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The effect of cooking with retort pouch system on lipid and phaseolin composition of Pinto Saltillo beans (*Phaseolus vulgaris*)

Mayra MARTINEZ-CENICEROS[1], Karen FERNANDEZ-MONREAL[1], Lenin Efraín DOMÍNGUEZ-ORDAZ[1], Juan Guillermo AYALA-SOTO[1], David CHAVEZ-FLORES[1], Teresita RUIZ-ANCHONDO[2], Fabiola SANDOVAL-SALAS[3], David NEDER-SUAREZ[1], Leon HERNANDEZ-OCHOA[1]*

Abstract

The study objective was to evaluate the processing of pinto beans using the retort pouch process, thus avoiding damage to its nutritional composition. The lipid evaluation was: 1.74% in bean seed and 1.214 %, 1.226%, and 1.417% in the retort pouch treatments. The enzyme Candida Antarctica lipase was used to transesterify the oil and was analyzed by chromatography (GC/MS), identifying the acids: hexadecanoic, 9-octadecanoic, eicosanoic, and octadecanoic. Total protein content evaluation showed 15.88% in the bean seed and 4.75%,4.58%, and 5.18% in the retort pouch treatments with a one-factor analysis of variance (ANOVA) Tukey test $p < 0.05$. The Phaseolin protein was identified by the SDS-PAGE electrophoresis method. These results showed that the nutrients of retort pouch pinto beans were conserved despite the high temperature and constant pressure.

Keywords: retort pouch; Pinto beans; GC/MS; phaseolin.

Practical Application: The use of retouch punch in the processing of beans can preserve its nutritional properties favoring food nutrition.

1 Introduction

Human beings have sought to prepare, consume, and preserve food throughout history, intuitively or through practical learning; however, rapid urbanization has caused an increase in the demand for food with good nutritional and sensory quality (Herrero et al., 2012; Majumdar et al., 2017). Technology applications in packaging have gained attention recently, improving shelf life and minimizing food waste (Hsieh & Ofori, 2007; Mohebi & Marquez, 2015). The packaging technology, known as retort-pouch, promises to be a solution to preserving products due to its flexible and laminated packaging; it is ideal for food, pharmaceutical, cosmetic, and agrochemical products (Cruz et al., 2019; Misra et al., 2019; Pal et al., 2019). In the food industry, foods packed using the retort-pouch technique do not show overcooking, which means a better texture and flavor for the consumer and better handling of the product, avoiding deterioration of the food and minimizing its production costs (Majumdar et al., 2017), which gives it an advantage over metal cans and plasticized containers (Jun et al., 2006). This type of technology is a convenient, economical, and easy-to-use packaging solution for a wide variety of food products, especially legumes, which have a high nutritional value, which is why they are essential in animal feed and human consumption. They constitute the primary source of protein in developing countries, especially with the most vulnerable populations. However, in developed countries, the need for healthy foods has favored their consumption (Ali et al., 2005; Majumdar et al., 2017). There is evidence that the consumption of legumes helps prevent and reduce the risk of diseases associated with feeding as type II diabetes mellitus, obesity, and anemia (Conti et al., 2021; Kumar & Pandey, 2020; Moreno-Valdespino et al., 2020).

Among legumes, the best-known species is beans (Phaseolus vulgaris), an indigenous seed of Central America, considered an essential legume in the world. (Graham & Ranalli, 1997; Kalavacharla et al., 2011), Its long shelf life, accessible storage, and easy preparation make it viable for consumption in all countries (Shamseldin & Velázquez, 2020). Beans contain a high nutritional value of approximately 15% protein, 80% starch, and 2% fat, provide folic acid, dietary fiber, and complex carbohydrates (Svetleva et al., 2006). The main protein fraction is a glycoprotein called phaseolin which is present between 40 to 60% of the total protein and is the primary source of methionine available in the seed; however, phaseolin loses its stability during cooking by conventional methods that include thermal processes and high pressure (Montoya et al., 2010; Osborne, 1894). For this reason, the present research considers it essential to use the retort-pouch technique to process this legume, preserving its nutritional stability in terms of energy content, proteins, lipids, carbohydrates, and dietary fiber.

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[1] *Universidad Autonoma de Chihuahua UACH, Facultad de Ciencias Químicas, Campus Universitario #2, Chihuahua, Chihuahua, Mexico*
[2] *Universidad Autonoma de Chihuahua UACH, Facultad de Ciencias Agrotecnológicas, Campus Universitario #1, Chihuahua, Chihuahua, Mexico*
[3] *Tecnológico Nacional de México TecNM, Instituto Tecnológico Superior de Perote, Perote, Veracruz, Mexico*
*Corresponding author: lhernandez@uach.mx

2 Materials and methods

2.1 Bean (Phaseolus Vulgaris) variety Pinto Saltillo

Pinto Saltillo variety bean seeds obtained after their harvest were used, weighing approximately 36.07 g for every 100 seeds. Visual inspection of the lot used did not present darkening or hardening, which is why it is ruled out that there was oxidation in the grains. Physical characterization of *Phaseolus vulgaris* seeds was carried out by randomly choosing 50 whole seeds; they were weighed and measured with an analytical balance and Vernier caliper considering the width, length, and thickness of each selected seed.

2.2 Retort pouch processing

Retort pouches pre-fabricated with multilayer laminated (12 µm Polyester/12 µm aluminum foil/75 µm cast polypropylene/ biaxially oriented nylon 15.0 µm) of dimension 15 cm × 20 cm were used for treatments. 1.5 kg of Pinto Saltillo variety beans were weighed, the beans were cleaned, separated impurities, and left to rest for 2 h in water. Subsequently, 100 g of wet beans were placed in retort-pouch bags, varying the amount of water added in 100 mL, 120 mL, 140 mL; this was done in triplicate. The bags were sealed (Tew model THS-3305, United States) to 150 °C and placed in a pressure cooker (Presto, capacity 21L, SKU 79291) with 6 L of water for 40 min at a constant heat, once a pressure of 1.2 kgf/cm^2 was acquired, and a temperature of 121 °C, the pressure of air without modifying the established pressure, the fire was turned off, leaving the air pressure constant for 40 min. After that time, the preserves could cool to room temperature.

2.3 Bean lipid extraction

Extraction of the oil from the seed, 50 g of previously ground sample (bean flour) was used, and it was placed in a soxhlet extraction equipment, using hexane as a solvent for 20 min, after time the solvent was recovered, and the oil was collected.

2.4 Bean in retort-pouch lipid extraction

The oil extraction was carried out for the three preserves made in retort-pouch (100 g/100 mL, 100 g/120 mL, and 100 g/140 mL). All samples were placed in refrigeration for 48 h at 4°C before analysis. The preserves were ground, and 50 g of each sample were taken; they were placed in an oven at 65 °C for 1 h for dehydration, after time the dehydrated beans were placed in soxhlet equipment adding 200 mL of hexane as a solvent for 20 min, after the time the solvent and oil were recovered. The samples were made in duplicate.

2.5 Oil transesterification by Candida Antarctica lipase enzyme

Four vials of the samples were prepared with the oil collected by the Soxhlet method with Candida Antarctica lipase enzyme at 10% based on the weight of the sample oil. The vials with collected oil were weighed, and the calculations were made to obtain the grams of enzyme and methanol required for each sample (seed, 100 g/100 mL, 100 g/120 mL, and 100 g/140 mL). All the samples were incubated at a temperature of 36 °C with a speed of 200 rpm for 24 h plus 48 h in incubation, 5 mL of hexane were added to each sample to filter under vacuum and separate the lipase from the sample, later it was stored for analysis subsequently by gas chromatography-mass spectrometry (GC/MS).

2.6 Chromatography-mass Spectrometry (GC/MS) analysis

1 mL of hexane was added to the four samples obtained in the transesterification to dilute them, later aliquots of 1 µL were taken and placed in the injector. The analysis of FAME used a GC-MS Perkin-Elmer instrument (Auto System XL Gas Chromatograph Turbo Mass Gold Mass Spectrometer). Separations were achieved using an EquityTM-1 capillary column (30 m × 0.25 mm ID, 0.25 µm film thickness). The carrier gas was Helium at flow rates of 1.0 mL/min and a split ratio of 25 : 1. The injector temperature was 230 °C. The oven temperature was programmed 120 °C for a hold of 5 min, and continuing with 200 °C of temperature at a rate of 5 °C/min, remained for 5 min, and then re-programmed to reach 280 °C at a rate of 7 °C/min. As a control, the operation was used the turboMass software, GC-MS. MS spectra were obtained at range width *m/z* 60-450, interface temperature of 200 °C, ion source temperature of 200 °C, solvent cut time of 5 min, event time of 0.20, and a scan speed of 2500.

2.7 Evaluation of the total protein

The protein fractions of the treatments were analyzed by the determination of nitrogen with micro-kjeldahl, based on the method of Lang (1958) using nitrogen to a protein conversion factor of 6.25 and analyzed with a one-factor analysis of variance (ANOVA) and the Tukey test at $p < 0.05$ using Minitab 16 software (Minitab, 2010).

2.8 Extraction of phaseolin protein

The phaseolin extraction was carried out following the methodology of Montoya et al. (2008) 0.1 g of each of the samples of bean flour, and the samples of retort-pouch technique were taken, and 1.2 mL of a solution containing 0.5 M NaCl and 0.025 M HCl were added. These were incubated at room temperature with magnetic stirring for 1 h and centrifuged at 13,000 rpm for 15 min, separating the supernatant from the precipitate; five volumes of distilled water were added at 4 °C to the supernatant, obtaining the precipitation of the phaseolin fraction, it was centrifuged for 15 min at 13000 rpm to be analyzed by electrophoresis.

2.9 Analysis by electrophoresis

Electrophoresis analysis was performed using the protocol of Smith (1984). The conditions were an SDS-Acrylamide compactor gel 5% and separator gel 10% to 100 volts for 3 h; the four samples previously were placed in water for 10 min at 100 °C and taken in a 1 : 1 ratio (sample and loading buffer); 20 µL were placed in each well of the electrophoresis gel.

3 Results and discussion

3.1 Lipid extraction

Although the total oil content may vary depending on the variety, species, origin, locality, climate, and environmental conditions; The oil content in the bean seed before processing by retort pouch was 1.74%, which agrees with that reported by Mabaleha & Yeboah (2004) who obtained a fat percentage of 1.5 to 2.0% (w/w) in the same variety of beans. The percentage of oil in samples processed with retort pouch was 1.214 %, 1.226% and 1.417% with respect to the three treatments (100 g/100 mL, 100 g/120 mL, and 100 g/140 mL). Compared with the seed without processing, a decrease of 0.5% was obtained in treatments one and two (100 g/100 mL, 100 g/120 mL) and only 0.3% in the third treatment (100 g/140 mL). However, the processed beans with conventional methods such as canning contain 0.15 and 0.20% (w/w) in pinto beans, as reported by Rocha-Guzman et al. (2013). Audu & Aremu (2011) analyzed raw and processed (boiled, cooked, roasted, sprouted, and fermented) pinto bean seeds; the cooking methods showed a significant effect on bean seeds' chemical composition and mineral profile in thermal processing. The analysis on beans showed a content of proteins of 18.0%, carbohydrate content was 59.7%, and 14.4% of lipids, making beans an economical alternative to proteins such as phaseolin protein. Also, Sutivisedsak et al. (2011) analyzed the lipid content of Pinto, Black, Kidney, and Great Northern beans without thermal processing, finding 2% of triacylglycerols. Furthermore, they found between the primary fatty acids, hexadecanoic or palmitic acid with 10.7-12.7% by weight, corroborating the presence of this fatty acid in the treatments analyzed by the retouch pouch.

3.2 Chromatography-mass Spectrometry (GC/MS)

The ethyl esters were analyzed by GC/MS obtained after transesterification; the raw bean sample was standard. The three samples of the retort pouch treatment were analyzed under the same conditions as the standard, observing a decrease in the peaks; for visualization, the analyzed samples are shown in three scales 2.0 $_{x}$ 10^{9}, 1.5 $_{x}$ 10^{9}, and 2.5 $_{x}$ 10^{8}. The comparison of the peaks obtained between the standard and the analyzed samples is shown in Figure 1.

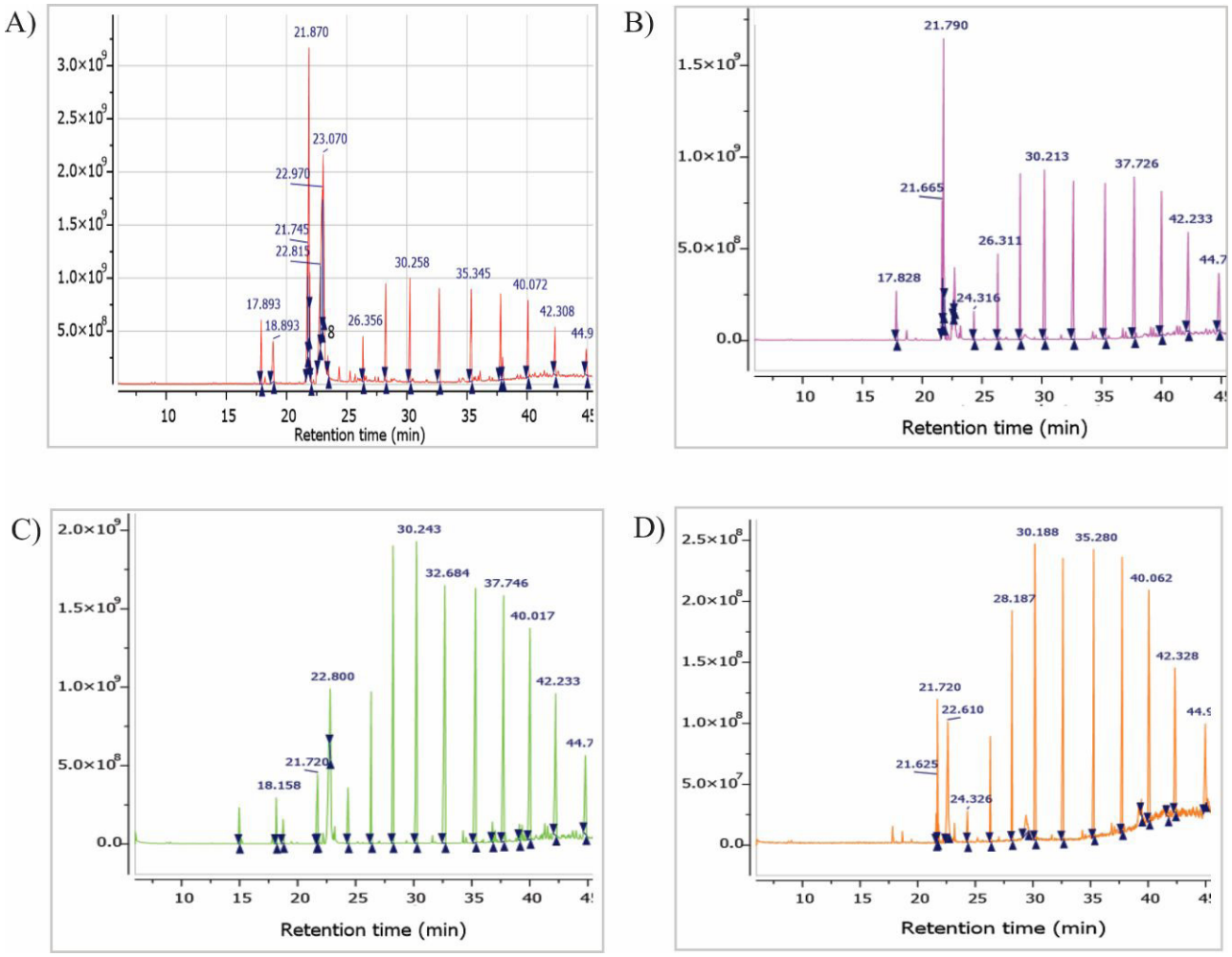

Figure 1. Chromatograms of the methyl esters obtained by GC/MS, the standard, and the three samples are listed: A) beans without processing B) beans in retort pouch 100 g/100 mL, C) beans in retort pouch 100 g/120 mL, D) beans in retort pouch 100 g/140 mL.

The results show the absence of eicosanoic acid methyl ester (arachidic) in the three samples processed with retort pouch, and the octadecanoic acid (stearic) was not identified in the samples of 100 g/120 mL and 100 g/140 mL; this may be because the yield obtained in the transesterification was 30%. There were slight variations in the retention time and the identified compound, as shown in Table 1. Chromatograms show the presence of other peaks belonging to replicas of the acids mentioned above and to unidentified peaks (four in beans without treatment, three in treatments 100 g/100 mL,100 g/120 mL, and two in the treatment 100 g/140 mL), though, according to what was reported by David et al. (2019), these peaks could correspond to the methyl esters of the pentadecanoic, myristic, and linoleic acids in accordance its retention time.

The results showed the presence of hexadecanoic acid methyl ester and 9-octadecanoic methyl ester, which are the present methyl esters of the fatty acids of the pinto bean (Phaseolus vulgaris). Furthermore, the hexadecanoic acid methyl ester has a high antimicrobial effect against clinical pathogenic bacteria, as Shaaban et al. (2021) mentioned. In addition, other studies such as that of Bharath et al. (2021) have found that hexadecanoic acid has a high inhibitory effect on cancer cells, constituting this as one more reason for the consumption of this seed.

3.3 Protein content

The results showed an average of 15.88% of total protein content in beans without treatment, which turns out to be the majority of what Rocha-Guzman et al. (2013) report, who obtained 15.1% of protein content in pinto beans, the differences in the results are due to the different conditions of thermal processing. In the retort pouch treatments, values of 4.75% [100 g/100 mL], 4.58% [100 g/120 mL] and 5.18% [100 g/140 mL] were obtained.

The statistical results show $p < 0.05$ for the ANOVA, which indicates no significant difference in the three treatments with retort pouch; however, there is a significant difference in the retort pouch treatments concerning beans without any treatment the Figure 2. show the Tukey simultaneous confidence intervals.

The structure of bean proteins is affected by heat treatment, which induces antinutritional factors inactivation, increasing their digestibility and biological values (Hayat et al., 2014).

Previous studies report the decreased protein in cooking common beans, mainly in the globulin fraction compared to raw beans. Cooking promotes physical and chemical changes in proteins, mainly in glutelins, generating a variation in its solubility caused by the thermodynamic equilibrium between protein-protein and protein-solvent interactions, which is associated with the hydrophobic and hydrophilic characteristics own of protein molecules (Carbonaro et al., 1993; Oliveira et al., 2017).

3.4 Identification of phaseolins by electrophoresis

Protein phaseolin is present in all the samples analyzed; however, a high concentration of phaseolin is observed in the beans without treatment because there is no processing that alters the protein; on the other hand, in the treatments with retort pouch, the sample 100 g/140 mL showed a higher concentration, see Figure 3. The results agree with the protein identification carried out by Toledo et al. (2013) and Fuente et al. (2012), who have the same band identified as phaseolin in Bean (Phaseolus Vulgaris).

In studies reported by Naozuka & Oliveira (2012), heating in the bean cooking process promotes a decrease in the concentration of total protein, particularly in globulins such as phaseolin, because the native conformation of proteins is altered by destabilizing non-covalent interactions, modifying the association and dissociation interactions between amino acids with opposite charges, and their protein subunits. Carbonaro et al. (1993) also mention that this dissociation alters the isoelectric point of the proteins and their solubility. On the other hand, it is essential to mention that the secondary structure of phaseolin is conserved in the common bean. In contrast, its tertiary and quaternary

Table 1. Identifiable main compounds by GC/MS in the different pinto saltillo bean treatments.

Bean treatment	Retention time (min)	Identified compound
Beans without processing (standard)	17.91	Hexadecanoic acid methyl ester
	18.90	Hexadecanoic acid
	21.93	9- octadecanoic methyl ester
	23.26	Octadecanoic acid
	26.60	Eicosanoic acid methyl ester
100 g/100 mL	17.81	Hexadecanoic acid methyl ester
	18.83	Hexadecanoic acid
	21.90	9- octadecanoic methyl ester
	23.26	Octadecanoic acid
100 g/120 mL	17.81	Hexadecanoic acid methyl ester
	18.65	Hexadecanoic acid
	21.81	9- octadecanoic methyl ester
100 g/140 mL	17.81	Hexadecanoic acid methyl ester
	18.68	Hexadecanoic acid
	21.84	9- octadecanoic methyl ester

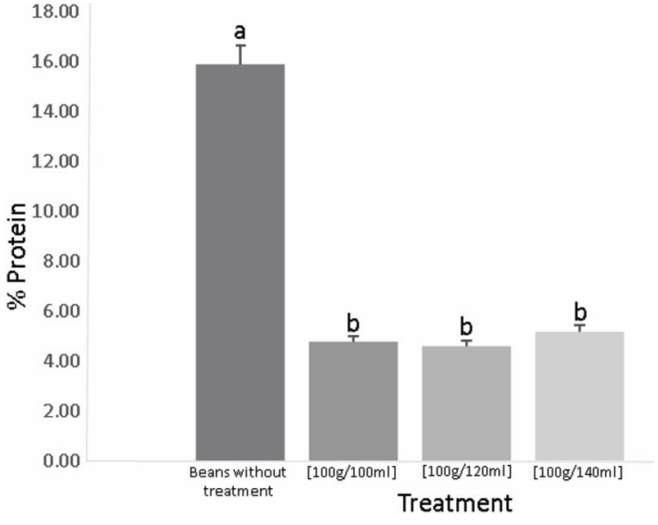

Figure 2. Protein content in the treatments of pinto beans; columns with the same letters do not present a significant difference (Tukey; $\alpha = 0.05$).

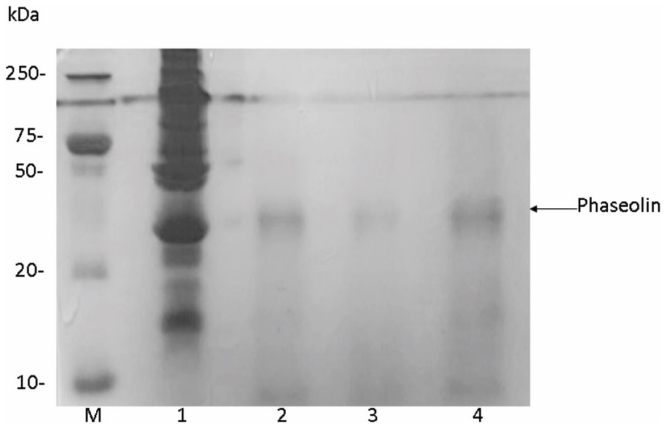

Figure 3. Electrophoresis gel with a concentration of 10% acrylamide. The first lane shows molecular weight marker; the four treatments are listed: 1) bean without treatment, 2) 100 g/100 mL, 3) 100 g/120 mL, 4) 100 g/140 mL, the band between 20 and 50 kDa corresponds to phaseolin.

structure undergoes an alteration, increasing the surfaces hydrophilic and breaking the phaseolin subunits interaction, which leads to further degree hydrolysis (Montoya et al., 2010).

4 Conclusions

The Pinto bean-based preserves in flexible retort pouches made in this project conserve significant oils and preserve phaseolin protein, suggesting that retort pouch treatment is a promising technique for preserving bean quality and providing nutritional value for their consumption. Furthermore, using the retort pouch system in common beans is crucial since it is necessary to cook the beans before consuming them. This research study provides essential nutritional information that will add value to this globally consumed food.

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