Production of tortillas from nixtamalized corn flour enriched with Andean crops flours: Faba-bean (*Vicia faba*) and white-bean (*Phaseolus vulgaris*)

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**ABSTRACT**

This study aims to produce corn nixtamalized tortillas enriched with faba-bean (25%, 50%, 75% w/w) and white-bean (25%, 50%, 75% w/w) flours. Faba-bean and white-bean are Andean crops (AC) rich in protein, carbohydrates, fiber, minerals, vitamins, and gluten-free. Tortillas were characterized in terms of proximal, physicochemical, sensorial, microbiological, and texture properties. Proximal composition shows that corn flour has 14.5 % less protein, 0.83 % less ash, and 1.39 % fatter than faba-bean flour, while in white-bean flour, the fiber content is three times higher. Moisture content was less than 14 %, which guarantees the control shelf-life; gluten content was approximately 5 ppm. Granulometry properties showed that flours have coarser than finesse particles, water absorption capacity showed a range of 60 to 80 g of water for 100 g of flour. In nixtamalized tortillas, high protein content was observed in samples with 25 % of corn and 75 % of two different Andean crops. Enriched tortillas showed lower lipid content, higher dietary fiber, and higher ash content than the control sample. The sensorial analysis showed that the best formulation based on overall acceptability was 25% (w/w) of corn flour and 75% of white-bean flour. The oil content showed that the samples absorbed about 8% of oil during the toasted. The hardness parameter showed that the sample CPB2575: Corn flour (25%) + White-bean flour (75%); was harder than the control. The microbiological evaluation established that the tortillas comply with the normative what indicates the absence of harmful microorganisms to public health. Color parameters showed that samples tend to lightness with a tendency to reddish color in enriched tortillas while in control are greenish. Andean crop flours are one alternative to increase the nutritional value of corn tortillas with acceptable sensorial characteristics.

**Keywords:** Andean crops; Legumes; Gluten-free; Nixtamalization; Enrichment.

**INTRODUCTION**

The nixtamalization process produces a high nutritional value and extraordinary functional changes (Gutiérrez-Cortez et al., 2010). This process is essential in producing the tortilla that has been the leading food in indigenous peoples’ diets and the basis of their survival for more than 3,500 years; it has been transmitted from generation to generation in Mesoamerican cultures and is still used in pre-Hispanic times (Rojas-Molina et al., 2007; Waliszewski et al., 2002). The process begins with adding two parts of a 1% lime solution or wood-burning ash to one portion of corn, which is the base of this process (Escalante-Aburto et al., 2020; Vaca-García et al., 2011). Also, nixtamalization has been used to refer to the alkaline process of cooking corn to turn it into a dough (a mixture of amylose and amyllopectin with partially gelatinized starch granules, intact granules, parts of endosperm, and lipids). This dough can be used in a wide range of preparations, among which tortillas are the most important (Bryant and Hamaker, 1997; Espinosa-Ramírez et al., 2020; Pérez et al., 2002). Since the middle of the 20th century, a series of studies have been carried out to understand the effect that the alkaline cooking process has on the nutritional quality of corn (Briones et al., 2000; Cabrera-Ramírez et al., 2020). For example, alkaline cooking alters corn proteins’ structure and solubility (Escarlante-Aburto et al., 2020; Larkins, 2019).

The nutritional value of corn depends on the genotype of the variety, the environment, and the sowing conditions; the protein content is 10% and is considered to have low nutritional quality because in the zeins, which are the main...
protein fraction of corn, the essential amino acids lysine and tryptophan are in low concentrations (Cháidez-Laguna et al., 2016; Hernandez-Chavez et al., 2019; Jiang et al., 2018). On the other hand, legumes as unconventional raw materials are very suitable for fortification due to its high protein content. Different studies have been developed for the fortification of tortillas with different flours. In some cases, the use of unconventional flours has been demonstrated significant advances; in others, fortification has altered the sensorial and textural properties (Reyes-Moreno et al., 2013). Hernández-Chavez et al. (2019) studied the use of lupin flour for the fortification of tortillas in Mexico, it was found that the addition of Lupinus flour produced higher adhesiveness and hardness in tortillas, and also there were no changes in color in contrast with control. Although tortillas have become the basis of a large population’s diet, Mexico continues to lead the consumption rate; however, in other countries, the trend of consumption is high. In this regard, it is essential to note that maize tortillas are high in calories but deficient in proteins; Argüello-García et al. (2017) examines the effect of fortification of maize tortillas with nontoxic *Jatropha curcas* flour. The results showed a slight modification, but as was expected, the protein content increased 10.8%, it was found that there was no change in color, and consumer acceptance was not affected. As was reported, legumes are an important source of protein and other nutrients; in this sense, Faba-bean (*Vicia faba*) is considered healthy due to its protein content, is recommended for vegetarians and vegans (Kumari and Sangeetha, 2017; Salamanca-Bautista et al., 2018). It is rich in iron, so its consumption is recommended in people suffering from anemia, also provides potassium that favors the proper functioning of the nervous system ( Crépon et al., 2010; Millar et al., 2019). Likewise, fiber content contributes to regulating intestinal transit, reducing cholesterol, and preventing cardiovascular diseases. Even knowing the good nutritional quality of this crop, its consumption has been reduced in the last time because people do not know the nutritional and biological value of this crop (Ambigaipalan et al., 2011; Salamanca-Bautista et al., 2018). On the other hand, the White-bean stands out for its content of magnesium, rich in proteins, carbohydrates, fiber, minerals, and vitamins; starch represents the main fraction in this crop, even though, during cooking, part of it is unavailable since it is transformed into the so-called resistant starch (Bryant and Hamaker, 1997). The role of white-bean fiber as a phytochemical is due to its hypocholesterolemic effect, which is because it reduces blood cholesterol by up to 10% (Cortes et al., 2006; Hughes, 1991). In this study, proximal, physicochemical, sensory, microbiological, and texture properties of tortillas made from nixtamalized corn with the addition of faba-bean and white-bean flours were analyzed.

### MATERIALS AND METHODS

#### Material

Corn, faba-bean, and white-beans were purchased in a local market of Ambato-Ecuador. Firstly, to obtain flours, the grains that had physical damage were discarded; the ash for corn nixtamalization was obtained from firewood’s combustion. The commercial nixtamalization was developed according to the procedure proposed by Gomez et al. (1989). White corn (10 kg), water (18.5 kg), and ash (0.63 kg) were cooked for 60 min (maximum temperature was 89 °C) and allowed to steep with air agitation for 20-24 hr.

The steeped corn was washed to remove waste and corn peel. The corn, faba-bean, and white-bean were uniformly distributed in trays and dried in a convective dryer (Gander MTN) at 60 °C for 24 hours or until reaching the minimum moisture (12%). Once dry, they were milled in an industrial mill (Inox Equip, Ecuador) (Fig. 1) and hermetically packed in aluminized bags at room temperature (25 °C). The mixed flours for the study were calculated according to the substitution levels (Table 1). For tortilla production, the dough was adjusted to portions of 180 g (100 g of flour and 80 g of water), and for preparing individual tortillas, the dough was weighed into approximately 10-gram portions (Fig. 2).

#### Flour Analysis

**Proximal Analysis**

A proximal analysis of the flours and tortillas was performed using the following AACC (2000) methods: Moisture (method 44-19); ash (method 08); Soxhlet method with petroleum ether for fat (method 20-30); acid and alkaline hydrolysis for crude fiber (AOAC method 962.09); and Kjeldahl method for protein content estimated by the nitrogen portion using a 6.25 factor. Carbohydrate content was calculated by difference. The analysis of gluten was carried out using the liquid chromatography method according to the methodology described by Wieser et al. (1998).

#### Table 1: Formulations for the production of tortillas

| Samples          | Corn flour (%) | Fava-bean flour (%) | White-bean flour (g) |
|------------------|----------------|---------------------|----------------------|
| Control          | 100            | -                   | -                    |
| CPB7525          | 75             | 25                  | -                    |
| CPB5050          | 50             | -                   | 50                   |
| CPB2575          | 25             | -                   | 75                   |
| CBF7525          | 75             | 25                  | -                    |
| CBF7525          | 50             | 50                  | -                    |
| CBF2575          | 25             | 75                  | -                    |

Control: Corn flour (100%); CPB7525: Corn flour (75%)+White-bean flour (25%); CPB5050: Corn flour (50%)+White-bean flour (50%); CPB2575: Corn flour (25%)+White-bean flour (75%); CBF7525: Corn flour (75%)+Faba-bean flour (25%); CBF7525: Corn flour (50%)+Faba-bean flour (50%); CBF2575: Corn flour (25%)+Faba-bean flour (75%);
Granulometry
The particle size was determined according to the Standard AOAC 965.22. One hundred grams of flour was placed in a set of 5 sieves (Tyler series, USA), the diameter of sieves decreases downward, and they were labeled as 40 (420 μm), 60 (250 μm), 100 (149 μm), 140 (106 μm), 200 (75 μm) and the pan collector. The sieves were mechanically vibrating in shaker equipment (Porter Sand, USA) for 5 minutes. Finally, each mesh flour was removed with a brush, and the flour retained in each sieve was weighed. The tests were carried out in triplicate.

Water absorption capacity
Water absorption capacity (WAC) was determined using five grams of sample, which was weighed into a centrifuge tube, and 30 ml of distilled water at a temperature of 25 °C was added. The mixture was left to rest for 30 min and centrifuged using a Hettich® centrifuge (D-78532, Hettich®, Germany), at 3000 rpm for 15 min. The supernatant was decanted, and the increase in weight was noted by weighing. The water absorption capacity was expressed as water absorbed (g/100 g dry sample). The determination was done in duplicate.

Tortillas analysis
Tortilla preparation
The dough was compressed into thin disks of approximately 8 cm diameter, 2 mm thickness, and 10 g weight, using a commercial tortilla press machine (Corempro SKU 13920, Guayaquil, Ecuador). Tortillas were toasted on a griddle at 190 °C with 1 ml of palm oil for two minutes, 1 minute by each side. The griddle’s temperature was measured with a non-contact portable infrared thermometer (PCE- 670, Spain).

Oil content on toasted tortillas
The tortillas’ total oil content was determined using Soxhlet extraction with petroleum ether (AACC, 1986a). The total fat content obtained in raw tortillas was subtracted from the fat value obtained in toasted tortillas to establish the gain percentage of fat in tortillas.

Texture profile analysis (TPA)
The texture of tortillas was determined in terms of the maximum extensibility force (N) and distance (mm) according to the method described by (Ruiz-Gutiérrez et al., 2012) using a texturometer (Pro CT3 Brookfield, USA); tortilla was formed and extended in the texturometer until breaking. The results are the average of three different trials, each with at least ten determinations.

Microbiological analysis
Microbiological analysis was developed using ten grams of sample, which were aseptically placed into a sterile
stomacher bag. It was then homogenized with 90 ml of sterile peptone water (Difco, Le Pont de Clai, France). For each treatment, appropriate serial decimal dilutions were prepared in sterile peptone water. Mold and yeast were incubated at 25 °C and evaluated using Potatoe Dextrose Agar (PDA) (Difco, Le Pont de Clai, France). Viable aerobic mesophilic microorganisms in PCA agar (Difco, Le Pont de Clai, France) incubated at 30 °C for 72 h, Enterobacteriaceae on a double layer of Violet Red Bile Glucose Agar (VRBG) (Acumedia, Michigan, USA). All analyses were performed in triplicate.

Color
Color of tortillas was measured by a colorimeter (ColorFlex EZ, HunterLab, USA) using CIELAB® color scale with the parameters L* (lightness), a* (red / green), b* (yellow / blue). The polar coordinate or saturation Chroma C* was calculated using the expression $C^* = \sqrt{(a^2 + b^2)}$, Hue angle ($^\circ$H) with the equation $(^\circ$H) = $\arctg (b*/a^*)$, and whiteness index (WI) with the equation $WI = L - 3b + 3a$. The flour samples were placed in small petri dishes at a depth of 0.5 cm to obtain a uniform distribution. Measurements were made in 5 sections of the box. At least 25 measurements were made in different parts of the sample by triplicate.

Sensory analysis
A semi-trained panel of 20 judges evaluated the changes at the sensory level of tortillas enriched with Andean crops flours. Judges were asked to assess attributes such as color, texture, taste, odour, and overall acceptability. The sensory test was performed using a 5-point hedonic scale (5 - liked very much; 4 - like moderately; 3 - neither liked nor disliked; 2 - disliked moderately; 1 - disliked very much). Six samples (one of each formulation, coded with three digits and presented in random order) and control samples were served to each panelist accompanied with water to cleanse their palates between sample tasting.

Experimental design
Statistical analysis was performed with the GraphPad Prism 5.0 program (GraphPad Software, San Diego, California, USA) with a bidirectional analysis of variance. The test of comparisons was carried out with the Tukey test with a significance level of $P \leq 0.05$.

RESULTS AND DISCUSSION

Flours characterization
Proximal Analysis
The proximal composition of corn, faba-bean, and white-bean flours are shown in Table 2. Corn shows 14.5 % less protein, 0.83 % less ash, and 1.39 % more fat than faba-bean flour. Values obtained in this study are in contrast to those reported by Reyes-Moreno et al. (2013) in tortillas from amaranin transgenic corn, also in white-bean flour, there was an interesting content of fiber (6.59 %), similar to those reported by Méndez-Albores et al. (2012) in corn tortillas produced with a modified tortilla-making process. Moisture content is in concordance with regulations that recommend a maximum of 15.5 % in flours to control shelf life according to Codex Standard 152-1985. The proximate composition of faba-bean and white-bean is comparable to those previously reported for Vicia faba-bean flour (Millar et al., 2019) and white-bean flour (Dzudie and Hardy, 1996). Likewise, the gluten of flours was approximately 5 ppm (data do not show); this content is an essential characteristic because the reduced content provides an excellent raw material source to produce food for the celiac population. The Food and Drug Administration (US-FDA) ruled that products labeled “gluten-free” cannot surpass a threshold of 20 parts per million.

Granulometry (fineness and uniformity module)
The importance of the particle size distribution is related to the quality of the final product. Granulometry properties of flours showed differences in particle size distribution (Table 3) probably due to the milling process; the fine fractions are responsible for increase water absorption capacity, and also influences the viscosity during mixing, being those characteristics fundamental in dough cohesiveness to obtain rollability in corn tortillas (Gomez et al., 1992; Vaca-García et al., 2011). The results in Andean crops flours and corn showed a high amount of coarse particles, probably due to the milling process and the fiber content responsible for coarse fractions. This study’s results were compared with the research of commercial vegetable flours of lentils and rice flour, which are rich in fiber and have particle size distributions between 430 and 150 micrometers (Rios et al., 2018).

Water absorption capacity
The results of water absorption (WA) of the mixed flours (w/w) are shown in Fig. 3; results showed a significant difference (p<0.05). The water absorption capacity was evaluated at room temperature (25 °C) to simulate the same temperature as the water is added when producing tortillas. Results showed that corn flour has the highest WA, while in mixed flour when the amount of corn is reduced, the water absorption capacity is less than the other samples. The WA capacity differences have been related to particle size distribution, the extent of starch gelatinization mainly affected by the lime or ash cooking, starch damage, kernel endosperm texture, and the presence of hydrocolloids (Espinosa-Ramírez et al., 2020). The amount of water in the tortillas must be kept between 15–30 % of final moisture to obtain the best textural properties with sufficient flexibility and reheating ability (Almeida-Dominguez et al., 1996); in
contrast, the decrease in final moisture could produce a brittle tortilla (Arámbula-Villa et al., 2001).

**TORTILLAS CHARACTERIZATION**

**Proximal composition**

The proximal composition of tortillas is shown in Table 4. The results indicate that there are significant differences between treatments (p < 0.05). High protein content was observed in CPB2575 and CBF2575 samples; these values are related to the high protein content protein in white-bean and faba-bean flours (Crépon et al., 2010). The protein content is important to characterize the product as a source of significant biological and nutritional value. The control sample’s protein content is similar to those reported by Bedolla and Rooney (1984) in Mexican nixtamalized corn flours, who reported 8.5 and 10.27%. Other researchers reported 7 to 10 % protein contents in nixtamalized and extruded corn tortillas (Chaidez-Laguna et al., 2016). Enriched tortillas with Andean crops flours had lower (P < 0.05) lipid content, higher (P < 0.05) dietary fiber content, and higher (P < 0.05) ash content than control sample. The high protein, reduced fat, and reduced gluten content (5 ppm in all samples, data do not show) could be an indication that tortillas are a nutritional and safe product for people with celiac disease because they can consume up to 20 ppm of gluten in food (FDA, 2014; Haraszi et al., 2011; Llorens-Ivorra et al., 2019).

**Sensory evaluation**

It was possible to establish significant differences in color, texture, taste, odour, and overall acceptability (P < 0.05). However, the statistical differences, it is essential to note that the judges’ scores show that they like the enriched tortillas since the qualification is
Table 4: Proximal composition of tortillas of Corn, White-bean, and Vicia-faba

| Sample          | Moisture (%) | Ash (%)  | Fat (%)   | Protein (%) | Fiber (%) |
|-----------------|--------------|----------|-----------|-------------|-----------|
| Control         | 25.04±0.01   | 0.42±0.01| 1.89±0.08 | 8.12±0.05   | 2.07±0.08 |
| CPB7525         | 24.45±0.02   | 0.57±0.03| 1.77±0.07 | 11.86±0.07  | 3.43±0.08 |
| CPB5050         | 20.54±0.01   | 0.76±0.05| 1.68±0.07 | 16.32±0.01  | 4.59±0.08 |
| CPB2575         | 19.85±0.02   | 0.94±0.04| 1.59±0.07 | 20.17±0.01  | 5.58±0.08 |
| CBF7525         | 20.08±0.03   | 0.78±0.07| 1.35±0.08 | 13.54±0.01  | 2.14±0.08 |
| CBF5050         | 20.66±0.02   | 0.80±0.09| 1.38±0.09 | 14.33±0.01  | 2.25±0.08 |
| CBF2575         | 20.08±0.01   | 0.98±0.01| 1.07±0.09 | 17.18±0.07  | 2.07±0.08 |

Control: Corn flour (100%); CPB7525: Corn flour (75%)+White-bean flour (25%); CPB5050: Corn flour (50%)+White-bean flour (50%); CPB2575: Corn flour (25%)+White-bean flour (75%); CBF7525: Corn flour (75%)+Fava-bean flour (25%); CBF5050: Corn flour (50%)+Fava-bean flour (50%); CBF2575: Corn flour (25%)+Fava-bean flour (75%)

above three points. Tortillas made with Andean crops flours had good acceptability; the judges qualify with the highest overall acceptability and taste to treatment CPB2575. The control sample showed similar to odor with treatment CPB2575; however, color in enriched tortillas is slightly affected by different flours. The fact that tortillas with Andean crops had good sensory evaluation is vital to take advantage of the high nutritional quality of the fava-bean and white-bean, contributing to improving the nutritional status of people that consume tortillas as their primary source of energy and protein, with the additional advantage of an alternative of gluten-free products.

Oil content
The percentage of oil content of the samples is shown in Figure 4. The samples absorbed about 8% of oil during the toasted treatment (2 min - 1 minute for each side). The amount of oil absorbed at the moment of toasting showed a significant difference (P<0.05); however, the difference is slight between samples. The oil absorption by deep-frying is reported around 18% in corn nixtamalized tortilla chips (Topete-Betcourt et al., 2020); in this sense, the results reported are high compared to the present study; nevertheless, it is important to note that results are reported by deep-frying. In this sense, toasting treatment reduces the percentage of oil absorption compared to deep-frying treatment, and sensorial attributes are suitable qualified by testers.

Analysis of the best treatment
Textural, microbiological, and color analysis were developed only in the best treatment obtained by the sensorial qualification and correspond to sample CPB2575 [Corn flour (25%)+ White-bean flour (75%)]. Textural parameters such as hardness and distance showed significant differences (P<0.05) between the CPB2575 sample with control. The hardness value is lower than typically reported for this type of product (Chávez-Santoscoy et al., 2016; Rangel-Meza et al., 2004), the distance was lower than reported by Bueso et al. (2004) in corn tortillas. Bean solids contain polysaccharides and non-cellulosic compounds that are important for food's functional properties as thickening agents, stabilizers, emulsifiers, gelling agents, and film-forming agents; the polysaccharides present in white-bean could influence the hardness of nixtamalized tortillas (Agrahar-Murugkar et al., 2018). Also, the frying or toasting procedure, time, and temperature could cause physical changes (Arambula-Villa et al., 2001). The microbiological evaluation established that the tortillas comply with the normative what indicates the absence of harmful microorganisms to public health. The best treatment's shelf life was obtained by linearization of the concentration of molds and yeasts (ln (CFU / g) = 0.4606 * time - 0.4393; R² = 0.9711). The shelf life was calculated by the equation and was 16 days, which is an excellent value for enriched tortillas with no preservatives added in its formulation. CIELab coordinates (L*, a*, and b*) are shown in Table 5. Values of luminosity (L*) are above 70, which indicates that the samples tend to lightness. The control sample showed that the highest L* values were more than five units than the sample with white-bean flour. Similar values were found by Vaca-Garcia et al. (2011) in tortillas with triticale flour as a partial substitute of nixtamalized corn flour (L*: 74.40) and Reyes-Moreno et al. (2013) (L*: 876.3).

Regarding parameters a* and b*, all the results showed differences between the samples (P<0.05). It was observed that sample CPB2575 shows the value of a* in the positive range, which is indicative of their reddish color, while the control presents a more greenish tone (negative range of a*). As for parameter b*, the values have a clear tendency to yellow. The relationship between a less lightness value (L*) and a yellow tendency may be due to the crust's browning during toasting. Hue angle (˚H), chromaticity, and whiteness index (WI) show a significant difference between samples. The C* and H° parameters show that the yellow-red quadrant samples tend towards brown colors and show low saturation, probably attributable to the toasted treatment. The WI measures the degree of deviation of the floors concerning a clean white; the highest value corresponds to the control sample.
Table 5: Effects of inclusion of white-bean on textural and color properties of tortillas

| Parameter | CPB2575 | Control |
|-----------|---------|---------|
| Texture   |         |         |
| Hardness (N) | 7.07±0.53* | 4.10±0.53* |
| Distance (Mm) | 0.0273±0.33* | 0.0217±0.53* |
| Color     |         |         |
| L         | 75.20±0.44b | 83.40±0.57a |
| a*        | 3.60±0.54a  | -1.20±0.44a |
| b*        | 6.80±0.44b  | 10.00±0.71a |
| C*        | 7.69±0.01b  | 10.07±0.01b |
| H         | 1.08±0.01b  | -1.45±0.01b |
| IB        | 74.03±0.01b | 80.58±0.01b |

Control: Corn flour (100%); CPB2575: Corn flour (25%)+White-bean flour (75%). * Different letters in the same row are significant different (p < 0.05)

CONCLUSIONS

The proximal composition of the white-bean and faba-bean flour indicated an important nutritional value; it could also guarantee people with celiac disease because of the reduced gluten content. The inclusion of white-bean and faba-bean flour to nixtamalized corn flour showed an effect on physicochemical, microbiological, and sensory properties. The best formulation obtained by sensorial characteristics was the sample with 25% corn flour and 75% white-bean flour. Microbiological characteristics allowed to establish shelf life in the best formulation, which was 16 days. Results indicated that it was possible to incorporate white-bean and vicia-faba flours effectively.

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Authors’ contributions

Diego Salazar and Mirari Arancibia contributed equally to the writing of this paper. They were also involved in the experiments’ overall work: Diego Salazar and Mayra Rodas—production and evaluation of proximal, physicochemical, and sensorial parameters of enriched tortillas. Mirari Arancibia developed microbiological and textural analysis. Diego Salazar and Mirari Arancibia—Analysis of data and drafting the manuscript.

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