

Impact of Cooking and Extrusion Processing on Nutritional, Antinutritional, and Techno-Functional Characteristics of Indigenous Bean (*Phaseolus coccineus*)

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ABSTRACT: Ayocote bean (*Phaseolus coccineus*) is an underutilized pulse rich in protein, lipids, and carbohydrates; however, the impact of thermal processing has not been studied. Thus, the objective of this research was to assess the nutritional, antinutritional, and techno-functional properties of ayocote beans processed by conventional cooking and extrusion. Thermal processing had a positive impact on total dietary fiber increasing after cooking (35.49%). Trypsin inhibitors were inactivated after processing; however, lectin activity was still observed after extrusion (0.2 mg/mL). Extrusion showed a higher impact on TPC (75% retention) and TFC (88% retention) than cooking (61 and 74% retention, respectively). Cooking and extrusion caused significant losses of antioxidant activity when compared to raw flour, though cooking showed higher AOX retention (ORAC = 88.5/ABTS = 87.3%) than extrusion (ORAC = 74.4/ABTS = 77.5%). The results suggest that ayocote bean is a legume that could be used to develop novel food formulations with improved health benefits for the wider population.

KEYWORDS: *Phaseolus coccineus*, cooking, extrusion, antinutrients, lectins, antioxidant, techno-functional properties

INTRODUCTION

Bean (*Phaseolus* spp) seeds are a family of legumes that play an important role in human nutrition, especially among low-income populations in developing countries including Mexico, which represents a rich and consuming tradition throughout history and has even become a staple food of cultural identification.^{1,2} Although in Mexico there are about 70 species of *Phaseolus* reported in the country, only five have been domesticated, including *P. vulgaris* (common bean), *P. lunatus* (lima bean), *P. acutifolius* (teparty bean), *P. polyanthus* (year bean), and *P. coccineus* (ayocote bean).^{3,4}

Ayocote bean (*Phaseolus coccineus* L.) (Figure 5) is an underutilized legume native from Mexico with great potential for a wide variety of food purposes by its foliage, flowers, pods, and seeds.⁵ This legume is used as an ornamental plant in some parts of the world due to its large size and diverse colors (beige, brown, violet, and black). Likewise, ayocote bean can tolerate colder climates compared to other *Phaseolus* species. In Mexico, small growers and indigenous communities cultivate this species on a small scale and use it to prepare different soups, bean purees, and other dishes.^{6–8}

On the other hand, colored raw beans have shown the presence of phytochemical compounds such as polyphenols, anthocyanins, carotenoids, and tannins. These compounds have been linked to their nutraceutical properties, mainly for the prevention of chronic diseases (cancer, diabetes, and cardiovascular diseases).⁹ However, the bioactive compounds present in raw foods are modified during processing (dehulling, steeping, germination, domestic cooking, extrusion,

among others).¹⁰ Sometimes these changes improve palatability, nutritional value, and nutraceutical properties.¹¹

Domestic cooking is one of the most traditional methods used for bean consumption. It is defined as the percentage of bean cooking required to reach an acceptable texture for the consumer, which has been subjected to a constant boiling temperature (100 °C) in water, at a ratio of 1:3 (w/v) each time.¹² Different processing methods have been used for cooking beans (steaming, boiling, pressure cooking, and microwave). Corzo-Ríos et al. (2020)⁴ used a high-pressure cooking method for ten different bean varieties, reporting that, after cooking, an increase in the dietary fiber in all beans was observed; moreover, a decrease in non-nutritional compounds, particularly in trypsin inhibitor content (95–100%), was found; also total phenolic compounds exhibited a reduction between 3.2 and 40.9% after cooking. Hydrothermal heating causes gelatinization, thus increasing the starch digestibility, and upon cooling, retrograded starch (less digestible) is formed, thus affecting the nutritional composition of legumes.¹³ Moreover, cooking can modify the composition of pulses, increasing the concentration of resistant starch and total dietary fiber (soluble and insoluble).^{4,14,15} Also, a significant reduction in antinutritional compounds like phytic

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acid, tannins, trypsin inhibitors, and saponins, among others, has been observed.^{4,14,16} Luo and Xie (2013)¹⁷ reported that cooking fava bean did not affect total phenolics, while phytic acid was increased. Phenolic compounds and anthocyanins have been reported to decrease after cooking by different processing methods.^{18,19}

Extrusion is a high-temperature/short-time, simple, versatile, and modern method that has already been used to process legumes and cereals, obtaining favorable results for the generation of novel food formulations.²⁰ Extrusion seems to be suitable to produce ready to eat legume-based foods.²¹ The temperature, pressure, and moisture conditions used during extrusion can cause changes at structural, nutritional, and functional levels, thus causing lipid oxidation, inactivation of antinutritional compounds, improvements of sensorial attributes, and modifications on the bioactive profile.²² Furthermore, extrusion has been used to reduce antinutritional factors²³ and to increase the protein and carbohydrate digestibility.²⁴

There is scarce information regarding ayocote bean properties, and furthermore, to our knowledge, any previous research on the changes that may occur during processing, as well as its potential to develop new food products for human consumption with enhanced health benefits, has been reported for this bean species. Thus, it is essential to study the impact of cooking and extrusion on the ayocote bean. The objective of this research was to assess the nutritional, antinutritional, and techno-functional properties of ayocote beans processed by conventional cooking and extrusion, to understand the impact that dry and wet heating may have on ayocote bean, and thus provide knowledge on the potential use of this underutilized pulse for human consumption.

MATERIALS AND METHODS

Chemicals. Folin–Ciocalteu reagent, gallic acid, fluorescein, 2,2'-azobis(2-methyl-propinamide) (AAPH), and Trolox were obtained from Sigma Chemical Co. Ethanol, sodium hydroxide, hydrochloric acid, boric acid, potassium sulfate, cupric sulfate, methyl red, petroleum ether, hexane, isopropanol, sodium carbonate, and potassium chloride were purchased from Sigma-Aldrich (St. Louis, MO, USA). The available carbohydrates dietary fiber assay kit, resistant starch rapid format, and damaged starch were purchased from Megazyme. The sheep hemagglutination kit was purchased from Rockland. All reagents used were of analytical grade.

Materials. For this study, black ayocote bean (*Phaseolus coccineus*) seeds were collected in 2019 from Teacapa Puebla, Mexico. The seeds were thoroughly cleaned and sorted to remove foreign materials; then, the seeds were stored in plastic bags until further use at 8 °C.

Methods. Physical Characteristics of Ayocote Bean Seeds. Physical characteristics of seeds were determined by using standard procedures: Physical dimensions were measured according to the methodology reported by Milán-Carrillo et al. (2000).²⁵ The weight of 1,000 seeds was determined by the international method of ISTA (1985)²⁶ (International Seed Testing Association). To determine the hectoliter weight, procedure 55-10.01 of the AACCC (1995)²⁷ was used. The caliber and percent of testa determination was carried out according to Reyes-Moreno et al. (2000).²⁸

Preparation of Processed Ayocote Bean Flours. Cooking Time (CT). A Mattson bean cooker was used to carry out the cooking process following the method by Reyes-Moreno et al. (2001),²⁹ with modifications. For each batch, 25 bean seeds (whole grains, cotyledons, and seed coats) were used at a time. Cooking time is in the meantime, when 15 bean seeds were cooked, as indicated by stainless steel plungers (weight 75 ± 0.5 g, point diameter 2 mm) dropping and penetrating individual samples. The 60% cooked point corresponded to the sensorial preferred degree of cooking. The determinations were made in triplicate.²⁹ Once the cooking time was

obtained (2 h), the bean seeds were cooked in boiling water (100 °C) at a 1:3 (w/v) ratio, frozen overnight at -80 °C, then lyophilized (Freeze-Dry System Model 77520, LABCONCO) (including cooking liquor), and finally ground to obtain the cooked ayocote bean flour (CAF).

Extruded Ayocote Bean Flour. The extruded ayocote bean flour (EAF) was obtained following the procedure reported by Milán-Carrillo et al. (2012).³⁰ The bean seeds (1 kg batch) were ground (Thomas-Wiley laboratory Mill, Mod. 4) using a 2 mm sieve (particle size ~1–2 mm) and conditioned with purified water to reach a moisture content of 24%. Each batch was packed in a polyethylene bag and stored at 4 °C for 12 h. Before extrusion, the grits were tempered at 25 °C for 2–4 h. A single screw laboratory extruded Model 20 DN (CW Brabender Instruments, Inc., NJ, USA) with a 19 mm screw diameter, length to diameter of 20:1, nominal compression ratio of 2:1, and die opening of 3 mm was used. The extrusion operation conditions were an extrusion barrel temperature (ET) of 137 °C and a screw speed (SS) of 220 rpm. After extrusion, the flours were cooled, equilibrated under environmental conditions (25 °C), milled (UD Cyclone Sample Mill, UD Corp, Boulder, CO, USA) to pass through an 80-US mesh (0.180 mm) screen, packed in plastic bags, and stored at -4 °C until further use.

Nutritional Properties. Proximate Composition. The proximate composition of unprocessed ayocote beans flour (AF), cooked ayocote flour (CAF), and extruded ayocote flour (EAF) was determined according to the official methods of the AOAC (1999);³¹ moisture (44.15), protein (979.09), fats (920.39), ashes (923.03), total fiber, and available carbohydrates was determined according to the K-ACHDF (Megazyme) kit, resistant starch by the K-RAPRS (Megazyme) kit, and damaged starch by the K-SDABM (Megazyme) kit. The measurements were made in triplicate on a dry basis.

Fatty Acid Profile. 7 g of the samples was mixed with 15 mL of a hexane:isopropanol (3:2 v/v) solution. The mixture was stirred for 1 h in a rotator (300 rpm) (Thermolyne MIRAK). After that, it was centrifuged at 5000 rpm/10 min (Eppendorf brand centrifuge, Mod. 5804R), and the supernatant was collected. The precipitate was resuspended in 15 mL of hexane:isopropanol (3:2 v/v) and again stirred and centrifuged. The two supernatants were combined, and 2.5 mL of KCl 0.75% was added; after that, two phases appeared. The upper phase was collected and dried in a concentrator (50 °C/sample was protected from the light). Lipid transesterification was carried out by adding 0.0269 g of KOH and 500 μ L of MeOH. Then, the samples were sonicated (Hielscher, Ultrasound Technology, UP200S, 200 W, 24 kHz) for 5 min at 65% amplitude during one cycle followed by centrifugation at 4000 rpm/5 min. The supernatant was analyzed using a gas chromatography (Agilent 6890N, NY, USA) device with an Agilent 5973 mass spectrometer (Agilent 5973N, USA), Omega Wax 250 column, Helio as a carrier, and injector gas series 7683. The operating conditions were the following: injection volume, 1 μ L; flow, 1 mL/min; column temperature, 50–270 °C (10 min) with a heating rate of 5 °C/min. The samples were analyzed by gas chromatography-mass spectrometry (GC-MS).³²

Amino Acid Analysis. Protein samples (2 mg) were hydrolyzed in 6 N HCl (4 mL) at 110 °C for 24 h in tubes sealed under nitrogen. Tryptophan was analyzed by HPLC after basic hydrolysis according to Yust et al. (2004).³³ Amino acids were determined after derivatization with diethyl ethoxymethylenemalonate by HPLC according to the method of Alaiz, Navarro, Girón, and Vioque (1992),³⁴ using D,L- α -aminobutyric acid as an internal standard and a 300 mm \times 3.9 mm i.d. reversed-phase column (Novapack C18, 4 μ m; Waters, Milford, MA, USA).

Mineral Content. 2 g of samples was cremated in a Phoenix microwave furnace overnight at 780 °C or until the sample did not present black spots. This was followed by cooling down the sample; then 1 mL of concentrated nitric acid/10 min incubation and 10 mL of Milli-Q water were added until a volume of 25 mL was reached. Finally, the samples were filtered through a 0.45 μ m nylon syringe filter. Then the samples were analyzed by an ICP-OES iCAP 7000

DUO, Serial number: IC76DC151510, Model: Cetac ASX-520 autosampler.

Physicochemical Characteristics. Total Color Difference (ΔE). The surface color of the samples was measured using a Minolta color-difference meter Model CR-210 (Minolta LTD, Osaka, Japan). The parameters L (0 = black, 100 = white), a (+ value = red, - value = green), and b (+ value = yellow, - value = blue) were recorded. The L , a , and b values of a white standard (std) tile used as reference were 97.63, 0.78, and -2.85, respectively. ΔE was calculated (SI Formula 1) in triplicate for each sample.

Water Activity (a_w). The measurement of a_w was carried out using the methodology reported by Milán-Carrillo et al. (2002),³⁵ in a calibrated Aqualab instrument (Mod Series 3TE) (using distilled water). A sample of ayocote bean flour of approximately 2 g was placed in the sample cell of the equipment until equilibrium was reached (40–60 min). The measurements were made in triplicate.

pH. The pH was determined according to the methodology of the AOAC (1999)³¹ (Association of Official Analytical Chemists). 5 g of flour was added to 50 mL of boiled and cooled distilled water; the suspension was stirred on an orbital shaker (Thermolyne, MIRAK, US) (10 min/240 rpm), and then the pH of the suspension was measured with a potentiometer (Hanna Instruments, Mod. HI3221). The measurements were made in triplicate on a dry basis.

Functional Properties. Water Absorption (WAI) and Solubility Index (WSI). For this determination, the method described by Anderson et al. (1969)³⁶ was used with some modifications.³⁷ A 2.5 g flour sample was suspended in 30 mL of water at 30 °C in a tube previously calibrated; the suspension was stirred in an orbital shaker (OVAN NRS0-E brand) at 30 rpm for 30 min. The suspension was centrifuged at 3000g/30 °C/10 min (Eppendorf brand centrifuge, Mod. 5804R). The supernatant was poured into a calibrated evaporating dish. The WAI was calculated from the weight of the remaining gel and expressed as g of gel/g of solid. The WSI expressed as g of solids/g of original solids was calculated from the weight of dry solids recovered by evaporating the supernatant overnight at 110 °C. The measurements were made in triplicate on a dry basis.

Oil Absorption Capacity (OAC). The oil absorption capacity was determined according to the methodology reported by Marie et al. (2015)³⁸ with minor modifications. Samples of 0.5 g of flour with 3 mL of vegetable oil were placed in a graduated centrifuge tube, stirred for 1 min in a vortex, and allowed to stand for 30 min. Finally, they were centrifuged at 1600g/25 min (Eppendorf brand centrifuge, model 5804R), and the free oil volume was measured. The results were expressed as mL of absorbed oil/g of flour (SI Formula 2). The test was performed in triplicate.

Dispersibility Index (DI). The DI was determined according to the technique reported by Mora-Escobedo et al. (1991).³⁹ 1 g of flour samples was mixed in a conical graduated tube with 10 mL of distilled water. The samples were homogenized in an ultra Turrax (IKA brand, Mod. T18 basic) at 10,000 rpm/5 min. The phase separation (sediment and liquid) was measured after 30 min of rest. Samples were made in triplicate. The results were expressed as a percentage of dispersibility in triplicate (SI Formula 3).

Foaming Capacity (FC) and Foam Stability (FS). FC and FS were determined by the method of Huffman et al. (1975)⁴⁰ and Khattab et al. (2009),⁴¹ respectively. In a 100 mL test tube, 0.5 g of the sample and 50 mL of distilled water were added. The mixture was stirred in an Ultraturax (IKA brand, Mod. T18 Basic) at 12,000 rpm/1 min. Subsequently, the volume of the foam formed was measured. Foaming capacity was reported as a percentage (% FC) and carried out in triplicate (SI Formula 4.1). The material was allowed to stand for 30 min; at the end of this time, the volume of the residual foam was measured, and the foam stability was expressed as percent foam stability (% FS) (SI Formula 4.2).

Emulsifying Capacity (EC) and Emulsion Stability (ES). EC and ES were determined using the method described by Huffman et al. (1975)⁴⁰ and Butt and Batool (2010).⁴² 0.7 g of samples was added to 10 mL of distilled water and 10 mL of vegetable oil. Subsequently, the samples were stirred in an Ultraturax (IKA brand, Mod. T18 basic) homogenizer at 12,000 rpm for 1 min. The mixture was centrifuged at

1,300g for 5 min (Eppendorf brand centrifuge, Mod. 5804R). The EC (SI Formula 5.1) was calculated by dividing the height of the emulsion layer by the total height of the emulsion and multiplied by 100. ES was determined by heating the emulsion at 80 °C for 30 min in a water bath and then centrifuging (1300g/5 min). ES was measured and calculated (SI Formula 5.2), dividing the height of the emulsion layer after heating by the height of the emulsion before heating. Both results were expressed as a percentage (%).

Antinutritional Factors. Phytic Acid Content (PAC). The PAC was determined according to the colorimetric procedure reported by Vaintraub and Lapteva (1988)⁴³ with some modifications. PAC was calculated (SI Formula 6) and expressed as mg equivalents of sodium phytate (ESP)/100 g of sample.

Saponins (SC). Saponins extraction was performed as follows: 0.5 g of sample flours was weighed into centrifuge tubes, and 10 mL of 80% methanol was added. The tubes were mixed for 16 h at room temperature on an orbital shaker, and then the extracts were recovered by centrifugation (10 min/5,000 g/25 °C) (Eppendorf, model 5804R). The remaining pellet was re-extracted twice by vortexing with 5 mL of 80% methanol and further centrifugation. The resulting supernatants were pooled together. The total saponins content (SC) was quantified based on the vanillin–sulfuric acid assay proposed by Hiai et al. (1976).⁴⁴ SC was expressed as mg of diosgenin equivalents/100 g of sample (SI Formula 7).

Condensed Tannins Content (CTC). This analysis was carried out according to the methodology reported by Xu and Chang (2007).⁴⁵ CTC was calculated (SI Formula 8) and expressed in mg equivalents of catechin (EC)/100 g db of sample.

Trypsin Inhibitor Activity (TIU). The trypsin inhibitor activity was determined according to the method reported by Welham and Domoney (2000).⁴⁶ The trypsin inhibitor activity was expressed in terms of trypsin units inhibited per milligram of sample, where one trypsin inhibitor unit (TIU) was defined as a decrease of 0.01 absorbance units at 410 nm in relation to the trypsin control reaction. The trypsin inhibitor activity was calculated (SI Formula 9) and expressed as trypsin inhibitor units (TIU)/mg of sample.

Hemagglutinin Activity. To detect lectin activity in samples, the sheep Hemagglutination Kit (KPA-39913) was used. Lectins were extracted according to De Mejía et al. (2005).⁴⁷ 1 g of sample was mixed with 10 mL of 10 mM phosphate buffered saline (PBS) pH 7.4 (1:10 w/v) and stirred overnight at 4 °C, and then the mixture was centrifuged at 12,000g/30 min/4 °C; supernatant was brought to 80% (NH₄)₂SO₄ saturation. Stirring was performed 1 h/4 °C, making sure salt was dissolved. The precipitate was recovered after centrifugation at 12,000g/30 min/4 °C, dissolved in PBS (1:10 w/v), dialyzed against distilled water for 1 h/5 times, and finally lyophilized.

Nutraceutical Properties. Free Phenolics Extract. Phenolic compounds in AF, CAF, and EAF were extracted as previously reported by Mora-Rochin et al. (2010).⁴⁸ Briefly, 5 g of ground samples was stirred with 20 mL of cold ethanol–water (80:20 v/v) for 10 min. After centrifugation at 3000g for 10 min, the supernatant was evaporated to dryness at 45 °C. The resulting extracts were frozen at -20 °C and stored until use.

Total Phenolic Content (TPC). Briefly, 20 μ L of diluted extracts was oxidized with 180 μ L of Folin–Ciocalteu reagent. After 3 min, 50 μ L of 7% sodium carbonate was added and the mixture incubated for 90 min. Then, absorbance was measured at 750 nm (Microplate Reader, SynergyTM HT, BioTek, Inc., Winooski VT). Total phenolics were expressed as milligrams of gallic acid equivalents (mg of GAE)/100 g db of sample.⁴⁹

Flavonoids. In a 96-well microplate, 20 μ L of sample and standard were added with 100 μ L of distilled water and 6 μ L of NaNO₂ 5% and then incubated for 5 min, and after, 12 μ L of AlCl₃ 10% was added. After 5 min of incubation, 40 μ L of 1 M NaOH and 22 μ L of distilled water were added and incubated for 30 min at 25 °C. Absorbance was measured at 510 nm (Synergy HT Multidetector, Biotek, Inc., Winooski, VT). The standard was catechin (CA), and the content of total flavonoids (TF) was expressed as mg equivalents of catechin/100 g of sample in dry basis (mg of ECA/100 g db). Determinations were made in triplicate.⁵⁰

Anthocyanins. A 1 g sample was weighed, and 1 mL of cold acidified methanol was added (95% methanol/1 N HCl, 85:15 v/v). Subsequently, sample was centrifuged at 3,000 rpm/10 min, the supernatant was recovered, and the absorbance of the samples was read at 535 and 700 nm (SynergyTMHT Multidetector, Biotek, Inc., Winooski, VT). Anthocyanin content (AC) was calculated (SI Formula 10) following the formula by Abdel-Aal and Hucl (1999).⁵¹ The determination was carried out in triplicate.

Antioxidant Capacity. Determination of Oxygen Radical Absorbance Capacity (ORAC) Assay. The ORAC method was determined by the assay described by Ou et al. (2001).⁵² The assay was performed using fluorescein as the fluorescent probe, AAPH as a radical generating agent, and Trolox as the standard. Extracts, Trolox, and AAPH were diluted with sodium phosphate buffer (75 mM, pH 7.4). Extract aliquots (25 μ L) were transferred into a 96-well plate, loaded into the plate holder on the Synergy Microplate Reader (SynergyTM HT Multi-Detection, BioTeK, Inc., Winooski, VT) for the kinetic reading. Results of the protective effects of the extracts and control were calculated based on the differences in the area under the sodium fluorescein decay curves (AUC) between the blank and the sample.⁵³ Results were expressed as micromoles of Trolox equivalents/100 g db.

Determination of ABTS Radical Scavenging. Antiradical activity by ABTS radical assay was determined by the method described previously by Re et al. (1999).⁵⁴ A stock solution of the ABTS radical cation was generated by reacting the ABTS solution with 2.45 mM potassium persulfate, and the mixture was allowed to stand in the dark for 12–16 h before use. An aliquot of the radical ABTS solution was diluted in PBS (pH 7.4) until an absorbance of 0.7 ± 0.02 at 734 nm was reached. 7.5 μ L of the blank, extracts, and curve was transferred into a transparent 96-well plate; the test started when 292.5 μ L of ABTS solution was added and then recorded after 10 min at 734 nm. The ABTS loss of absorbance was calculated against the blank. A calibration curve with Trolox was used, and the data were expressed as μ mol equivalents of Trolox/100 g of sample on a dry basis (μ mol of ET/100 g db of sample).

Statistical Analysis. Data were reported as the mean \pm standard deviation (SD) in triplicate. A one-way analysis of variance (ANOVA) was used for statistical analysis of the experimental results. Differences among treatments were determined using Fisher's pairwise *t* test comparison. A *p*-value <0.05 was considered significant. All statistical analyses were performed with the use of Minitab 16 software.

RESULTS AND DISCUSSION

Physical Characteristics of the Seed. Table 1 shows the physical characteristics of black ayocote bean (*P. coccineus*)

Table 1. Physical Characteristics of Ayocote Bean¹

Physical Characteristics	Ayocote Bean
Dimensions (mm)	
Length	16.21 \pm 1.41
Width	10.21 \pm 0.96
Thickness	7.11 \pm 0.81
1,000 seeds weight (g)	785.96 \pm 27.88
100 seeds weight (g)	78.59 \pm 2.78
Hectoliter weight (kg/hL)	75.69 \pm 0.58
Caliber	37.40 \pm 0.89
Percent of testa (%)	11.50 \pm 0.26

¹Values represent the mean \pm standard error of three determinations.

seeds. The results of the seed dimensions were registered as length = 16.21 mm, width = 10.21 mm, and thickness = 7.11 mm. Recently, Corzo-Ríos et al. (2020)⁴ reported the following dimension range: length, 16.1–20.9 mm; width, 10.7–13.7 mm; thickness, 6.8–8.8 mm for ayocote bean varieties. The weight of 100 seeds was 78.59 g/100 seeds,

hectoliter weight 75.69 kg/hL, and caliber 37.40 seeds/30 g, respectively. Ayala Garay et al. (2006)⁵⁵ reported values of hectoliter weight of 68–83 kg/hL for ayocote bean, similar to those obtained in this investigation. Considering the classification reported by Singh et al. (1991),⁵⁶ black ayocote beans could be classified as “large” seeds (large: >40 g/100 seeds). On the other hand, hectoliter weight is a useful value for pulse producers as large seeds make large holes during transportation or grain storage; thus, too many empty spaces will result in low hectoliter weight.⁵⁷ The importance of measuring physical attributes in bean seeds allows evaluating the commercial quality of varieties with potential to increase high yields during sowing, which are of interest both for the agriculture sector and the institutions responsible for seed certification, since they determine the value of the seeds therefore benefiting the farmer.²⁶

The percent of testa for ayocote beans was 11.5%; this is higher than values reported for different *P. vulgaris* varieties (9.2–9.7%)⁵⁸ and previous reports for ayocote bean seeds (8.7%).⁵⁹ The high content of testa has been related to the protection of the seed against different pathogens, higher cooking time, and water absorption capacity; it also contains fiber, phenolic compounds, and minerals.^{60–62}

Nutritional Properties. Proximate Composition of Raw, Cooked, and Extruded Flours. The proximal composition of raw and processed ayocote beans is shown in Table 2. The protein contents for AF, CAF, and EAF flours were 18.82, 19.69, and 19.75%, respectively; these results were similar to those reported for different raw ayocote bean varieties (18.07–18.93%).⁶³ After processing, the protein content did not show significant differences (*p* > 0.05). Corzo-Ríos et al. (2020)⁴ obtained an increase of protein content in some *P. vulgaris* beans (0.5–2.2%) after traditional cooking; this has been explained by different factors such as grain hardness, cooking time, and lixivation of soluble solids during cooking similar to those obtained in this study. The fat content was 2.78, 2.50, and 1.74%, respectively, in the different flours. Corzo-Ríos et al. (2020)⁴ reported a range of fat content between 1.7 and 4.3% for different varieties of beans, with similar results to this study. Nevertheless, a decrease after cooking was reported, attributing this to lipolysis which is catalyzed by the processing of food in the presence of water and high temperatures, where the ester linkages of triglycerides and phospholipids are hydrolyzed, thus releasing fatty acids.⁶⁴ Margier et al. (2018)⁶⁵ reported the content of fat for different pulses after household cooking and canning, observing that the method and the pulse used influenced the fat content; for instance, kidney beans had no changes in fat content after using both methods (0.28 and 0.29 g/100 g), while chickpeas showed a significant change between these (2.04 and 1.78 g/100 g); this could be related to differences in the lipid profile.

A decrease in fat content was found in the extruded flour; this could be the result of high temperature that can reduce the lipase and lipoxygenase activity and moisture level, thereby favoring free fatty acid development and fatty acid oxidation; extrusion can enhance the oxygen exposure, contributing to the oxidation of lipids; furthermore, fatty acids could form complexes with amylose and proteins.^{66–68} Moreover, in instances where the extrusion duration is extended and/or the fat content of the samples exceeds 5%, the shear stress exerted upon the sample within the extrusion barrel leads to the depletion of fat from the vegetal tissues, consequently yielding

Table 2. Chemical Composition, Physicochemical, and Techno-Functional Properties of Raw, Cooked, and Extruded Ayocote Bean Flours

Proximate Composition (% db ²)	Ayocote Bean ¹		
	AF	CAF	EAF
Protein	18.82 ± 0.69 a	19.69 ± 0.09 a	19.75 ± 0.30 a
Fat	2.78 ± 0.31 a	2.50 ± 0.00 a	1.47 ± 0.05 b
Ash	4.45 ± 0.07 a	4.51 ± 0.05 a	4.54 ± 0.05 a
Carbohydrates	73.93 ± 0.72 ab	73.27 ± 0.05 b	74.22 ± 0.37 a
Total dietary fiber	30.38 ± 0.17 b	35.49 ± 0.34 a	29.85 ± 1.15 b
Insoluble	30.31 ± 0.64 a	31.83 ± 0.07 a	25.87 ± 0.95 b
Soluble	0.07 ± 0.46 b	3.66 ± 0.26 a	4.66 ± 0.20 a
Available carbohydrates	35.47 ± 0.20 a	33.81 ± 0.10 b	36.11 ± 0.36 a
Total starch	33.24 ± 0.46 c	42.99 ± 0.31 a	39.49 ± 0.54 b
Resistant starch	8.53 ± 0.20 b	18.86 ± 0.06 a	3.97 ± 0.29 c
Non-resistant starch	24.71 ± 0.25 c	24.12 ± 0.25 b	35.52 ± 0.84 a
Damaged starch	0.94 ± 0.03 c	5.28 ± 0.17 b	16.76 ± 0.20 a
Physicochemical Properties			
Color			
Hunter value "L"	78.40 ± 0.64 a	46.72 ± 1.54 c	55.40 ± 0.90 b
Total color difference	19.42 ± 0.64 c	51.79 ± 1.50 a	42.81 ± 0.87 b
Aqueous activity (aW)	0.33 ± 0.00 b	0.07 ± 0.00 c	0.36 ± 0.00 a
pH	6.36 ± 0.00 b	6.28 ± 0.00 c	6.56 ± 0.00 a
Techno-Functional Properties			
Water absorption index ³	2.87 ± 0.04 b	3.47 ± 0.39 a	3.46 ± 0.03 a
Water solubility index ⁴	20.41 ± 0.15 b	23.07 ± 0.69 ab	27.45 ± 0.69 a
Oil absorption index ⁵	1.60 ± 0.12 a	0.87 ± 0.11 b	0.78 ± 0.09 b
Dispersibility (%)	71.66 ± 2.88 b	100.00 ± 0.00 a	93.33 ± 5.77 a
Foaming (%)	8.08 ± 0.11 a	2.00 ± 0.04 b	ND
Foam stability (%)	4.04 ± 0.05 a	ND	ND
Emulsion capacity (%)	27.85 ± 2.57 a	27.04 ± 1.03 a	13.42 ± 0.76 b
Emulsion stability (%)	50.72 ± 1.25 a	50.00 ± 0.00 a	25.92 ± 1.60 b

¹Results are expressed as mean ± standard deviation of three determinations; applying the Fisher multiple range test; different letters in the lines indicate statistically significant differences ($p < 0.05$). ²db: dry basis. ³g of gel/g of db. ⁴g of solids/100 g of db. ⁵g/g of db. AF = raw ayocote bean flour, CAF = cooked ayocote bean flour, EAF = extruded ayocote bean flour, ND = not detected.

fat loss and the manifestation of visible die drip fat on the end plate.⁶⁹

No differences ($p > 0.05$) were found in the ash content of AF, CAF, and EAF flours (4.45, 4.51, and 4.54%, respectively), being similar to those reported by Sahasakul et al. (2022)⁷⁰ for 10 bean varieties from Thailand (3.51–5.13%). On the other hand, Corzo-Ríos et al. (2020)⁴ reported a decrease in ash concentration after cooking, except for one bean variety, with no correlation between cooking time and the decrease in ash content. Changes in the ash content during thermal processing have been related to hardness of the bean and its composition.

The content of total dietary fiber (TDF) of AF, CAF, and EAF was 30.38, 35.49, and 29.85%, respectively, presenting an increase after cooking; this could be due to an increase of soluble fiber (3.59%), which is related to the formation of resistant starch or tannin–protein complexes, contributing to the increase of TDF.⁷¹ Other studies have found similar behaviors in TDF after cooking; for instance, Corzo-Ríos et al. (2020)⁴ obtained a TDF increase between 1 and 6.6% after cooking for 10 different bean varieties, while Bento et al. (2022)⁷² also observed an increase of TDF (1.66%) in Dama bean after cooking.

Moreover, during extrusion, dietary fiber may be significantly modified; this is related mainly to the fiber content and solubility, depending on the extrusion conditions and composition of the material extruded.⁷³ In this study, we observed an increase in soluble fiber after extrusion around

66.5 times more than the AF sample. This effect could be related to different explanations, the use of high temperature, pressure, and shear force leads to a breakdown of glycosidic bonds of insoluble polysaccharides, converting them into smaller soluble units, and/or a release of the hemicellulose soluble fraction during the process.^{68,74} Chen et al. (2014)⁷⁵ reported similar results where the soluble dietary fiber content of soybean increased after the extrusion process.

The content of available carbohydrates (CAC) in AF, CAF, and EAF was 35.47, 33.81, and 36.11%, respectively. A significant decrease was found in the CAC after cooking; this result could be due to an increase of resistant starch content in the flour after cooking, decreasing the amount of CAC. Nakitto et al. (2015)⁷⁶ reported an increase in the CAC after cooking for dry beans (3.19%); these differences could be because of the different methods used for cooking and the varieties of beans studied. Extrusion did not significantly ($p > 0.05$) affect the CAC content; similar results were reported by Ciudad-Mulero et al. (2022),⁷⁷ reporting the CAC content for chickpeas and lentils before and after extrusion without any significant effect. Kowalski et al. (2018)⁷⁸ reported a decrease in starch and available carbohydrates after extrusion in waxy wheat flour, due to high shear forces, heat, and pressure during extrusion, thus promoting a breakdown of starch resulting in dextrinization. Arribas et al. (2019)⁷⁹ described that the effect caused by extrusion treatment depends on the extrusion conditions as well as the food matrix.

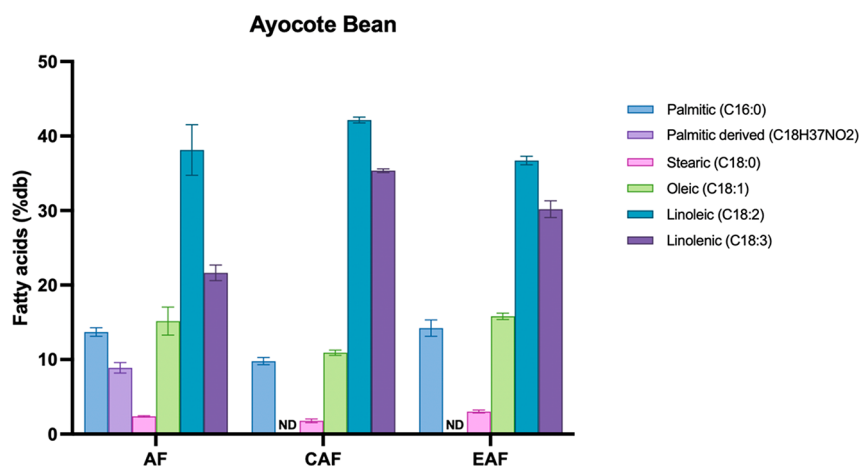


Figure 1. Fatty acids content of raw, cooked, and extruded ayocote bean flours. AF = raw ayocote bean flour, CAF = cooked ayocote bean flour, EAF = extruded ayocote bean flour, ND = not detected.

Starch is a polymeric carbohydrate that serves as the major source of energy in the human diet, which is composed of amylose and amylopectin.⁸⁰ Starch can be classified by its time of digestion, as rapidly digestible (20 min), slowly digestible (>2 h), and resistant starch that generally remains undigested by the small intestine; this last one needs to be fermented by the microbiota in the large intestine.⁸¹ Since starch can be modified during processing, the content of total starch (resistant and non-resistant) and damaged starch in raw and processed ayocote bean is shown in Table 2. Resistant starch (RS) is the portion of starch that is not broken down by human enzymes in the small intestine. The measurement of RS can provide information about starch reorganization; high values of RS might indicate a retrogradation. Besides, damaged starch (DS) is related to the susceptibility of starch to α -amylase hydrolysis; high values of DS in processed foods indicate starch gelatinization and/or disintegration.⁸²

The resistant starch (RS) contents in AF, CAF, and EAF were 8.53, 18.86, and 3.97%, respectively. The content of RS had a significant increase ($p \leq 0.05$) after cooking (10.33%). During cooking, starch gelatinization can be produced, thus increasing starch digestibility, and upon cooling, retrograded starch can be formed (less digestible); this could be due to RS increase after cooking, as samples were freeze-dried. However, the formation of RS is dependent on the starch composition.⁷² Bento et al. (2022)⁷² reported the content of RS in raw (9.0–29.0%) and cooked (31.1–8.3%) carioca bean flours. The RS results for raw beans were higher than the ones obtained in this study, and for processed bean flours, some of them increased (2.11%) while others decreased (0.8%). However, the content of RS could be influenced by different factors, like composition (fiber), starch characteristics (crystallinity of starch), and high content of amylose, which tend to make the major amount of RS related to the chain length.⁸³

The content of RS decreased significantly after extrusion (4.56%). Escobedo et al. (2020)⁸⁴ reported a reduction of RS in black beans after extrusion, attributing that this could correspond to the destruction of RS1 and RS2 (native RS); besides during thermal treatments, protein amino groups could interact with starch carbonyl groups through hydrogen and covalent bonds, thus hindering complete starch retrogradation, because of the protein–starch interactions, resulting in reduction of RS content. Gulzar et al. (2021)⁸⁵ studied how the extrusion process and parameters used during extrusion

could affect the RS content in rice-flour-based extruded snacks, finding that the different parameters used during extrusion affected differently the RS content; for instance, the increase of temperature from 130 to 180 °C increased significantly from 6.84% to 7.78%, while the RS content decreased from 6.40 to 6.21% with an increase in screw speed from 50 to 150 rpm. Furthermore, during extrusion starch gelatinization can be generated, resulting in an increase in starch digestibility (90%); in addition, extrusion can cause a cleavage of amylose and particularly of amylopectin molecules induced by the shear, resulting in smaller and more digestible fragments (dextrins and reducing sugars).²¹ The interest in RS is growing because of its potential role as a protective factor against colon cancer and its capability to reduce the rate of glucose release into the bloodstream.⁸¹

Present findings in this research showed differences in DS in unprocessed sample (AF) and processed ones (CAF and EAF) with a content of 0.94, 5.28, and 16.76%, respectively. The content of DS in raw flour is similar to those reported by Blandino et al. (2023)⁸² in three different flour pulses of 0.99–1.8% (chickpeas, red lentils, and green field peas). DS content in raw flours can be due to the effect of milling on starch granules; the final product provides information about relevant changes to starch; the higher value, the greater the degree of gelatinization.⁸⁶ Both processes increased DS, cooking increased DS 4.6 times compared to raw sample, while extrusion was increased 16.8 times. It is known that during extrusion cooking can cause a complete breakdown of the starch structure due the high shear forces and high temperatures, thus promoting almost complete gelatinization of starch, leading to a decrease in RS.⁸⁷ This is in accordance with the results obtained in this research where an increase in DS and a decrease in RS were observed after extrusion processing. On the other hand, the increase of DS in CAF was lower than that in EAF because, even though gelatinization occurs during cooking, a retrogradation of starch can take place after cooking and during freeze-drying processes and most of the starch fraction can change into RS.

Fatty Acid Content. The fatty acid content in AF, CAF, and EAF is shown in Figure 1. For AF the values of palmitic acid 13.71%, stearic acid 2.38%, oleic acid 15.16%, linoleic acid 38.12%, and linolenic acid 21.64% were close to the values that have been reported previously by Grela and Günter (1995)⁸⁸ for ayocote beans (16.40, 3.30, 13.30, 31.40, and 21.80%,

respectively). However, linoleic acid values are higher than those reported by other researchers for other bean varieties (15–33.7%).^{89,90} Linoleic acid is known for its potential beneficial properties for human health, such as prevention of atherosclerosis, cancer, and hypertension and improvement of bone mineralization, insulin sensitivity, and immune response.⁹¹ Furthermore, linoleic and linolenic acids increased after cooking processing. Heat processes can cause cell membrane disruption, thus releasing of pro-oxidant, and/or isomerization reactions that produce small amounts of trans fatty acids, particularly linoleic and linolenic acids.⁹² On the other hand, significant ($p \leq 0.05$) differences were found in CAF; a decrease in palmitic, stearic, and oleic fatty acids was observed. This behavior was reported by De La Cruz Garcia and Simal Lozano (2000),⁹³ in which processed beans by different cooking methods (pressure-cooking, steaming, boiling, and microwave) showed slight decreases in palmitic acid (3.6, 4.8, 5.2, and 4.2%, respectively) after processing. Also, the compound palmidrol, also known as palmitoylethanolamide ($C_{18}H_{37}NO_2$), was found in AF. This compound is present as well in egg yolk, peanuts, and soy seeds, being an endogenous lipid chemically related to endocannabinoid anandamide.⁹⁴ Palmitoylethanolamide (PEA) is a saturated fatty acid amide derived from the naturally occurring N-acylethanolamine (NAE) in which the carboxylate group is amidated by the primary amine of ethanolamine. PEA is recognized for its extensive effects, believed to play protective and homeostatic roles across both animal and plant domains. This compound is present in a variety of organisms, from animals to plants, and is linked to a host of beneficial functions.⁹⁵ No significant differences ($p > 0.05$) were found for EAF, except for a decrease in palmidrol and an increase of linoleic acid. This behavior could be explained by the absence of palmidrol in the flour processed by extrusion. There was a change in the proportion of linoleic acid. Ushakumari et al. (2004)⁹⁶ found an increase in unsaturated fatty acids in a foxtail millet based extruded product. This could be as a result of lipids released from cells due to high temperature and physical disruption of plant cell walls owing to severe shear actions, as discussed above.⁹⁷

Amino Acids Content. The amino acid (AA) contents of AF, CAF, and EAF flours are summarized in Table 3. Overall, Leu, Lys, and Phe were found to be the major essential AAs, while Asp and Glu were the two most abundant nonessential AAs. On the contrary, Met, Trp, and Cys were the less abundant AAs. These results were similar to those reported by Alvarado-López et al. (2019)⁶³ for 4 different varieties of ayocote bean, with Leu (1.74–1.84 g/100 g) being the most abundant essential AAs, followed by Glu (nonessential AAs, 3.22–3.58 g/100 g), while the limiting AAs were the sulfur-containing AAs (Cys and Met, 0.23 and 0.28 g/100 g, respectively).

Cooking changed the AA content, with an increase in Glu (3.70%), Ala (3.47%), Val (3.03%), Ile (6.42%), and Leu (2.4%). On the other hand, a decrease in Tyr (17.18%), Cys (66.66%), and Met (56.25%) was observed. The reason for an increase in some AAs could be attributed to the breakdown and degradation of some other AAs.⁹⁸ On the other hand, proteins not only exist alone but also form complexes with carbohydrates and lipids; for instance, AAs with a side chain can be associated with reducing substances and form complexes with phytates; nevertheless, these compounds are partially or completely destroyed during hydrothermal treat-

Table 3. Amino Acid Profile (g/100 g of Protein) of Raw, Cooked, and Extruded Ayocote Bean Flours¹

Amino Acid	Ayocote Bean (g/100 g of Protein)		
	AF	CAF	EAF
Asp	2.75 ± 0.03 a	2.83 ± 0.03 a	2.75 ± 0.00 a
Glu	3.24 ± 0.04 b	3.36 ± 0.01 a	3.06 ± 0.00 c
Ser	1.49 ± 0.01 a	1.51 ± 0.00 a	1.49 ± 0.00 a
His*	0.65 ± 0.00 a	0.64 ± 0.03 a	0.65 ± 0.01 a
Gly	1.03 ± 0.01 a	1.08 ± 0.05 a	1.12 ± 0.04 a
Thr*	1.30 ± 0.00 a	1.31 ± 0.01 a	1.29 ± 0.00 a
Arg	1.47 ± 0.04 a	1.44 ± 0.00 a	1.43 ± 0.02 a
Ala	1.15 ± 0.00 b	1.19 ± 0.00 a	1.10 ± 0.00 c
Pro	0.96 ± 0.00 a	0.92 ± 0.04 a	0.86 ± 0.06 a
Tyr	0.64 ± 0.00 a	0.53 ± 0.00 c	0.57 ± 0.00 b
Cys	0.21 ± 0.00 a	0.07 ± 0.00 c	0.13 ± 0.00 b
Val*	1.32 ± 0.00 ab	1.36 ± 0.01 a	1.30 ± 0.01 b
Met*	0.16 ± 0.00 a	0.07 ± 0.00 b	0.02 ± 0.00 c
Phe*	1.48 ± 0.00 a	1.50 ± 0.00 a	1.47 ± 0.01 a
Ile*	1.09 ± 0.01 b	1.16 ± 0.01 a	1.08 ± 0.00 b
Leu*	2.08 ± 0.00 b	2.13 ± 0.01 a	2.05 ± 0.01 b
Lys*	1.70 ± 0.01 a	1.70 ± 0.01 a	1.64 ± 0.00 b
Trp*	0.19 ± 0.01 a	0.18 ± 0.00 a	0.18 ± 0.01 a

¹Results are expressed as mean ± standard deviation of three determinations; applying the Fisher multiple range test; different letters in the lines indicate statistically significant differences ($p < 0.05$). *Essential amino acids. AF = raw ayocote bean flour, CAF = cooked ayocote bean flour, EAF = extruded ayocote bean flour.

ment, which is likely to increase the amino acid content of boiled beans compared with the raw ones.^{99,100} Shamkova et al. (2022)¹⁰⁰ studied the effect of cooking of beans (*P. vulgaris* L.) using three different solutions (water, 0.25% whey solution, and 0.25% calcium bicarbonate solution), observing that after cooking mainly with water a significant increase in total AA content was obtained, similar to our results. This may be related to the loss of carbohydrates (oligosaccharides) during heat treatment; these oligosaccharides could form bonds with other macromolecules such as proteins or polysaccharides, and these bonds may get affected during the cooking process, resulting in leaching of the oligosaccharides into the cooking medium.¹⁰⁰ However, the available data about changes in AA content of beans during processing is contradictory; some studies found that most of the processing methods have a little effect on protein quality, while others found that even under gentle technological processes the proteins are easily damaged.^{100,101} For example, Aremu et al. (2010)¹⁰² reported a reduction in all essential AAs in cranberry bean (*Phaseolus coccineus* L.) after cooking, attributing this effect to transamination and deamination reactions that might be responsible for the changes in the amino acid profile of this bean after cooking. Heat treatment can cause considerable nutritional damage, particularly in methionine and cysteine because these are heat sensitive; thus, prolonged cooking times in legumes may result in destruction and/or racemization of amino acids.¹⁰³

Extrusion of the ayocote bean significantly reduced the concentration of Glu (5.5%), Ala (4.3%), Tyr (10.9%), Cys (38.0%), Met (87.5%), and Lys (3.52%), respectively. The relation between AAs and temperature in Maillard's reaction is considered the principal reason for the reduction of AA concentration observed in grain legume extrudates.¹⁰⁴ Free sugars might be produced from starch during extrusion and

Table 4. Composition of Minerals of Raw, Cooked, and Extruded Ayocote Bean Flours[†]

Minerals (mg/kg)	Ayocote Bean		
	AF	CAF	EAF
	Macrominerals		
K	8420.94 ± 224.48 a	7755.20 ± 133.45 a	8285.48 ± 789.74 a
Mg	1673.28 ± 73.94 a	1708.58 ± 5.42 a	1694.96 ± 9.33 a
Na	29.17 ± 10.65 b	64.42 ± 10.56 a	29.16 ± 3.24 b
Ca	1169.00 ± 61.25 b	1253.68 ± 22.54 a	1290.16 ± 0.75 a
	Trace Minerals		
Cu	5.08 ± 0.10 b	4.95 ± 0.23 b	6.59 ± 0.12 a
Fe	55.24 ± 0.04 c	57.46 ± 1.44 b	83.51 ± 0.01 a
Mn	14.91 ± 0.13 b	16.63 ± 0.37 a	16.88 ± 0.12 a
Zn	26.14 ± 1.25 b	27.92 ± 0.46 a	28.76 ± 0.20 a
Se	0.010 ± 0.00 a	0.016 ± 0.00 a	0.003 ± 0.00 a

[†]Results are expressed as mean ± standard deviation of three determinations; applying the Fisher multiple range tests; different letters in the lines indicate statistically significant differences ($p < 0.05$). AF = raw ayocote bean flour, CAF = cooked ayocote bean flour, EAF = extruded ayocote bean flour.

react with amino groups of AAs, starch, and dietary fiber fragments, as well as with sucrose hydrolysis products, which are available for Maillard reaction.¹⁰⁵ The loss of AAs depends mainly on the extrusion conditions; for instance, some studies have shown that higher temperatures than 100 °C during extrusion increase the loss of most AAs in three different legumes (Bambara groundnut, pigeon pea, and African yam pea).¹⁰⁴ These results are similar to this study considering that the extrusion process used a temperature of 137 °C. Marsolais et al. (2010)¹⁰⁶ investigated the AA profile of different storage proteins (globulins, albumins, glutelins, and prolamins) in *Phaseolus* spp. demonstrating that the modification of the ratio of these protein fractions altered the AA composition; this is why it is possible that during thermal processing (baking/cooking/extrusion) the protein fractions ratio can result in different AA profiles in the final product, and the use of different varieties of beans, cropping years, and environmental conditions can alter the AA profile.¹⁰¹

Minerals. The mineral contents of AF, CAF, and EAF samples are shown in Table 4. Minerals have a key role in our body for vital functions, from building strong bones to transmitting nerve pulses. For example, trace minerals (Cu, Fe, Mn, Mg, Se, and Zn) play a vital role as a structural part in many enzymes, while macrominerals (Ca, Mg, P, Na, and K) have a lot more considerable functions in nerve cells.¹⁰⁷

The most abundant macrominerals in AF were K, Mg, and Ca, while the highest trace minerals were Fe and Zn. These results were lower for K, higher for Mg, and similar to Ca (12,834–14,433, 1,467–1563, and 1205–1229 mg/kg, respectively) than those reported by Alvarado-López et al. (2019)⁶³ for four varieties of ayocote bean; these differences might be attributed to the genetic composition of varieties, different soil conditions at different habitats during growth, etc. Despite this, similar studies have reported that major minerals present in beans are K, Mg, and Ca.⁷⁰ Redondo-Cuenca et al. (2022)¹⁰⁸ reported contents of macro and micro minerals of three different colors of ayocote bean, with higher results in Na (400–300 mg/kg) and K (14,000–13,000 mg/kg), lower results in Mn (12.8–10.7 mg/kg), and similar values for Mg (1,900–1,600 mg/kg), Ca (1,100–900 mg/kg), Cu (6.9–6.1 mg/kg), Fe (53.6–52.5 mg/kg), and Zn (26.4–24.7 mg/kg). It has been reported that the variation in mineral composition may be related to pedoclimatic conditions, genotypic

characteristics, plant maturity, and analytical techniques used in the analysis.¹⁰⁹

The mineral content in CAF had a minor effect (with the exception of Na), with an increase in Na (120.84%), Ca (7.24%), Fe (4.01%), Mn (11.53%), Zn (6.80%), and Se (60%) and a decrease in K (8%). The augmentation in mineral content may arise as a consequence of the retention of cooking water and subsequent freeze-drying thereof in conjunction with the cooked beans. This process results in the incorporation of minerals that were originally dissolved in the water, which become concentrated post freeze-drying. Moreover, the observed reduction in potassium (K) levels may be attributed to the concomitant increase in the relative proportion of other minerals. In this study, there was a notable increase in mineral content after the cooking process. The potential reasons for this phenomenon could be attributed to the reduction of phytic acid upon heating, which might result in enhanced mineral extractability and bioavailability. Alternatively, this could be due to modifications in the food matrix, such as the disruption of plant cell walls.^{110,111} In this study, the cooking liquor was retained. Consequently, it is plausible that certain minerals might have been introduced via the cooking water, leading to the final concentrations described above. On the other hand, some studies have observed that mineral loss can occur due to minerals leaching into soaking and/or cooking water.¹⁰⁸ Additionally, some losses can be related to mineral binding to proteins causing less extractability.¹¹⁰

Extrusion increased Ca (10.36%), Cu (29.72%), Fe (51.17%), Mn (13.2%), and Zn (10.0%) and decreased K (2.0%) and Mg (1.29%). The main mineral that changed was Fe, which is most likely a result of wear of metallic pieces, mainly screws, and the barrel of the extrusion equipment.¹¹² Minerals are heat stable and unlikely to be lost in the steam-distillate at the die.¹⁰⁵ Even though the content of minerals is not significantly affected, other studies have shown that extrusion can improve the absorption of minerals by reducing other factors like antinutritional factors that inhibit absorption; further research in this area is necessary.¹⁰⁵

Physicochemical Properties. Table 2 shows the physicochemical properties of raw and processed ayocote bean flours. The raw and processed flours showed significant differences ($p \leq 0.05$). The results for total color difference (ΔE) in AF, CAF, and EAF were 19.42, 51.79, and 42.81,

respectively. Cooking and extrusion caused an increase ($p \leq 0.05$) in ΔE of raw ayocote bean flour. Furthermore, a decrease in the hunter L value from 78.4 to 46.72 (CAF) and 55.40 (EAF) was observed. Higher ΔE and lower L values mean darker flours. During the extrusion process, the color changed; this has been related to Maillard reactions, where sugar reacts with proteins, thus producing melanoidins and making extrudates turn dark brown.¹¹³ These changes could be a result of the drive power or the specific mechanical energy as the source for the changes in extrusion due to heating and shear forces produced. CAF color was darker than EAF; this may be because traditional cooking had a longer reaction time compared to the extruded one.¹¹⁴

Water activity (a_w) from 2 g of AF, CAF, and EAF samples was 0.33, 0.07, and 0.36, respectively. Borjindakul and Phimolsiripol (2013)¹¹⁵ reported similar values for lablab (*Dolichos lablab*) beans (0.24–0.58); values below 0.6 reduce the growth probability of harmful microorganisms. The lowest water activity observed was 0.07 for CAF. This result may be due to the drying method used, as CAF was lyophilized, while EAF was dried at room temperature (25 °C).

Regarding pH, AF, CAF, and EAF were 6.36, 6.28, and 6.56, respectively. Dzudie et al. (1996)¹¹⁶ reported acidity values in raw black and white bean flours grown in France, with values of 5.9 and 6.2, respectively. A pH increase ($p \leq 0.05$) in EAF was observed; this may be due to the solubilization of basic amino acids during extrusion.¹¹⁷ The determination of pH in flours has been associated with some functional properties such as solubility and emulsion properties, which are extremely affected by pH fluctuations.¹¹⁶

Functional Properties. The functional properties of raw and processed ayocote beans are shown in Table 2. The water absorption index (WAI) for CAF and EAF was higher ($p \leq 0.05$) than that of AF, 3.47, 3.46, and 2.87, respectively. Other studies have found an increase in WAI for beans subjected to cooking¹¹⁷ and extrusion processing.¹¹⁸ Researchers argue that the cooking temperature increases the WAI of different pulses. During extrusion, protein denaturation (mainly globulins as the major protein fraction present in beans)¹¹⁹ increases the surface area to mass ration and exposes some previously hidden peptide bonds and pole side chains which leads to increased ability to entrap and retain water molecules; starch gelatinization could also increase the WAI due the large loss of crystalline structure and molecular order, and swelling of raw fiber occurs. This behavior could be responsible for the increased WAI in extruded products.^{67,119}

Significant differences ($p \leq 0.05$) were found regarding WSI results; the increase for processed samples was noticeable, and even more for EAF, findings are consistent with those reported by other authors¹²⁰ for raw and extruded bean (*P. acutifolius*) flours (20.21 and 27.45 g of solids/100 g of dry sample). It is suggested that this behavior is caused by starch degradation due to the cutting pressure and thermal process during extrusion.⁶⁷ Water solubility index (WSI) indicates the number of solids dissolved in water when a flour sample is subjected to an excess of liquid and shows the degree of cooking. WSI is associated with the presence of soluble starch molecules, which is related to dextrinization.⁶⁷

In this study, OAI results obtained for AF were 1.60 g/g (25.5%) of dry sample; on the other hand, a decrease ($p \leq 0.05$) was found in processed flours (CAF = 0.87 (14.4%) g/g of dry sample and EAF = 0.78 (12.4%) g/g of dry sample, respectively). These results agree with those reported by

Granito et al. (2007),¹²¹ observing a reduction of OAI during cooking. The OAI is related to the number of hydrophobic groups present in the proteins, thus increasing the fat bonding interactions. When the cooking process is applied, protein denaturation and aggregation are produced, which causes interactions among hydrophobic groups, decreasing the free hydrophobic groups exposed to oil bonding.¹²¹ Oil absorption index (OAI) is the mechanism of this property due to the physical retention of oil by capillary attraction. Additionally, our results agree with Lazou and Krokida (2010);¹²² in this study, a decrease in OAI after extrusion in a sample of corn/lentils was reported, with increasing feed moisture (6, 13, 19%); in this study, a 24% feed moisture was used. The rise of feed moisture content decreases the degree of cooking, and the OAI of extrudates should be lower because small amounts of molecules of starch are present, which favor OAI. On the other hand, it has been reported that OAI can vary depending on the extrusion conditions used; for example, high temperatures increase the degree of cooking of extrudates, resulting in a formation of smaller molecules due to starch dextrinization; the presence of these molecules may be responsible for the increased OAI.¹²³ The increase of protein content present in extrudates can decrease the OAI and could be related to the increased presence of nonpolar amino acids, thus conferring different conformational features and that starch–protein–lipid complexes could all be in some or less extent related to different oil retaining capabilities.¹²⁴

The percentages of dispersibility for AF, CAF, and EAF were 71.66, 100, and 93.33%, respectively. CAF and EAF had higher values ($p \leq 0.05$) than AF. Milán-Carrillo et al. (2000)²⁵ found an increase (38.7–145%) in dispersibility values in extruded chickpea flours. The increase in dispersibility could be attributed to the degree of damaged starch due to gelatinization during thermal processing, extrusion induced fragmentation, and thus a molecular weight reduction of amylose and amylopectin molecules.¹²⁵ Other authors have attributed that cooking and extrusion processing slightly increased the protein denaturation, meaning the more denaturation, the higher the dispersibility.⁶⁷ Dispersibility is a useful property for the formulation of beverages, since it is a means of comparing the solubility of the protein in water.

AF and CAF presented foaming percentages (F) of 8.08 and 2.00 and foam stability (FS) of 4.04 and ND (not detected), respectively, while EAF showed non-foaming capacities (Table 2). For both parameters, a significant decrease ($p \leq 0.05$) was observed after both processes were applied. The effect of temperature on the reduction of F and FS has been reported for other bean varieties (Tepary bean).¹²⁶ This effect is attributed to the fact that high temperatures used in both processes (cooking and extruding) probably affected the molecular and physicochemical characteristics of the components responsible for foam formation¹²⁷ and extrusion is the one that has mostly reduced both parameters. Batista et al. (2010)¹²⁷ evaluated the percentage of foam formation in raw and extruded cowpea beans (51.16 to 0.00%), finding no foam formation after extrusion (ET = 150 °C, SS = 150 rpm), which corresponds to the results obtained. Potentially, proteins may be unfolded by shear forces during extrusion, while the high temperature could generate protein cross-links into macromolecular aggregates reducing F and FS.¹²⁸ Foaming percentage (F) is a measure of the maximum level of foam generated by a solution, while foam stability (FS) provides a measure of the foam's resistance to destabilization.

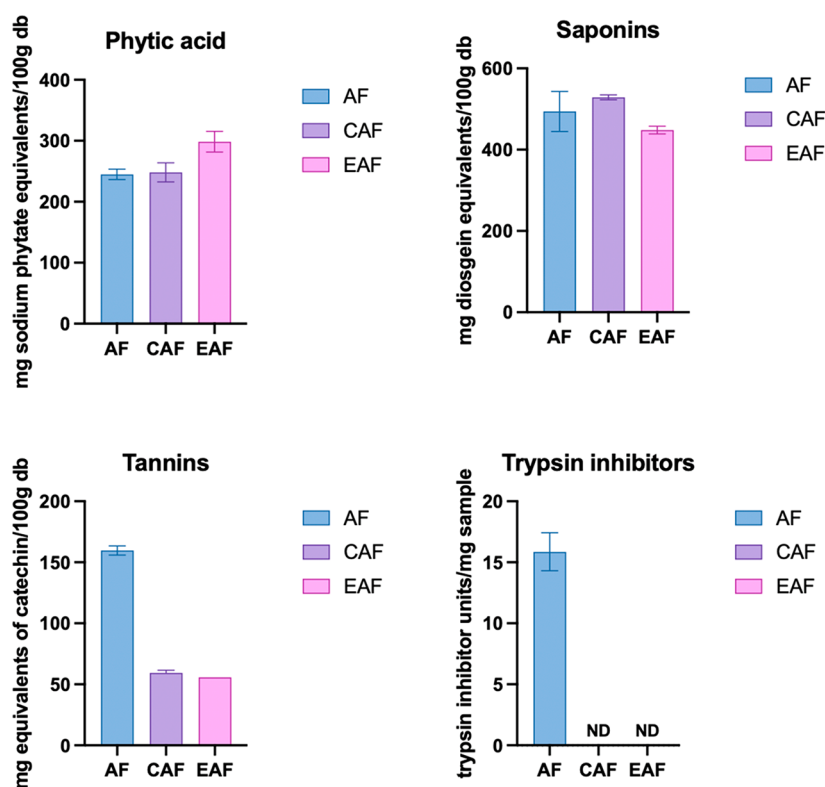


Figure 2. Antinutritional factors content of raw, cooked, and extruded ayocote bean flours. AF = raw ayocote bean flour, CAF = cooked ayocote bean flour, EAF = extruded ayocote bean flour, ND = not detected.

AF, CAF, and EAF showed emulsion capacity (EC) values of 27.85, 27.04, and 13.45% as well as emulsion stability (ES) values of 50.72, 50.00, and 25.92%, respectively. The extrusion process causes a decrease ($p \leq 0.05$) in the pH levels in EC and ES. The emulsifying properties of flours are known to be influenced by the quality and quantity of soluble proteins.²⁰ Lin et al. (2020)¹¹⁹ reported a decrease in EC of common bean flours after heat treatments, attributing this behavior to a reduced ability of processed bean protein to rapidly absorb, unfold, and reorient at the interface; this may be due to a change in the distribution pattern of hydrophilic/hydrophobic residues on the protein surface, favoring a higher proportion of hydrophilic groups on the surface. Singh (2007)¹⁰⁵ reports that heat treatment causes marked reductions in emulsifying capacity in processed legume flours. Emulsion capacity (EC) is a measure of the interface area that stabilizes per unit weight of protein. In contrast, emulsion stability (ES) is a measure of the resistance of the emulsion to decompose.¹²⁹

Functional properties are physicochemical properties that provide information on how a particular ingredient (compound) in particular (protein, carbohydrate, etc.) could behave in a complex or simple food matrix. These properties are established by the composition and molecular structure of the individual components and the interactions established between them.¹¹⁷

Antinutritional Factors. Antinutritional compounds are secondary metabolites that act as the opposite of nutrient functions.¹³⁰ These are synthesized during plant growth, playing a protecting role in the plant from diseases and pests. Nevertheless, these compounds mainly interfere with the release or absorption of nutrients, reducing the bioavailability of nutrients.¹³¹

The PAC in AF sample ayocote bean (244.91 mg of ESP/100 g) was similar to the results reported by Shang et al. (2016)¹³² (200–500 mg of ESP/100 g) in 56 bean varieties (Figure 2). However, these were lower than those reported by Corzo-Ríos et al. (2020)⁴ in two different varieties of ayocote bean, 1035 and 1038 mg of ESP/100 g samples.

The PAC was not affected after cooking; phytates are relatively heat stable. Corzo-Ríos et al. (2020)⁴ reported that PAC after cooking for eight varieties of *P. vulgaris* and two *P. coccineus* decreased for some varieties, while in others it was not significantly affected; this could be attributed to the food matrix microstructure, as the formation of insoluble phytic acid can form complexes with proteins and minerals, thus reducing the probability of phytic acid loss during hydrothermal processing.

On the other hand, extrusion significantly increases PAC (21.9%) in ayocote bean (Figure 2); it could be due to a release of these compounds during the extrusion process, which make it more detectable. Rathod and Annapure (2016)¹³³ reported that extrusion can affect PAC mainly by the processing conditions such as moisture, temperature, and pressure in different pulses.

Saponins (SC) are compounds present in legumes as amphiphilic glycosides of steroids and triterpenes, playing a protective role against insects.¹³⁴ SC in AF was 494.19 mg of diosgenin equivalents/100 g (mg of DE/100 g) (Figure 2). Corzo-Ríos et al. (2020)⁴ reported similar results in eight varieties of *P. vulgaris* and two *P. coccineus*, 279–482 and 477–514 mg of DE/100 g, respectively.

After cooking, saponins were not significantly affected ($p \leq 0.05$). Sánchez-Velázquez et al. (2021)¹⁰ reported a decrease in SC after cooking in 10 different pulses, attributing this effect to the migration and/or solubilization of these into the soaking/

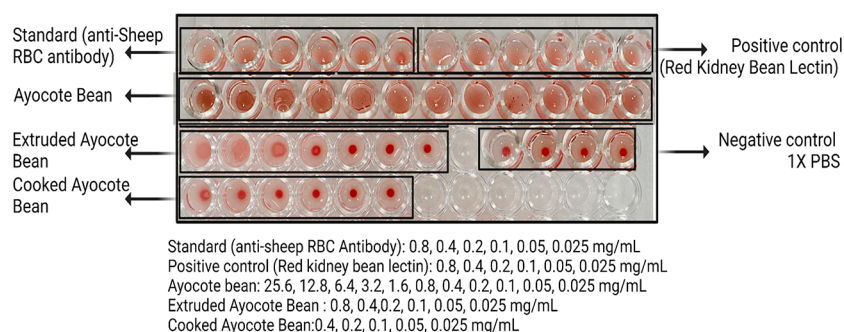


Figure 3. Hemagglutinin activity of raw, cooked, and extruded ayocote bean flours.

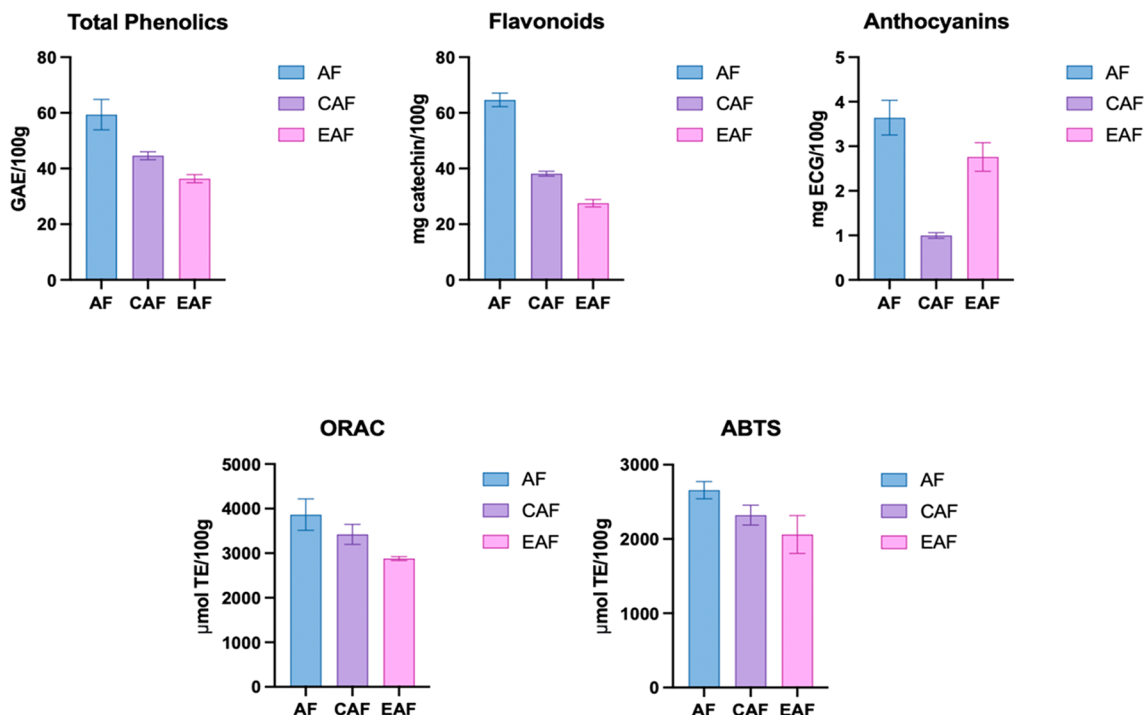


Figure 4. Phenolic compounds and antioxidant activity of raw, cooked, and extruded ayocote bean flours.

cooking water. However, some researchers reported that saponins are relatively heat-stable components.¹³⁵ Barakat and Rohn (2015)¹³⁶ reported that different cooking conditions differently affected the saponin content in chickpea, soy, and fava beans. Furthermore, saponins were identified; particularly saponin B increased after cooking; therefore, the type of saponin could have an impact on the concentration and types of saponins that could be found in different legumes. Also, hydrothermal processing can destroy and/or change the original structure of saponins into smaller fragments.¹³⁷

Meanwhile, extrusion generated a decrease in SC (9.31%). Barakat and Rohn (2015)¹³⁶ reported SC in quinoa was subjected to different parameters of extrusion, demonstrating that moisture, temperature, and screw speed had significant linear effects in SC; for example, increased heat decreased SC, and lower moisture content also decreased SC. On the other hand, Sánchez-Velázquez et al. (2021)¹³⁸ reported that the impact of the extrusion process on saponins did not show a clear pattern, stating that this effect will depend on different factors such as pulse type, saponins location in the seed, processing conditions, etc. However, the shear and thermal energy present during extrusion may be sufficient to destroy

the original structure of saponins, resulting in the formation of smaller chemical fragments.¹³⁷

Additionally, as saponins are mainly found in the testa, milling and/or decortication before extrusion could be used as pretreatment methods to reduce SC, due to the removal of the testa and particle size reduction during the milling process.

Tannins are antioxidant compounds in foods with multifunctional properties related to health benefits, but these also exhibit antinutritional properties decreasing the digestion of various nutrients.¹³⁹ The tannin content (TC) in AF was 159.69 mg of EC/100 g (Figure 2); these results are within the range reported by Wang et al. (2010)¹⁴ for seven different varieties of beans (3.00–1,990.00 mg of EC/100 g). Cooking significantly ($p \leq 0.05$) reduced TC (62.82%); other studies reported a decrease in TC after cooking in beans and pulses.^{14,72,140} Corzo-Ríos et al. (2020)⁴ reported a decrease in TC after cooking processing in two species of *Phaseolus* (*P. vulgaris* and *P. coccineus*), attributing the reduction to the high temperatures that alter the chemical structure of polyphenols, promoting polymerization and/or decomposition of their aromatic structure. Reduction in TC could also be explained by the thermolability of tannins; besides the variation in TC reduction

rates depends on the plant material and the cooking time and temperature used.¹⁴¹ TC was reduced after the extrusion process (65.06%); this could be a result of decarboxylation that promotes polymerization, leading to declined extractability.⁷²

In the case of the trypsin inhibitor activity, AF presented 15.86 TIU/mg (Figure 2); this result is higher than that reported by Corzo-Ríos et al. (2020)⁴ for eight varieties of *Phaseolus vulgaris* and two *P. coccineus*, i.e., 5.97–8.40 and 6.70–6.90 TIU/mg, respectively. The TIU in common beans is related to seed protein content, planting year, and genotypes; this could be the reason for the differences between studies.¹⁴² Cooking and extrusion caused a 100% loss of the activity of TIU; these are thermolabile proteins that undergo denaturation during thermal processing, which causes a loss of activity due to protein denaturation and unfolding.⁴

Hemagglutinin Activity. Hemagglutinin or lectins are glycoproteins that have been found in more than 600 genus of legumes; the total protein of pulse seeds contains around 2–10% lectins, and these can inhibit digestive enzymes.^{143,144} Figure 3 shows the presence of hemagglutinin activity in AF, CAF, and EAF samples. Wells with uniform red color or diffuse suspension are positive to hemagglutinin activity, while those with a red button or dot shape at the bottom of the well are negative. Different concentrations of lectin extracts were assayed to determine the dose-dependent activity. AF showed hemagglutinin activity at all concentrations tested, while CAF showed no activity at all concentrations and EAF showed minor activity after 0.2 mg/mL of protein. Therefore, the cooking process was the most effective in eliminating hemagglutinin activity.

González-Cruz et al. (2022)⁸ reported that, in extracts of lectins from two varieties of *P. coccineus* treated at different temperatures (12–95 °C) for 30 min, the hemagglutination activity was lost up to 90 °C. Changes in hemagglutination activity by thermal treatment are associated with modifications in the secondary structure of lectins,¹⁴⁵ which occurred by the conditions used during the cooking and extrusion processes, time of exposure, water/substrate ratio, pressure, temperature applied, and shear rate.

Nutraceutical Properties. Total Phenolic Content. The total phenolic compound content for the raw and processed beans is shown in Figure 4. AF, CAF, and EAF showed total phenolic compounds of 59.3, 44.6, and 36.3 mg of GAE/100 g, respectively. Both cooking and extrusion processes caused significant ($p \leq 0.05$) losses of phenolics when compared to AF. However, a higher percentage of retention was observed in CAF (75.2%) than in EAF (61.7%). Corzo-Ríos et al. (2020)⁴ evaluated the content of phenolic compounds in raw and cooked common bean (*P. vulgaris*) and ayocote bean (*P. coccineus*), reporting a decrease in these compounds after cooking and similar retention percentages for common (59.1–96.6%) and ayocote (66.4–74.7%) beans. The results after the cooking process could be due to the high heating temperatures that altered the chemical structure of polyphenols, promoting polymerization or decomposition of their aromatic structures.¹⁴⁶ Abd El-Hady and Habiba (2003)¹⁴⁷ studied the effects of extrusion processing variables such as barrel temperature and feed moisture in total phenolics content of whole meal peas, chickpeas, faba, and kidney bean flours. A significant decrease was observed in total phenolics for extruded products, and these behaviors were attributed mainly to the individual effect of both temperature and moisture content. Other authors mentioned that this decrease may be

due to the decarboxylation of phenolic acids during extrusion.⁶⁸

Flavonoids. FC is shown in Figure 4. AF, CAF, and EAF showed 64.65, 38.14, and 27.57 mg of CA/100 g of sample, respectively. The FCs of AF were lower than those reported by Alvarado-López et al. (2019)⁶³ in four varieties of ayocote bean, 108.4–161.2 mg of CA/100 g. In general, the content of flavonoids was impacted by the thermal process; after cooking and extrusion, a significant ($p \leq 0.05$) decrease (41% and 57.35%, respectively) of FC was found. Flavonoids are secondary metabolites from plants that are present in legumes; they are water-soluble compounds, and during cooking, these phenolics can be leached and degraded, depending on the chemical structure, time, and temperature used during the processing applied.¹⁴⁸ In this study, extrusion generated a significant ($p \leq 0.05$) decrease (57.35%) in FC; this could be due to the high content of starch in the sample produced after extrusion; additionally gelatinized starch can form complexes with lipids, thus trapping phenolics and reducing the extraction efficiency.¹⁴⁹ Moreover, high temperature and pressure in extrusion may result in decarboxylation of phenolics, promoting polymerization.¹⁵⁰

Anthocyanins. Anthocyanin content (AC) is shown in Figure 4. Anthocyanins are an important type of flavonoids in pigmented common beans; the AC in AF was 3.64 mg equivalents of cyanidin-3-glucoside/100 g (mg of ECG/100 g); this result is lower than those reported by Alvarado-López et al. (2019)⁶³ for black and purple ayocote bean varieties (108.2 and 119.3 mg of ECG/100 g, respectively).¹⁵¹

Cooking caused a significant reduction ($p \leq 0.05$) in anthocyanin content (72.58%); the loss of anthocyanins could be due to different factors. Anthocyanins are highly water-soluble; therefore, boiling could increase leaching; also these are sensitive to high temperatures, thus causing their rapid degradation.¹⁵² On the other hand, extrusion caused a loss of anthocyanins (24.17%) but not as noticeable as cooking, even though anthocyanins are thermally sensitive; during extrusion, starch is affected due to interactions between starch and nonstarch components like lipids, proteins, and phenolics including anthocyanins, causing prevention of the degradation and/or a reduced impact to these complexations.¹⁵³

Antioxidant Activity. Antioxidant activity for the raw and processed bean samples is shown in Figure 4. Antioxidant capacity was determined using the ORAC and ABTS methodologies, obtaining values for AF of 3,866.37 and 2,657.94 μmol of TE/100 g, respectively. The antioxidant capacity for CAF was 3,422.02 (ORAC) and 2,321.84 (ABTS) μmol of TE/100 g, while the antioxidant capacity of EAF was 2,879.72 (ORAC) and 2,062.09 μmol of TE/100 g, respectively. The cooking process generated a retention percentage of antioxidant capacity of 88.50% (ORAC) and 87.35% (ABTS) for CAF. EAF flours showed retention percentages of 74.48% (ORAC) and 77.58% (ABTS), respectively. However, a higher percentage of retention was observed in CAF than EAF; this could be explained because, during the cooking process, the cooking liquor was freeze-dried with cooked beans, avoiding the loss of compounds that may have been leached during cooking. Korus et al. (2007)¹⁵⁴ evaluated the antioxidant activity in raw and extruded common beans, reporting a decrease in antioxidant activity (1–43% loss of antioxidant activity) once processed by extrusion, presenting the same behavior as the present study. However, Anton et al. (2009)¹⁵⁵ reported an increase in antioxidant activity (DPPH)

for cornstarch/common bean extrudates. Alam et al. (2016)⁶⁸ concluded that the antioxidant capacity is dependent not only on the amount of bioactive compounds but also on their bioactives composition.

To evaluate a possible association between changes in phenolic compounds and antioxidant capacity, a correlation analysis was performed. A significant correlation was found between phenolic compounds and ORAC ($r^2 = 0.925$; $p \leq 0.05$) and between phenolic compounds and ABTS ($r^2 = 0.909$; $p \leq 0.05$). These findings suggest that phenolic compound content is a positive predictor for *in vitro* antioxidant activity. Salas-López et al. (2018)¹⁵⁶ reported a simultaneous relationship between total phenolic content and antioxidant capacity (DPPH, ABTS) in sorghum after extrusion. Cooking and extrusion processes caused significant ($p \leq 0.05$) losses of antioxidant activity when compared to that of raw AF.

This study elucidates the modifications in various aspects of ayocote beans (*Phaseolus coccineus* L.), an indigenous and underutilized pulse, concerning nutritional, antinutritional, and techno-functional characteristics following traditional (cooking) and alternative (extrusion) processing methods, as illustrated in Figure 6. Notably, carbohydrates emerged as

the primary macronutrient subject to modification during processing, with each method inducing distinct changes: a notable increase in resistant starch (RS) was observed in the cooked sample, whereas the extruded sample exhibited an opposing trend. Moreover, mineral and amino acid contents exhibited notable variations between both processing methods. Techno-functional properties, for the most part, displayed similar trends in response to both processes, with the exception of emulsion capacity and stability, which were exclusively influenced by extrusion. It is noteworthy that thermal processing resulted in a reduction of antinutritional factors, as evidenced by a decrease in tannin and trypsin inhibitor levels in both processed samples. Conversely, cooking proved to be the more efficient method for eliminating the presence of hemagglutinins. Furthermore, cooking exhibited a more pronounced impact on anthocyanins content, whereas extrusion exerted a greater influence on flavonoids content. Nevertheless, ayocote beans showed high antioxidant activity retention following both processing techniques. This suggests that ayocote beans represent a promising legume source for the development of novel food formulations that offer enhanced health benefits.



Figure 5. Flower and seeds of ayocote bean (*Phaseolus coccineus*).

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsfoodscitech.2c00416>.

Formulas used to calculate physicochemical properties (total color difference), oil absorption capacity, dispersibility index, foaming capacity and foam stability, emulsifying capacity and emulsion stability, antinutritional (phytic acid content, saponins, condensed tannins content, trypsin inhibitor activity), and anthocyanin content in ayocote bean flours (PDF)

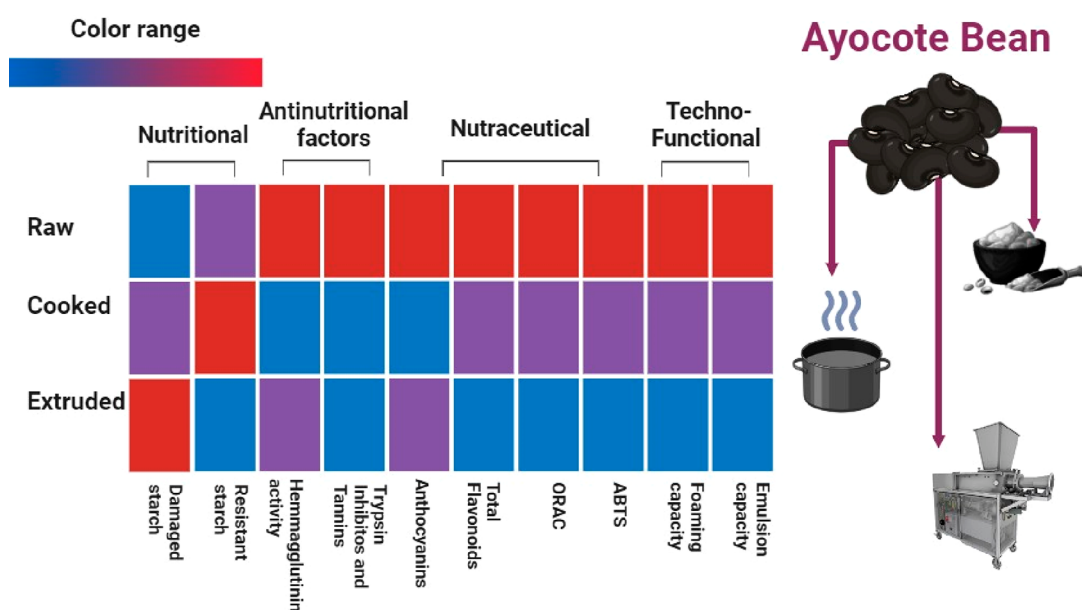


Figure 6. Overview heatmap of the different nutritional, antinutritional, nutraceutical, and techno-functional properties of raw, cooked, and extruded ayocote bean flours.

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Author Contributions

E.I.O.-G. performed bean processing (cooking and extrusion), nutritional composition (proximate analysis, fatty acid content, mineral content), physicochemical characteristics (total color difference ΔE , water activity, pH), functional properties (water absorption and solubility index, oil absorption capacity, dispersibility index, foaming capacity and foam stability, emulsifying capacity, and emulsion stability), hemagglutinin activity, nutraceutical properties (total phenolic content, ORAC, and ABTS), test data, interpreted results, and drafted the manuscript; E.O.C.-R. participated in the general design of the study and supervision and helped on the manuscript drafting and reviewing; C.I.S.-G. performed antinutritional tests (phytic acid content, saponins, condensed tannin content, trypsin inhibitor activity) and anthocyanins and flavonoids tests; C.R.-M. contributed with the sample (ayocote bean) and helped on the manuscript; L.L.-L. designed the antinutritional test and helped on the manuscript drafting; R.H. participated in the damaged starch determination and helped on the manuscript; A.J.H.-A. participated in the general design of the study and supervision and helped on the manuscript drafting and reviewing.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

AAs, amino acids; AAPH, 2,2'-azobis(2-methyl-propinamide); AC, anthocyanin content; AF, ayocote beans flour; AUC, area under the sodium fluorescein decay curves; a_w , water activity; BAPNA, $N\alpha$ -benzoyl-DL-arginine *p*-nitroanilide hydrochloride; CA, catechin; CAC, content of available carbohydrates; CAF, cooked ayocote bean flour; CT, cooking time; CTC, condensed tannins content; DI, dispersibility index; EAF, extruded ayocote bean flour; EC, emulsifying capacity; ECA, equivalents of catechin; ES, emulsion stability; ESP, equivalents of sodium phytate; ET, extrusion temperature; F, foaming percentage; FC, foaming capacity; FS, foam stability; GAE, gallic acid equivalents; NAE, *N*-acylethanolamine; OAC, oil absorption capacity; ORAC, determination of oxygen radical absorbance capacity; PAC, phytic acid content; PAE, palmitoylethanolamide; RS, resistant starch; SC, saponins content; SS, screw speed; TDF, total dietary fiber; TE, Trolox equivalents; TF, total flavonoids; TIU, trypsin inhibitor activity; TPC, total phenolic content; WAL, water absorption; WSI, solubility index; ΔE , total color difference

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