Highly nutritional cookies based on a novel bean-cassava-wheat flour mix formulation

Galletas con alto valor nutricional basadas en una nueva formulación de mezclas de harinas de fríjol, yuca y trigo

Diana Carolina Cabal G.[1], Aslenis Melo R.[2], Sofía Lissbrant[3], Sonia Gallego C.[4], María de la Luz La O Hechavarría[5], and Adriana Tofiño R.[2, 6]

ABSTRACT

Nutritional deficiencies are common among children in Colombia, and innovative strategies and supplements are needed in order to effectively address this problem. For example, in Colombia, when measured as ferritin, iron deposits are deficient in 58.2% of children between two and eight years of age. If a formulation is made with highly nutritional ingredients, cookies will have the potential to be used as supplements in children's diets because of their simple manufacturing process, long shelf life, and high acceptability. This study aimed to develop biofortified cookies, based on a bean-cassava-wheat flour mix, for children. The methodology grouped several studies in order to define the best treatment for the production of bean flour and the flour mix to produce cookies, prioritizing the nutritional content and the microbiological and sensorial quality. A production procedure for bean-based flour, suitable for the production of cookies with adequate nutritional, sensorial and microbiological characteristics was obtained. Additionally, the rheological characteristics of the proposed flour mixes permitted other possible uses for the bread-making industry, substituting cereal flours with flours with higher micronutrient contents. However, further studies are needed to determine the nutritional effects of the regular ingestion of biofortified cookies on children.

Key words: biofortification, nutrient deficiency, viscosity analysis, sensory acceptance.

RESUMEN

Las deficiencias nutricionales son comunes entre los niños colombianos y se requieren estrategias y suplementos innovadores para direccionar apropiadamente este problema. Por ejemplo, en Colombia, los depósitos de hierro según ferritina muestran un déficit del 58,2% en niños de dos a ocho años. Si se realiza una formulación con ingredientes de alto valor nutricional, las galletas tendrán potencial para su uso como suplementos dietarios en los niños debido a la simplicidad en su elaboración, durabilidad y aceptabilidad. Este estudio tiene como fin el desarrollo de galletas biofortificadas para niños, a partir de una mezcla de harinas de fríjol, yuca y trigo. La metodología, agrupó un conjunto de ensayos para definir el mejor tratamiento para la producción de harina de fríjol y la mezcla para la formulación de la galleta priorizando el contenido nutricional, calidad microbiológica y sensorial. Se obtuvo un procedimiento para la elaboración de harina de fríjol con características adecuadas a nivel nutricional, microbiológico y sensorial, utilizable en la elaboración de galletas. Las características reológicas de la mezcla de harinas propuesta también permiten otros posibles usos en la industria panificadora, sustituyendo la harina de cereal por harinas de alto contenido de micronutrientes. Sin embargo, se requieren estudios minuciosos en los niños para determinar el efecto de la ingesta regular de las galletas.

Palabras clave: biofortificación, deficiencia nutricional, análisis de viscosidad, aceptación sensorial.

Introduction

The low-level ingestion of micronutrients affects three billion people around the world. One of the reasons is the consumption of poor-quality diets, which are based on high amounts of staple foods (wheat, corn and rice) and low amounts of complementary food products (fruits, legumes, vegetables, animal products and fish). The latter two are especially high in bioavailable minerals and vitamins (Dibb *et al.,* 2005). Numerous microelements and vitamins are considered essential to human nutrition and even minor deficiencies markedly increase the risk of serious diseases and even death. A lack of iron (Fe), iodine (I), vitamin A, and zinc (Zn) are currently the most important deficiencies for human health in the developing world. In Colombia, when measured as ferritin, iron deposits are deficient in

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1 Faculty of Engineering and Technology, Universidad Popular del Cesar (UPC). Valledupar (Colombia).
2 Motilonia Research Center, Corporacion Colombiana de Investigacion Agropecuaria (Corpoica). Codazzi (Colombia). atofino@corpoica.org.co
3 Corporacion Observatorio del Caribe Colombiano. Cartagena (Colombia).
4 Corporación CLAYUCA, International Center for Tropical Agriculture (CIAT). Palmira (Colombia).
5 Instituto de Investigaciones de la Caña de Azucar. La Habana (Cuba).
6 Extension, Universidad de Santander (UDES). Valledupar (Colombia).

58.2% of children between two and eight years of age, and 23% of children between one and five years of age are anemic according to their hemoglobin levels. In the department of Cesar, 34.3% of children five to seven years of age are anemic with a greater incidence in rural areas and in the populations belonging to SISBEN (*Sistema de Identificacion y clasificacion de Potenciales Beneficiarios de Programas Sociales*) levels 1 and 2 (Fonseca *et al*., 2010).

Therefore, the biofortification of crops, such as the bean (*Phaseolus vulgaris* L.), is being proposed as an alternative to combat malnutrition. Moreover, legume-based flours have the potential to increase the nutritional value when compared to other types of flour and are of interest in the production of food products for children, who constitute the most vulnerable population in regards to a lack of minerals, such as Zn and Fe. One commonly used strategy in combating malnutrition is through the use of dietary additives. Cookies are highly sought after snacks, for children as well as for adults, not only because they are appetizing, but also due to their simple manufacturing process, long shelf life and potential for containing high nutrient components (Noor Aziah *et al*., 2012). Therefore, they can be used as nutritional supplements. The rich composition of whole and fractioned grains, together with their high content of dietary fiber, have motivated numerous nutritional interventions focused on exploiting their potential in order to obtain healthier and more nutritious food products. Diets rich in whole grains and other plant-based food products and low in lipids, such as saturated fats and cholesterol, can reduce the risk of coronary diseases, some types of cancers and other chronic diseases (Sanz, 2012). However, although wheat is a good source of calories and other nutrients, its protein content is of low nutritional quality when compared to milk, soy bean, pea and lupine because it is deficient in essential amino acids, such as lysine and threonine (Ndife *et al.,* 2011). The increased use of legume-based flours in different formulations of food products has raised great interest among researchers looking for high-quality sources of vegetable protein due to the limited availability and high cost of animal-based proteins in developing countries (Kohajdová *et al*., 2013; Raya-Pérez *et al*., 2012). The addition of legumes to cereal-based products could be an efficient way to increase the consumption of these products. Furthermore, legume proteins are rich in lysine but deficient in sulfur-containing amino acids; whereas, cereal proteins are deficient in lysine but have adequate levels of sulfur-containing amino acids. Thus, the combination of cereal and legume proteins would provide a more complete spectrum of essential amino acids, which is of great importance in a balanced diet (Kohajdová *et al*., 2013).

In a global context, cassava follows cereals (maize, rice and wheat) and potatoes in importance as a staple food. In Africa, the contribution of cassava as a food supply is much higher than in Latin America, where the local population does not depend on one single carbohydrate staple as the backbone of its diet. However, cassava contributes to a fundamental part of the basic food basket of the population of the seven departments of the Colombian coast, with great acceptability in all of its presentations (Fonseca *et al*., 2010; Charles *et al*., 2004).

In addition to the nutritional properties of raw materials used in foodstuffs, the functional properties, such as pasting properties of flour and starch, must be considered in the food industry because they greatly influence the characteristics of the products. However, in Colombia, research on the production of flour from beans is limited despite the fact that the consumption of this legume is well-established. Cassava, another potential substitute for traditional wheat flour, has been studied with respect to the characteristics of its flour and starch. However, the existing literature regarding the physicochemical and functional properties of these industrially obtained materials is limited. Cassava flour is made by washing and chipping roots, then drying and milling/refining them. These processes may affect the properties of cassava flour. The physicochemical and functional properties of cassava flour from different crops have previously been reported (Charles *et al*., 2004; Sandoval and Fernández, 2013). Cassava can be processed into high-quality flour for use as a partial substitute for wheat flour and other flours, such as maize and rice, and it can be used in the formulation of food products, such as bread, pasta, cake mixes, pastries, and in flour mixes for creams and soups. In addition, it can be used as a thickening agent and as an extensor in dehydrated soups, spice mixes, baby cereals, candy and processed meats (Henao, 2004; García *et al*., 2012).

The wheat consumed in Colombia is mainly imported because little is produced locally. However, previous studies have indicated the potential use of cassava-based flour in the partial substitution of traditional flours, without losing the organoleptic characteristics of the products coming from the bread-making industry (Henao, 2004). Furthermore, the addition of legumes to cereal-based products could be an efficient way to increase the nutritional content, especially if the flour is produced from biofortified raw material.

Given this context of (a) nutritional need, (b) the attractiveness of cookies for children, (c) the lack of wheat production in Colombia, and (d) the undemonstrated potential of bean-based flour, the objectives of this research were to develop biofortified cookies based on a bean-cassava-wheat flour mix and to assess their acceptability in a population of 7-11 year-old children.

Materials and methods

The methodology described a sequence of studies that have been conducted, grouped in order to initially define the best treatment for producing bean flour and, thereafter, identify the proper mix of the bean-cassava-wheat flour for the formulation of cookies, prioritizing those with the highest nutritional content and microbiological and sensorial quality.

Supplies

The biofortified bean cultivar SMN18, containing 175.3 mg kg^{-1} of Fe and 37.15 mg kg^{-1} of Zn (Tofiño *et al.,* 2011), was selected for the biofortified cookie preparation. This bean variety is expected to be released as a commercial variety in October of 2014 (Rodríguez, 2014; Vargas, 2014). The selected cassava variety HMC-1 has a high starch content and can expand more than traditional cassava, which makes it suitable for the bread-making industry (Henao, 2004). Commercial wheat flour (Haz de Oros®, Harinera del Valle, Cali, Colombia) was used.

Preparation of bean and cassava flours

Two flour treatments were developed, for which 8 kg of beans, previously classified, were used. The beans were divided into two batches of 4 kg each and washed with clean water to remove any impurities and to control the quality of the grains. The first batch (bean flour-1 treatment) was then pre-cooked for 1 h at 40ºC at a bean:water ratio of 1:4 (4 L of potable water/kg of beans), mashed and oven dried for 4 h at 100ºC. The second batch (bean flour-2 treatment) was pre-soaked for 14 h at a bean:water ratio of 1:3 (3 L of potable water/kg of beans), then allowed to drain and then left to oven dry at 60ºC for 16 h.

The milling was done after the pre-treatments, using sieves of three different diameters. Thereafter, the milled beans were passed through a 212 µm diameter sieve in order to obtain a fine and homogenous flour.

For the formulation of the cassava flour the stump of the root was removed and washed with clean water for at least 5 min in order to remove the soil and outer peel. The cassava was then passed through a chipping machine in order to cut the roots into smaller pieces, allowing for a more efficient drying process. The cassava pieces were distributed evenly on a fixed-bed dryer and dried for 8 h at 60ºC. The dried cassava pieces were left to rest and then passed through a milling and refining machine with a 177 µm sieve, which is the optimal size for the bread-making industry.

The dry-weights of the bean, cassava, and wheat flours were determined using 5 g of each sample in crucibles. The samples were oven dried for 4 h at 105ºC. Dry samples were kept in a dehumidifying chamber until the moment of weighing in order to avoid water absorption.

Nutritional quality of the bean and cassava flours

The nutritional quality of the raw materials, bean flour-1, bean flour-2, cassava flour and commercial wheat flour was determined.

These tests were done in the research laboratories of the International Center for Tropical Agriculture (CIAT) in Cali (Colombia). The protein content was determined using the Kjeldahl method, Fe and Zn through atomic absorption, and the ethereal extract, crude fiber and ashes were measured using the methods described by Horwitz (2006). Anti-nutritional metabolites, such as phenols, were analyzed according to Makkar (2003). Trypsin inhibitors and soluble, insoluble and total condensed tannins were evaluated following Terril *et al.* (1992).

Preparation of mixed flours

Two flour mixes of bean (bean flour-2), cassava and wheat with weight proportions (%, w/w) of (1) B(15)-C(15)-W(70) (bean-cassava-wheat) and (2) B(20)-C(15)-W(65), respectively, were prepared for further analysis.

Viscosity analysis

The analysis was done in the Root and Tuber Quality Laboratory at CIAT. A Rapid Visco Analyzer, series No. 4 (RVA-4) (Newport Scientific, Warriewood, Australia), was used to evaluate the pasting properties of the flours. A suspension of flour in distilled water at a concentration of 10% (w/w) was prepared and then exposed to heating and chilling in order to create viscogram profile/pasting curves that showed the relationship between time, viscosity and temperature during the cooking processes. The suspension was heated and kept at 90°C for 5 min; thereafter, it was chilled to 50°C and maintained at this temperature for 5 min. The temperature changes were done at a speed of 1.6°C min^{-1}. The two flour mixes of bean-cassava-wheat, based on bean flour-2, (% w/w of (1) B (15)-C (15)-W(70) (bean-cassava-wheat) and (2) B(20)-C(15)-W(65)), were analyzed, along with the pure flour from cassava, bean, wheat, and bean-cassava and the bean-wheat mixed flours (50-50).

Formulation and production of biofortified cookies

Cookies were made using the two mixed bean-cassava-wheat flours, as well as controls based on the traditional wheat flour. The formulation for the production of the cookies was as follows: 400 g of flour (cookie 1: B(15)-C(15)-W(70) mix, cookie 2: B(20)-C(15)-W(65) mix or control: pure wheat flour), 250 g of melted butter, 30 g of baking powder, 100 g of sugar, two eggs, 60 g of milk and 100 g of chocolate chips.

All ingredients, except the chocolate chips, were stirred together in a bowl until a homogeneous dough was obtained. The chocolate chips were then added and the dough was spread out evenly at a thickness of about 0.5 cm using a rolling pin. Once the dough was ready, it was divided into equal parts and molded to a desirable shape. Thereafter, each cookie was carefully placed on a previously floured tray. The biofortified cookies were baked for 15 min at 180°C. When the cookies started to brown, they were removed from the oven and left to cool before packaging.

Sensory acceptance

The sensory acceptance was studied following the principles of the Helsinki declaration (WMA, 1964), as approved by the Ethics Committee for Scientific Research at Universidad de Santander in Valledupar-Colombia.

The biofortified cookies made from the two flour mixes (cookie 1: B(15)-C(15)-W(70) mix, cookie 2: B(20)-C(15)-W(65) mix) were evaluated according to their appearance, color, aroma, flavor, softness, resistance, hydration ability, perception of granules, and final residues. The evaluation was done with children ranging from 7 to 11 years of age. Cookies made from pure wheat flour were used as a control. A five-point hedonic scale ranging from 1 to 5 was provided to indicate a sensory judgment of "dislike a lot" (1 point), "dislike a little" (2 points), "not sure" (3 points), "like a little" (4 points) and "like very much" (5 points).

Nutritional quality of the biofortified cookies

These tests were performed at the Nutritional Quality Laboratory of CIAT. Total phytates were determined using the methodology described by Latta and Eskin (1980), Zn was determined according to Hotz and Brown (2004), Fe was dialyzed as described by Argyri *et al.* (2009), and the protein digestibility was determined by the protocol used by McDonough *et al.* (1990).

Microbiological quality of the biofortified cookies

The determination of the microbiological quality of the biofortified cookies was done in the Bacteriological Laboratory at the Universidad de Santander (Valledupar). The mixed flour was analyzed for total aerobic mesophylls, count of moulds and yeasts, most probable number of total and faecal coliforms, presence of *Salmonella* sp. and *Staphylococcus* sp., positive test for Coagulase and count of *Bacillus cereus*, according to the Colombian Technical Norms parameters for evaluating food quality (Icontec, 2007).

Statistical analyses

All measures were done with a minimum of three replicates. The percentage of high valuations in the sensorial analysis was calculated. The sensorial acceptance study and the determination of antimetabolites were evaluated using the Tukey test, the rheological properties study using the Duncan test, and the preference study using the Z-test. Other parameters were analyzed using descriptive statistics, means and standard deviations. The analyses were done using Statgraphics® Software v. 9 (StatPoint®, Herndon, VA).

Results

The results are presented in the sequential order that was followed when elaborating the biofortified cookies based on the bean-cassava-wheat flour: 1) production of bean flour; 2) determination of the proportion of each of the components of the mixed flour; 3) preparation of cookies. In each step, the treatments with low nutritional and microbiological indicators were discarded and the best treatment was maintained for the following phase of the research scheme.

Nutritional quality of the bean flours

The treatments applied to the bean flour during processing did not affect the nutritional quality when compared to the crude grain. Bean flour-2 treatment maintained the highest contents of protein (35.9 g kg^{-1}), Zn (44.7 mg kg^{-1}), and crude fiber (34.8 g kg^{-1}), while bean flour-1 treatment kept the highest concentration of Fe (128.4 mg kg^{-1}) (Tab. 1). Although bean flour-2 presented the lowest Fe content, it was selected for further studies and use in the elaboration of mixed flour due to the preservation of the other nutrients.

The treatments applied to the bean grain in the process of obtaining the flour reduced the antimetabolites by up to 27%, resulting in an improvement of the nutritional quality (Tab. 2). However, the concentration of phenols as a

TABLE 1. Nutritional content of the wheat, cassava and bean flours.

Description	Protein (g kg^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Ethereal extract (g kg^{-1})	Crude fiber (g kg^{-1})	Ashes (g kg^{-1})
Wheat flour (control)	93.0±0.7 a	11.0±0.2 c	8.0±0.3 c	23.6±0.7 a	8.6±0.4 d	7.2±0.5 d
Cassava flour	13.4±0.4 d	-	-	6.0±0.4 d	29.6±0.3 b	22.5±0.6 c
Bean flour-1	34.7±0.3 c	128.4±0.3 a	43.8±0.1 b	9.9±0.5 c	28.2±0.2 c	49.9±0.4 a
Bean flour-2	35.9±0.3 b	113.5±0.2 b	44.7±0.2 a	21.5±0.4 b	34.8±0.2 a	47.6±0.4 b

Means with different letters in the same column indicate significant differences according to the Tukey test ($P \leq 0.05$).

TABLE 2. Content of anti-nutritional metabolites in the bean flours.

Treatment	Total phenols (%)	Trypsin inhibitors (mg kg^{-1})	Condensed soluble tannins (%)	Condensed insoluble tannins (%)	Condensed total tannins (%)
Bean flour-1	5.4±0.3 b	129.5±0.1 b	4.6±0.0 b	1.4±0.0 b	6.0±0.0 b
Bean flour-2	2.6±0.4 c	101.4±0.1 c	4.4±0.0 c	1.1±0.0 c	5.6±0.0 c
Bean grain	7.3±0.4 a	186.5±0.3 a	6.4±0.1 a	2.3±0.1 a	6.7±0.1 a

Means with different letters in the same column indicate significant differences according to the Tukey test ($P \leq 0.05$).

percentage of dry matter was higher in bean flour-1 (5.4%), as compared to bean flour-2 (2.6%). The physical treatment of the bean grain during processing affected the content of antimetabolites in the bean flour. A greater temperature in the pre-cooking and a greater exposure time to sunlight decreased the anti-nutrient content, as compared to cooked and ground beans. The percentage of antimetabolites, such as total phenols, trypsin inhibitors, insoluble and total condensed tannins, were lower in bean flour-2, as compared to bean flour-1. However, the content of soluble condensed tannins was stable in the two treatments, although below 10%, which indicates acceptable nutritional quality.

Viscosity analysis

The RVA-4 allowed the properties of the bean flour-2, cassava and wheat flours, and those of different mixes to be analyzed. There were no significant differences in the retrogradation among the various flour mixes. Their retrogradation values were found to be between those of the wheat flour (lowest value) and the cassava and bean flours (highest values) (Tab. 3). The viscosity values were found to be different between all the flours, with the cassava flour showing the highest values and the bean flour the lowest, followed by the wheat flour and mixed flours with intermediate values. Given that greater viscosity is associated with a better tolerance of shear stress, ease of working the dough, and durability of the final product, the mix of 15% bean flour-2, 15% cassava flour and 70% wheat flour, with a final viscosity of 848 cP, would be the most appropriate for producing cookies in order to obtain a desirable stable texture, brittleness and adherence (Mínguez, 2012). Based on the results, the mix of 20% bean flour-2, 15% cassava flour and 65% wheat flour, and the mix of 50% bean flour and 50% wheat flour, with lower levels of final viscosity, may be appropriate for bread-making (Tab. 3).

The bean flour and the cassava flour corresponded to the two extremes of the spectrum as the bean flour presented a very low viscosity, while the cassava flour had a viscosity that was 17.5 times higher than the viscosity of the bean flour and that was 8 times higher than the viscosity of the wheat flour. The tendency to revert was very low in the bean flour and, although it was somewhat higher in the cassava pastes and mixes, it continued to be low. The viscosity of

TABLE 3. Analysis of the hardening and viscosity of the mixed flours and the controls composed of pure bean, cassava and wheat flour.

Identification	Hardening temperature (ºC)	Viscosity of warm paste (cP)	Viscosity of cold paste (cP)	Minimum viscosity (cP)	Final viscosity (cP)	Retrogradation (cP)
Bean flour	78 a	123 g	203 g	93 g	237 g	1,440 a
Cassava flour	67 c	3,266 a	3,882 a	3,240 a	4,142 a	902 b
Wheat flour	67 c	645 f	505 f	470 f	519 f	49 d
Mixed flour B(15)-C(15)-W(70)	69 c	919 c	828 c	722 c	848 c	126 c
Mixed flour B(20)-C(15)-W(65)	73 b	891 d	804 d	680 d	819 d	139 c
Mixed flour B(50)-C(50)	68 c	1,494 b	1,654 b	1,490 b	1,756 b	266 c
Mixed flour B(50)-W(50)	79 a	765 e	671 e	598 e	696 e	98 c

Means with different letters in the same column indicate significant differences according to the Tukey test ($P \leq 0.05$).

the cassava flour tended to increase, even after reaching its first peak, showing its instability when compared to the wheat flour.

The rheological analysis of the flours showed that the hardening temperature of the evaluated samples ranged widely, from 67°C in the case of the wheat and cassava flours to 78°C for the bean flour, with intermediate temperatures for the mixed flours when aqueous suspensions at 10% starch concentration were used.

Sensory acceptance

The sensorial evaluation of the cookies based on the biofortified bean flour with cassava and wheat flour (Tab. 4) showed that both treatments presented sensorial characteristics superior to those of the control ($P \leq 0.05$), regarding aroma and flavor, while appearance, color, softness and final residues did not reveal a significant difference between the biofortified cookies and the control.

Nutritional quality of the biofortified cookies

The biofortified cookies prepared with the mixed flour B(15)-C(15)-W(70) (cookie 1) presented a significantly higher percentage of Fe when compared to the control, indicating that the proportion of ingredients in this treatment provided a higher content of Fe for further absorption by consumers. On the other hand, the phytate content, Zn, and protein digestibility analysis did not show any significant differences in the biofortified cookies when compared to the control (Tab. 5).

According to these results, significant differences were not found among the three cookies in terms of protein digestibility or in casein concentration, which in the control was 90%, indicating a high nutritional quality.

Microbiological quality

The microbiological analysis of the bean flour showed that it fulfills the optimal hygienic conditions for human

TABLE 4. Sensory acceptance of the biofortified cookies.

Treatment	Sensory attributes								
	Appearance	Color	Aroma	Flavor	Softness	Resistance	Hydration ability	Perception of granules	Final residues
Cookie 1	3.9 a	4.0 a	4.2 a	4.0 a	3.7 a	3.6 a	4,0 a	4.0 a	3.6 a
Cookie 2	4.3 a	4.5 a	4.3 a	4.3 a	3.7 a	3.9 a	3.8 a	4.0 a	3.7 a
Control	3.9 a	4.0 a	3.3 b	2.7 b	3.1 a	3.0 b	3.2 b	3.2 b	3.2 a

Cookie 1, 15% bean flour, 15% cassava flour and 70% wheat flour (w/w); cookie 2, 20% bean flour, 15% cassava flour and 65% wheat flour (w/w); Control, pure-wheat flour.
Means with different letters in the same column indicate significant differences according to the Tukey test ($P \leq 0.05$).

TABLE 5. Determination of antimetabolites, total phytates, phytic acid to Zn mole ratio, dialyzed Fe and protein digestibility in two types of biofortified cookies based on mixed flours and in control cookies based on wheat flour.

Sample	Total phytates (mg g^{-1})	Phytic acid to Zn mole ratio	Dialyzed Fe (%)	Protein digestibility (%)
Control	2.572±0.23	38.921±3.58	5.807±0.17b	74.933±0.73
Cookie 1	2.775±0.46	31.576±5.24	6.662±0.40a	74.258±0.45
Cookie 2	2.922±0.45	33.254±5.17	5.781±0.24b	74.336±0.94
Casein				88.472±0.32

Means with different letters in the same column indicate significant differences according to the Tukey test ($P \leq 0.05$).
Total phytate concentration on a dry weight basis.

TABLE 6. Microbiological analysis of the flour used in the production of the biofortified cookies.

Analysis	Method	Reference limit	Result
Total count of aerobic mesophylls cfu/g	NTC4519	200,000-300,000	21,000
Mold count cfu/g	NTC4132	1,000-2,000	400
Yeast count cfu/g	NTC4132	1,000-2,000	<10
MPN total coliforms/g	NTC4516	43-150	93
MPN fecal coliforms at 45°C/g	NTC4516	<3	<3
Salmonella in 25 g	NTC4574	Absent	Absent
Coagulase positive *Staphylococcus* CFU/g	NTC4779	<100	<100
Bacillus cereus count CFU/g	NTC4679	700-1,000	300

MPN, most probable number; cfu, colony forming units; NTC, Colombian Technical Norm (*Norma Tecnica Colombiana*) (Icontec, 2007).

consumption, according to the Colombian Technical Norms, with microbiological counts below the established limits (Tab. 6).

Discussion

The nutritional content of the bean flours exceeded the expected values established by the CIAT Biofortification Program of 100 mg kg^{-1} of Fe and 40 mg kg^{-1} of Zn (Rodríguez, *et al*., 2009). This indicates the potential of biofortified beans and flour for use in the development of innovative food products that combat malnutrition.

The high dietary fiber content of the bean and cassava flours made them functional foods, considering that the daily requirement of fiber in a diet of 2,000 Kcal is 25 g (Díaz *et al*., 2012). Numerous studies have indicated that crude fiber can play a major physiological role in maintaining good health and general wellbeing in consumers (Lamsal and Faubion, 2009; Angioloni and Collar, 2011; Chandrasekara and Shahidi, 2011). Food products with a high content of dietary fiber improve gastrointestinal flow, help reduce cholesterol levels, and are excellent sources of probiotics, which contribute to increased beneficial microbiota in the human body. Moreover, these food products have a glycaemic index that is lower than in similar products with a lower fiber content, which helps in regulating the levels of glucose in the blood and reduces the risk of diseases and gastrointestinal disorders.

The lower concentration of total proteins in the bean flour as compared to the wheat flour was compensated for by higher contents of Fe, Zn, crude fiber and ashes, which makes it an alternative raw material for diversification in the production of food products, contributing to an increased nutritional status of populations with mineral and crude fiber deficiencies. Additionally, the consumption of cereal grains and legumes in one single food product increases the quality of consumed protein due to the complementing amino acids from both raw materials (Ndife *et al*., 2011).

The reduction in antimetabolites with the pre-treatment of the bean flour can be explained by the trypsin inhibitors, which are heat labile and can be inactivated by heat treatments, such as steaming and extrusive cooking (Liener, 1994). Raya-Pérez *et al.* (2012) obtained similar results regarding the inhibition of trypsin enzyme activity, which was greatly reduced after a thermic treatment of black cherry (*Prunus serotina*) seeds, without affecting their nutritional quality.

The rheological properties of the dough can predict the performance of the finished product if the applied force and deformation are in the same range as those used in the actual processing. Also, the characteristics of the strength of the gel are correlated to the ageing of the bread (Rodríguez *et al*., 2009). In the development of food products, the peak and final viscosities are important in order to understand their behavior during and after processing. Mixes that present a greater viscosity and lower retrogradation are more stable and of higher potential for use in the production of cookies, given the lower level of water absorption which results in durability and firmness in the product for a longer time (Singh *et al*., 2011; Mínguez, 2012). Although they had a significantly lower viscosity and higher retrogradation than the wheat flour, the mixed flours showed intermediate values, indicating a high potential for the production of bread products, with mixed flour B(15)-C(15)-W(70) displaying a higher viscosity than mixed flour B(20)-C(15)-W(65).

Sajilata *et al*. (2006) reported that the gelatinization properties of starches depend on the type, granular structure, botanical origin, and amylose/amylopectin ratio. The difference between the flour types in viscosity might be due to the weakness of the intermolecular network, which may cause the flour granules to fall apart when gelatinized in hot water, thus forming a paste of relativity low viscosity. It might also be due to changes in the flour protein interaction or a result of the reduction in protein because of carbohydrate and protein interactions (Balogun and Olatidoye, 2010). The unique bread-making properties of the wheat flour can be attributed mainly to the gluten protein's ability to form a viscoelastic network when mixed with water. The reduction of viscoelectric properties of the wheat flour dough seen with substitution by starch, or non-wheat flour, reduced the bread-making potential. This phenomenon can be explained as a reduced capacity of the gluten network to slow down the rate of carbon dioxide diffusion (Defloor *et al*., 1993).

The nutritional characterization of the biofortified cookies based on the mixed flour indicated their high quality, considering that the percentage of dialyzable Fe has been described as a reliable indicator of its availability in food products (Sanz, 2012). However, the bioavailability of Zn, estimated by means of the phytate content, showed low values in the biofortified cookies based on the mixed flour, as well as the control treatment based on the wheat flour, since the phytic acid to Zn mole ratios were higher than 15 (WHO, 2013).

Phytic acid (or phytate), an organic form of P found in the seeds of higher plants, may interact with trace elements. Many studies have shown that a diet rich in phytates causes mineral deficiencies (Zn and Fe), especially in unbalanced diets and in populations at risk because of diets based on animal protein (Afify *et al*., 2011). However, beneficial health effects have also been shown. The consumption of phytic acid can have a positive impact on certain diseases, such as coronary heart disease, diabetes, arteriosclerosis, and kidney stones. Furthermore, phytic acid is the precursor of molecules that provide protection against a variety of cancers (Sanz, 2012). The chelating properties of phytic acid not only result in the binding of cations in seeds, when released during food or feed processing or in the gut, phytic acid also binds minerals and makes them unavailable as nutritional factors. Iron and Zn uptake have both been shown to be inhibited when the phytic acid to metal ratio is above 10 (Gharib *et al*., 2006). Numerous experimental studies with animals have shown that the phytic acid content in their feed reduces Zn bioavailability. Results from research on humans have shown the same, showing that high levels of this anti-nutrient may cause Zn deficiency (Martínez *et al*., 2002). However, the majority of food products that present elevated contents of phytic acid are good sources of dietary fiber, which has a high affinity for minerals. Although the phytates and fiber were separated and evaluated independently, it was difficult to attribute the negative effects in the bioavailability of the minerals to only the presence of phytates. Also, a genetic selection of seeds low in phytates would result in them having a lower phosphorous (P) content as phytic acid aids in the efficiency of P utilization. However, genetic improvement programs should not be directed towards reducing the content of phytic P, but maintaining the total content of P in the plant and in the seeds, as well as other desirable constituents (Martínez *et al.,* 2002).

According to the results, significant differences were not found among the three cookies (cookie 1: B (15)-C (15)-W(70), cookie 2: B (20)-C (15)-W (65), control: pure wheat flour) in terms of protein digestibility or in casein concentration, which in the control was 90%, indicating a high level of nutritional quality in the formulations. Similar results were obtained by Pastuszka *et al*. (2012) despite the fact that, in this study, the new preparations had a greater protein content, as compared with the wheat flour, due to the addition of amino acids (7.5-10% oat protein).

Regarding the sensory evaluation, our results are in agreement with those published by (Balasubramanian *et al*., 2012), which showed that up to 15 % of the legume content can be incorporated in extrudate without losing sensory characteristics. Also, Abou-Zaid *et al.* (2012) concluded that wheat flour could be replaced by up to 30 % with different types of substitutes, in their case milled mushroom micelles grown on sorghum or wheat grains, to obtain food products that had small differences compared to the control.

Studies related to the occurrence of microorganisms and metabolites with potential toxicity in children's food have received limited attention in developing countries (Shadlia-Matug *et al*., 2008). Products containing cereals in the form of grains or flours are rich in sugars and protein, which serve as nutrient sources for airborne microorganisms, as well as for those originating from the soil, insects and tools that frequently cause cross contamination with a wide variety of yeasts, fungi, and bacteria. These microorganisms also provide the risk of further contaminating the final product by producing toxins. Among the pathogens frequently found are *Salmonella, Staphylococcus aureus* and *Clostridium perfringens,* depending on the product (Ray and Bhunia, 2013). Coliform bacteria and *Escherichia coli* counts are important as these are indicative of the general hygienic properties of the foodstuffs. Moreover, the presence of *E. coli* in a finished, ready-to-eat, product is a public health concern, indicating deficiencies in the microbiological control of the process (inadequate processing conditions or post-process contamination).

Some research has also shown the occurrence of *Enterobacteriaceae* in different types of food intended for children, although *Salmonella* sp. was not detected in any of the samples analyzed by several authors cited by Iversen and Forsythe (2004). However, pathogens, such as *Listeria monocytogenes* and *Salmonella* sp., have been shown to be tolerant of the dispersion and drying processes in the production of some formulas for children (In't Veld *et al*., 1991). In their study regarding the microbiological quality of cookies based on cassava flour and bovine plasma, Benítez *et al*. (2011) showed an absence of microorganisms of interest from a health standpoint after storage of the product for 15 d at 25 to 28°C without preservatives.

Many epidemic outbreaks with toxins in foodstuffs destined for children have been caused by *B. cereus.* This bacterium is naturally found in the soil, grows at room temperature in starchy environments, and produces a toxin that is heat resistant and easily transmitted through vegetables and other crops (Okahisa *et al.,* 2008). Additionally, the inappropriate storage of cereal-based food products in humid conditions, warm temperatures, and poor ventilation, can cause contamination by mycotoxins produced by fungus, which may be carcinogenic (Shadlia-Matug *et al*., 2008).

According to the WHO (2013), many countries do not comprehensively address malnutrition in all its forms, including the vicious circle of malnutrition and food-borne and other infectious diseases. Food security policies should not be limited to the area of availability of food since, although the criteria of availability may be achieved, the biological usefulness of the food products can be affected if food safety is not considered and, therefore, the adequate beneficial effects for consumers are no longer guaranteed.

Conclusions

A procedure for obtaining bean flour with high contents of protein, Fe, Zn and fiber was obtained alongside the development of a new formulation for cookies based on bean-cassava-wheat mix flours with a significantly higher Fe content than the wheat flour-based control, with a satisfactory microbiological quality and superior sensory acceptance. This new formulation could contribute to increasing the nutritional welfare of Colombian children through the consumption of biofortified cookies. Additionally, the rheological categorization of the flours that were developed suggests other possible uses in the bread-making industry, substituting cereal-based flours with mixed flours (bean-cassava-wheat) with a higher nutritional quality. However, further studies are needed to determine the nutritional effects on children as a result of the regular ingestion of biofortified cookies.

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Literature cited

Abou-Zaid, A.A.M., M.A.S. El-Bandy, and H. Ismaeil. 2012. Rheological properties and quality evaluation of pan bread and biscuits supplemented with mushroom micelles flours. Aust. J. Basic Appl. Sci. 6, 237-245.

Afify A.E.-M.M.R., H.S. El-Beltagi, S.M. Abd El-Salam, and A.A. Omran. 2011. Bioavailability of iron, zinc, phytate and phytase activity during soaking and germination of white sorghum varieties. PLOS One 6, e25512. Doi: 10.1371/journal.pone.0025512

Angioloni, A. and C. Collar. 2011. Physicochemical and nutritional properties of reduced-caloric density high-fibre breads. LWT-Food Sci. Technol. 44, 747-758. Doi: 10.1016/j.lwt.2010.09.008

Argyri, K., A. Birba, D.D. Miller, M. Komaitis, and M. Kapsokefalou. 2009. Predicting relative concentrations of bioavailable iron in foods using *in vitro* digestion: new developments. Food Chem. 113, 602-607. Doi: 10.1016/j.foodchem.2008.07.089

Balasubramanian, S., A. Borah, and C.L. Mahanta. 2012. Rheological and nutritional properties of legumes incorporated corn extrudates. Int. Food Res. J. 19, 971-975.

Balogun, I.O. and O.P. Olatidoye. 2010. Functional properties of dehulled and undehulled velvet beans flour (*Mucuna utilis*). J. Biol. Sci. Bioconserv. 2, 1-10

Benítez, B., K. Ferrer, A. Archile, Y. Barboza, L. Rangel, E. Márquez, and M.L. Delmonte. 2011. Calidad microbiológica de una galleta formulada a base de harina de yuca y plasma de bovino. Rev. Fac. Agron. (LUZ) 28, 260-272.

Chandrasekara, A. and F. Shahidi. 2011. Bioactivities and antiradical properties of millet grains and hulls. J. Agric. Food Chem. 59, 9563-9571. Doi: 10.1021/jf201849d

Charles, A.L., Y.H. Chang, W.C. Ko, K. Sriroth, and T.C. Huang. 2004. Some physical and chemical properties of starch isolates of cassava genotypes. Starch-Stärke 56, 413-418. Doi: 10.1002/star.200300226

Defloor, I., M. Nys, and J.A. Delcour. 1993. Wheat starch, cassava starch, and cassava flour impairment of the breadmaking potential of wheat flour. Cereal Chem. 70, 526-530.

Díaz M., C., C. Zuluaga, C. Morales, and M. Quicazán. 2012. Determinación de fibra dietaría en polen apícola colombiano. Vitae 19(sup. 1), S454-S456.

Dibb, D.W., T.L. Roberts, and R.M. Welch. 2005. De la cantidad a la calidad: la importancia de los fertilizantes en la alimentación humana. Inf. Agron. 28, 1-8

Fonseca C., Z., A.P. Heredia V., P.R. Ocampo T., Y. Forero T., O.L. Sarmiento D., M.C. Álvarez U., A. Estrada R., B. Samper K., J. Gempeler R., and M. Rodríguez G. 2011. Encuesta nacional de la situación nutricional en Colombia 2010 ENSIN. ICBF; Profamilia; Instituto Nacional de Salud, Ministerio de la Protección Social, Bogota.

García, J.A., L. Alonso, S. Gallego, J.A. Aristizábal, S. Henao, A.M. Bonilla, and A. Giraldo. 2012. Production and uses of refined cassava flour. pp. 442-462. In: Ospina, B. and H. Ceballos (eds.). Cassava in the third millennium. Modern production, processing, use and marketing systems. International Center for Tropical Agriculture (CIAT); Latin American and Caribbean Consortium to Support Cassava Research and Development (Clayuca), Cali, Colombia.

Gharib, A.G., S.G. Mohseni, M. Mohajer, and M. Gharib. 2006. Bioavailability of essential trace elements in the presence of phytate, fiber and calcium. J. Radioanal. Nucl. Chem. 270, 209-215. Doi: 10.1007/s10967-006-0330-3

Horwitz, W. 2006. Official methods of analysis of AOAC International. 18th ed. Association of Official Analytical Chemists (AOAC), Gaithersburg, MD.

Henao, S.O. 2004. Estudio tecnológico de la utilización de harina de yuca en panificación. Undergraduate thesis. Faculty of Agricultura Sciences, Universidad Nacional de Colombia, Palmira, Colombia.

Icontec, Instituto Colombiano de Normas Tecnicas y Certificacion. 2007. Norma Técnica Colombiana. Productos de molinería. Galletas. NTC 1241. Bogota.

In't Veld, P.H., P.S.S. Soentoro, E.H.M. Delfgou-Van Asch, and S. Notermans. 1991. Influence of reconstitution on isolation and enumeration of *Listeria monocytogenes* from milk powder used for reference samples. J. Food Prot. 54, 124-126.

Iversen, C. and S. Forsythe. 2004. Isolation of *Enterobacter sakazakii* and other Enterobacteriaceae from powdered infant formula milk and related products. Food Microbiol. 21, 771-777. Doi: 10.1016/j.fm.2004.01.009

Kohajdová, Z., J. Karovicová, and M. Magala. 2013. Rheological and qualitative characteristics of pea flour incorporated cracker biscuits. Croat. J. Food Sci. Technol. 5, 11-17.

Lamsal, B.P. and J.M. Faubion 2009. The beneficial use of cereal and cereal components in probiotic foods. Food Rev. Int. 25, 103-114. Doi: 10.1080/87559120802682573

Latta, M. and M. Eskin. 1980. A simple and rapid colorimetric method for phytate determination. J. Agric. Food Chem. 28, 1313-1315. Doi: 10.1021/jf60232a049

Liener, I.E. 1994. Implications of antinutritional components in soybean foods. Crit. Rev. Food Sci. Nutr. 34, 31-67. Doi: 10.1080/10408399409527649

Makkar, H.P.S. 2003. Quantification of tannins in tree and shrub foliage. A laboratory manual. Springer, Dordrecht, The Netherlands.

Martínez D., B., M.V. Ibáñez G., and F. Rincón L. 2002. Ácido fítico: aspectos nutricionales e Implicaciones analíticas. Arch. Latinoam. Nutr. 52, 219-231.

McDonough, F.E., F.H. Steinke, G. Sarwar, B.O. Eggum, R. Bressani, P.J. Huth, W.E. Barbeau, G.V. Mitchell, and J.G. Phillips. 1990. In vivo rat assay for true protein digestibility: collaborative study. J. Assoc. Off. Anal. Chem. 73, 801-805.

Mínguez D., J. 2012. Caracterización de la harina de teff para su uso en panificación. MSc thesis. Escuela Técnica Superior de Ingenierías Agrarias, Universidad de Valladolid, Valldolid, Spain.

Ndife, J., L.O. Abdulraheem, and U.M. Zakari. 2011. Evaluation of the nutritional and sensory quality of functional breads produced from whole wheat and soya bean flour blends. Afr. J. Food Sci. 5, 466-472.

Noor Aziah, A.A., A.Y. Mohamad Noor, and L.H. Ho. 2012. Physicochemical and organoleptic properties of cookies incorporated with legume flour. Int. Food Res. J. 19:1539-1543. Doi: 10.13140/2.1.4554.8164

Okahisa, N., Y. Inatsu, V.K. Juneja, and S. Kawamoto. 2008. Evaluation and control of the risk of foodborne pathogens and spoilage bacteria present in Awa-Uirou, a sticky rice cake containing sweet red bean paste. Foodborne Path. Dis. 5, 351-359. Doi: 10.1089/fpd.2007.0073

Pastuszka, D., H. Gambuś, R. Ziobro, B. Mickowska, K. Buksa, and R. Sabat. 2012. Quality and nutritional value of wheat bread with a preparation of oat proteins. J. Microbiol. Biotechnol. Food Sci. 1(Special issue), 980-987

Ray, B. and A. Bhunia. 2013. Fundamental food microbiology. 5th ed. CRC Press, Boca Raton, FL.

Raya-Pérez, J.C., C.L. Aguirre-Mancilla, R. Tapia-Aparicio, J.G. Ramírez-Pimentel, and J. Covarrubias-Prieto. 2012. Caracterización de las proteínas de reserva y composición mineral de la semilla de capulín (*Prunus serotina*). Polibotánica 34, 203-215.

Rodríguez P., M.A. 2014. Cultivos biofortificados, un bocado contra el hambre. Vanguardia Liberal, 8 June 2014. Bucaramanga, Colombia.

Rodríguez, E., E. Lorenzo, A. Melgar, E. Palacios, F. Gonzáles, and A. Quintero. 2009. Biofortificación del frijol común (*Phaseolus vulgaris* L.) en Panamá con micronutrientes. IDIAP; SENACYT; CIAT, Panama.

Sajilata, M.G., R.S. Singhal, and P.R. Kukarni. 2006. Resistance starch - a review. Comp. Rev. Food Sci. Food Saf. 5, 1-17. Doi: 10.1111/j.1541-4337.2006.tb00076.x

Sandoval A., A. and A. Fernández Q. 2013. Physicochemical characterization of two cassava (*Manihot esculenta* Crantz) starches and flours. Sci. Agroaliment. 1, 19-25.

Sanz P., J.M. 2012. Nuevas estrategias para incrementar la calidad nutricional de productos de panadería. Efecto sobre el contenido de fitatos y la biodisponibilidad de hierro en caco-2. PhD thesis. Consejo Superior de Investigaciones Científica (CSIC), Valencia, Spain.

Shadlia-Matug, M., K.E. Aidoo, A.A. Candlish, and A.M. Elgerbi. 2008. Evaluation of some antibiotics against pathogenic bacteria isolated from infant foods in North Africa. Open Food Sci. J. 2, 95-101. Doi: 10.2174/1874256400802010095

Singh, G.D., C.S. C.S. Riar, C. Saini, A.S. Bawa, D.S. Sogi, and D.C. Saxena. 2011. Indian water chestnut flour-method optimization for preparation, its physicochemical, morphological, pasting properties and its potential in cookies preparation. LWT - Food Sci. Technol. 44, 665-672. Doi: 10.1016/j.lwt.2010.09.015

Terril, T.H., A.M. Rowan, G.B. Douglas, and T.N. Barry. 1992. Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereal grains. J. Sci. Food Agr. 58, 321-329. Doi: 10.1002/jsfa.2740580306

Tofiño, A., R. Tofiño, D. Cabal, A. Melo, W. Camarillo, and H. Pachón. 2011. Evaluación agronómica y sensorial de fríjol (*Phaseolus vulgaris* L.) mejorado nutricionalmente en cinco municipios del departamento del Cesar. Perspect. Nutr. Hum. 13, 60-71.

Vargas, O. 2014. Biofortificación, alternativa para la nutrición humana. Hoy Diario de Magdalena, 16 June 2014. Cartagena, Colombia.

WHO, World Health Organization. 2013. Global nutrition policy review: what does it take to scale up nutrition action? Geneva, Switzerland.

WMA, World Medical Association. 1964. World medical association declaration of Helsinki. Ethical principles for medical research involving human subjects. Helsinki, Finlandia.