to liked slightly) for this attribute. This sample presented the lowest hedonic score differing from the others.

The acceptance score attributed to aroma for the control bread and sample F10 was between 7 and 8 (liked moderately to really liked) and did not differ from each other. Sample F5 showed a hedonic average score between 6 and 7 (liked slightly to liked moderately). Sample F15 had an average score between 5 and 6 (indifferent to liked slightly) for this attribute. This sample presented the lowest hedonic score, differing from the others.

The acceptance score attributed to texture for the control bread was between 7 and 8 (liked moderately to really liked). The control sample presented the highest score for this attribute, differing from the others. Samples F5 and F10 had scores between 6 and 7 (liked slightly to liked moderately), but they differed from each other. Sample F15 presented an average between 4 and 5 (indifferent to disliked slightly) as the lowest score, differing from the others.

In terms of taste and overall impression, the control bread obtained a score between 7 and 8 (liked moderately to really liked) and presented the highest score for these attributes, differing from the others.

Samples F5 and F10 had a hedonic average score between 6 and 7 (liked slightly to liked moderately), but they did not differ from each other. Bread with 15% showed an average between 4 and 5 (indifferent to disliked slightly) as the lowest score, differing from the others.

The results showed that the consumer accepts sample F10 (Table No.6), revealing a good acceptance. The scores are close to those reported by Ronda *et al*, (2015)[33], who used mixed flour of wheat and tef (*Eragrostis tef*) to formulate ciabatta bread.

For the judges, the highest scores were attributed to the control bread, which ranged from liked moderately the liked very much. Samples F5 and F10 showed scores ranging from liked slightly to liked moderately and sample F15, disliked slightly to indifferent, which presented the smallest scores, differing from the others with less acceptance. These results are similar to those found by Borges *et al*, (2011)[34] that analyzed the attributes aroma, color, appearance, taste, texture and overall impression of breads with 10% and 15% of quinoa flour and found that both formulations had acceptance average higher than 7.0 by the judges.

Cavallini (2015)[35] formulated bread with mixed wheat flour and breadfruit and observed a statistically significant difference between samples for all acceptance attributes. Similar to our study, the control bread (F0) received the highest acceptance scores for the attributes appearance, aroma, texture and overall impression, however, the flavor attribute for samples F0, F5, F10 and F15did not differ statistically between each other.

Skrbic *et al*, (2009)[19] evaluated white bread and whole-wheat bread with barley flour added, and the control sample of white bread presented the lowest score for the flavor attribute, differently from the results obtained in our study.

Regarding the intent to purchase to the breads (Table No.6), there was a significant statistical difference between the samples. The control sample (F0) presented purchase intent of "possibly purchase" and sample F5 had the purchase intent between "may purchase and possibly purchase", while sample F10 showed purchase intent of "possibly purchase". Sample F15 was statistically different from the others showing the lowest score for purchase intent, near "possibly would not purchase". There was a trend to reduce the purchase intent with increased percentage of wheat flour replaced by bean flour.

CONCLUSION

The addition of bean flour (F) was effective to increase the contents of proteins, lipids and fiber. There was an increase of phytate in samples F10 and F15 and tannin in all treatmens diferent control. The addition of bean flour to bread was effective to increase the contents of Fe and Zn. However, in relation to the dialysis, the formulated bread showed more availability of Fe and Zn. Regarding the sensory analysis, among the samples with bean flour added, sample F10 was more accepted by judges in terms of the attributes flavor, aroma, texture and overall impression, in addition to having the greatest score for purchase intent. Therefore, the formulation of bread biofortified with bean flour is promising, since the chemical properties and sensory evaluation were considered satisfactory.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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