

TECHNO-FUNCTIONAL PROPERTIES OF FLOUR, STARCH, AND PROTEINS OF PEA SEED (*Pisum sativum* L.) CULTIVATED IN THE MIXTECA REGION OF OAXACA

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ABSTRACT

Currently, the food industry is interested in using plant-based ingredients because they can improve the nutritional and technological properties of products while also providing health benefits. The application of these ingredients in food formulations is dependent on their techno-functional properties, so determining them is critical. Pea seeds (*Pisum sativum* L.) are a good source of nutrients such as starch, protein, and fiber, which could also be used as functional food ingredients. It has been reported that differences in nutritional content and functional properties of pea seeds are caused by environmental conditions, cultivation methods, and processing. In Mexico, in the Mixteca Alta region of Oaxaca, creole peas are cultivated using rainfed agriculture, and the seeds are harvested when the plant is completely dry. There are currently no studies that evaluate the composition and properties of the main components of pea seeds grown under the environmental conditions of this region. The objective of this work was to determine the proximal composition and techno-functional properties of flour (PF), starch (PS), and protein (PPI) isolated from dry creole seeds cultivated in the Mixteca Alta region of Oaxaca. Results were compared using ANOVA and Tukey's test ($p \leq 0.05$). Significant differences ($p \leq 0.05$) were found in the proximal composition and the color of PF, PS, and PPI. The extracted starch contains 19.6 ± 1.3 % amylose. The granules were ellipsoidal in shape and measured 57.2 ± 11.0 nm in diameter. The FTIR spectra showed structural differences between the samples. Functional properties such as water absorption capacity, foam formation, and emulsifying capacity were significantly higher in PPI than in PF and PS ($p \leq 0.05$). The results demonstrate that pea seeds grown in the Mixteca Alta region of Oaxaca can be an unconventional source of functional ingredients for the food industry.

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INTRODUCTION

Pea (*Pisum sativum* L.) is an herbaceous plant that belongs to the legume family. Its seeds contain mainly protein (20–25 %), carbohydrates in the form of starch (24–49 %), and fiber (60–65 %) (Shanthakumar *et al.*, 2022). In addition to its nutritional value, this seed can be a source of food ingredients for the design of healthy and dietary foods. Pea cultivation takes place primarily in temperate zones with temperatures ranging from 16 to 20 °C, as well as conditions of residual humidity and irrigation (SIAP, 2023). In Mexico, the main producer of peas is the State of Mexico; however, in the Mixteca Alta region of Oaxaca, in the municipality of San Miguel Tulancingo, the cultivation of creole peas is one of the agricultural products that contribute to the self-consumption and economic support of this indigenous population, which is in a situation of poverty (SE, 2020). Pea cultivation in this region is not carried out under optimal conditions. The municipality is at an average altitude of 2200 m, and the climate is dry-temperate. The pea crop cycle lasts about five months, and because it is a rainfed crop, it is grown in the Mixteca Alta from June to October. The peasant agricultural producers harvest the pea seeds when the plant becomes completely dry; they are not harvested fresh. Although there are numerous reports on the extraction and functional properties of pea seed components (Pedrosa *et al.*, 2020), no studies have been conducted to evaluate dry creole pea seeds grown in the Mixteca Alta of Oaxaca, Mexico. Different authors have recognized that environmental conditions, cultivation, and harvest methods of legume-seeds generate differences in their nutritional content and functional properties. Yegrem *et al.* (2022) found significant differences in the fat, protein, and carbohydrate content, as well as the functional properties of chickpea flours grown in different seasons and environmental conditions. Marquezi *et al.* (2017) reported differences in the proximal analysis, water absorption capacity (WAC), and oil absorption capacity (OAC) of bean flour obtained from different cultivars. Regarding pea seeds, Nikolopoulou *et al.* (2007) evaluated the proximal composition of pea seeds grown in different areas and seasons of the year, finding that the location significantly affected the content of sucrose, starch, and non-starch polysaccharides. Wang *et al.* (2010) discovered that the nutritional content (protein, starch, fiber, fat, and ash) of pea seeds is influenced by the variety and environment in which they grow. For their part, García-Arteaga *et al.* (2021a) reported differences in the yield, color, and functional properties of pea protein obtained from different cultivars. Because legumes have health benefits, the food industry is very interested in using them in the development of new products. The use of these ingredients in food formulations depends on their functional properties. The hypothesis of this work was that creole pea seeds grown in the Mixteca Alta region of Oaxaca, Mexico, present potential nutritional and techno-functional properties for use in the food industry. Therefore, the objective was to determine the composition and techno-functional

properties of flour (PF), starch (PS), and protein isolate (PPI) of dry pea seeds grown in the Mixteca Alta region of Oaxaca, Mexico, in order to generate useful information that promotes its application in the food industry and encourage its cultivation for the benefit of families in the region.

MATERIALS AND METHODS

Pea seeds were collected by peasant agricultural producers in the municipality of San Miguel Tulancingo in the Mixteca Alta region of Oaxaca, México, which is located at 17° 44' 00'' N and 97° 26' 00'' W, with an average precipitation of 680 mm. The seeds were collected in October 2022.

Obtaining pea flour (PF)

Pea seeds were dried for 2 h at 60 °C. They were then ground and sieved through a 60-mesh sieve.

Pea starch (PS) extraction

Starch was extracted using the method of Beta *et al.* (2001). Pea flour was ground with distilled water (40 %). The suspension was filtered through an 80-mesh screen. The remaining material on the sieve was rinsed with 50 mL of distilled water. The filtrate was washed with NaOH (0.2 %), and the starch was washed with distilled water and dried for 24 h at 45 °C.

Pea protein isolate (PPI) extraction

Pea protein isolate was obtained with the method of Xu *et al.* (2020). Briefly, PF was dispersed with distilled water at a ratio of 1:15. The pH of the solution was adjusted to pH 9.5 and stirred for 1 h at room temperature. The solution was centrifuged (Eppendorf Centrifuge 5810 R, Eppendorf SE, Germany) at 6000 rpm for 20 min. The supernatant was adjusted to pH 4.5. The protein was collected by centrifugation at 6000 rpm for 10 min.

Proximate composition

Moisture, protein, lipid, and ash content were determined according to AOAC standard methods (AOAC, 1997). Carbohydrates were calculated by the difference between the other components. All analyses were performed in triplicate.

Amylose content in PS

The amylose content in PS was determined using the ISO-6647-1:2007 standard (ISO, 2007). The samples were defatted with methanol, and calibration solutions of potato amylose and waxy rice amylopectin were used.

Starch granule size and morphology

Starch was dissolved in distilled water (0.5 g mL⁻¹). A drop was placed on a slide, covered with a coverslip, and observed in a trinocular light microscope integrated with a Scopepad-LX97 device (VE-B6PAD, Velab. Co., USA). Starch granule size measurements (length and width) were performed with the S-EYE program version v1.10.9. A total of thirty particles were measured, and the average was obtained.

Color

The color of the samples was measured using a portable spectrophotometer (Colorspec, CS 520 Sphere, Hangzhou CHNSpec Technology, China). It was quantified using the L*, a*, and b* systems. Chroma values and hue angle were calculated using the following equations:

$$\text{Hue angle} = \tan^{-1} \frac{b}{a}$$

$$\text{Chroma} = \sqrt{a^2 + b^2}$$

Structural analysis by FTIR

The samples were characterized with FTIR spectroscopy (Vertex 70v, Bruker, Bremen, Germany) with an ATR accessory. Measurements were made from 4000 to 400 cm⁻¹.

Techno-functional properties

Density and porosity

The parameters loose bulk density (LBD), packed bulk density (PBD), and true density (TD) were evaluated under the procedure described by Falade *et al.* (2019). The volume was obtained by placing 1 g of each sample in a 10 mL measuring cylinder (bulk volume). The samples in the cylinder were struck 100 times on a flat surface and measured as tapped volume. LBD and PBD were calculated as follows:

$$\text{LBD (g mL}^{-1}\text{)} = \frac{\text{Weight of the sample}}{\text{Bulk volume}}$$

$$\text{PBD (g mL}^{-1}\text{)} = \frac{\text{Weight of the sample}}{\text{Tapped volume}}$$

To determine the TD, 1 g of the sample was added to a 10 mL graduated cylinder, 5 mL of *n*-hexane was poured in, and the displaced volume of the graduated cylinder was determined. TD was determined by following the equation:

$$TD \text{ (g mL}^{-1}\text{)} = \frac{\text{Weight of the sample}}{\text{Displaced volume}}$$

The porosity (%) of the powder was calculated as follows:

$$\text{Porosity (\%)} = \left(1 - \frac{LBD}{TD}\right) \times 100$$

Water absorption capacity (WAC) and oil absorption capacity (OAC)

The methodology proposed by Elkhalfa and Bernahardt (2010) was used, adding 0.5 g of each sample (PF, PS, and PPI) into 10 mL of distilled water. Samples were vortexed for 1 min and allowed to rest for 30 min at 25 °C, then centrifuged at 4000 rpm for 25 min. Excess water was removed by settling. To determine the OAC, 0.5 g of the samples were placed in a centrifuge tube and 6 mL of soybean oil was added. The WAC was expressed as grams of water per gram of sample and the OAC in grams of oil per gram of sample.

Foaming capacity (FC)

The FC of the PF, PS, and PPI was determined using the method described by Coffman and García (1977) with modifications. The samples were dissolved in distilled water at a concentration of 2 %. Subsequently, they were beaten with a high-speed turbo blender (Turbolicuador Turlic-280, Rhino, Mexico) at 20 000 rpm for 2 min. Foam volumes were recorded, and the FC was calculated as follows:

$$FC (\%) = \frac{\text{Final volume} - \text{Initial volumen}}{\text{Final volumen}}$$

Emulsifying properties (EC)

The emulsifying capacity was determined using the methodology proposed by Navaf *et al.* (2022) with slight modifications. The samples were dissolved in distilled water at a concentration of 2 %. Then, 50 mL of soy oil was added by stirring with a high-speed turbo blender for 2 min for emulsion formation. The emulsion was centrifuged at 1100 rpm for 5 min. The weight of the tube contents and the emulsion were noted. The EC was calculated as follows:

$$EC (\%) = \frac{\text{Height of the emulsified layer}}{\text{Total height of tube content}} \times 100$$

Swelling power (SP) and solubility (S)

SP and S were calculated using the method followed by Yu *et al.* (2012) with a slight modification. The samples (5 g) were suspended in distilled water at 25 °C and

centrifuged at 4500 rpm for 15 min. The supernatant was poured into an aluminum tray of known weight, dried at 110 °C for 12 h, and the final weight was taken. SP and S were determined with the following equations:

$$SP \text{ (g g}^{-1}\text{)} = \frac{\text{Weight of the wet residue}}{\text{Sample weight}}$$

$$S \text{ (\%)} = \frac{\text{Weight of dissolved solid}}{\text{Sample weight}} \times 100$$

Statistical analysis

Analyses were performed in triplicate. The results were expressed as mean $\pm$ standard deviation and were compared using ANOVA and Tukey's test ($p \leq 0.05$) in the GraphPad program (Dotmatics, Boston, MA, USA).

RESULTS AND DISCUSSION

Proximate composition

The analysis of the proximal composition of PF showed that its main constituent is carbohydrates (Table 1). The amount of carbohydrates, lipids, and ashes in PF was similar to that reported in pea seed flour grown in Jalisco, Mexico (Arriola-Guevara *et al.*, 2020). In this area, the climate is warm-subhumid, and its average annual temperature (20.5 °C) is lower (INEGI, 2023) than the Mixteca Alta region. Similar results were reported by Nikolopoulou *et al.* (2007) in pea seed flours grown in different regions of Greece. The environmental conditions did not affect the content of carbohydrates, lipids, and ash in pea seeds. Differences were found in the content of proteins. In the PF sample, a higher percentage of protein was quantified than that reported in pea seed flour grown in Jalisco, Mexico. It has been reported that lower rainfall in growing areas and high temperatures are responsible for the high protein content (Nikolopoulou *et al.*, 2007).

Table 1. Proximate composition of the flour (PF), starch (PS), and protein isolate (PPI) of dry creole pea seeds.

Sample	Moisture (%)	Proteins (%)	Lipids (%)	Carbohydrates (%)	Ash (%)
PF	4.0 $\pm$ 0.0a	23.4 $\pm$ 0.8a	1.2 $\pm$ 0.0a	68.3 $\pm$ 0.9a	2.9 $\pm$ 0.0a
PS	11.9 $\pm$ 0.6b	2.8 $\pm$ 0.0b	4.7 $\pm$ 0.4b	79.6 $\pm$ 0.9b	0.7 $\pm$ 0.3b
PPI	7.3 $\pm$ 0.3c	85.4 $\pm$ 0.0c	0.7 $\pm$ 0.0a	2.6 $\pm$ 0.5c	3.8 $\pm$ 0.2c

Values show mean $\pm$ standard deviation. Carbohydrates were calculated by difference of the other components. a,b,c Mean values per column with a different letter are different ($p \leq 0.05$).

Significant differences were found in the proximal composition of the PF, PS, and PPI of pea seeds (Table 1). PS presented higher moisture values compared to PF and PP. Daudt *et al.* (2016) described that starches generally have a higher moisture content, around 12 %, due to easy interactions with water. The protein content of PPI agrees with that reported by García-Arteaga *et al.* (2021a) in protein isolates obtained from pea seeds grown in different regions of Germany. Some authors mention that the protein content in the isolates depends on the extraction method used, not on the cultivars (Stone *et al.*, 2015). In this case, in both investigations, the isoelectric precipitation method was applied with some modifications in the pH used to solubilize the proteins. As expected, PPI has the highest content of protein and PF has the highest amount of carbohydrates. It was observed that in the extraction of starch and proteins, certain amounts of lipids were also isolated. García-Arteaga *et al.* (2021a) reported fat values of 4.7 to 9 % in protein isolates from pea seeds of different cultivars. In this research, the lipid content in PPI was lower. These differences may be due to the extraction methodologies applied. On the contrary, PS presented a greater amount of lipids. During the extraction of proteins and starches, there may be protein-lipid and carbohydrate-lipid interactions (Gao *et al.*, 2020), so the isolates obtained without defatting are generally accompanied by lipids. The extractions of these macromolecules are not completely pure, and many times the purification processes make the product more expensive.

In this study, a simple methodology for starch extraction was applied, which does not require sophisticated equipment, in order to transfer the starch extraction process to farmers so that they obtain added value from this crop. For this reason, the isolates obtained are not 100 % pure. On the other hand, PPI presented a greater amount of ash. The value was lower than that found by García-Arteaga *et al.* (2021a). In their study, a pH 8 to solubilize proteins was used. The pH adjustments can cause the formation of salts that favor the extraction of minerals. In the methodology applied in this research, the pH used to solubilize the proteins was 9.5, which may suggest that greater alkalinity during the extraction of these proteins can generate purer isolates.

Amylose content in PS

The amylose content in PS was 19.6 ± 1.3 %. The results were higher than those reported by Gao *et al.* (2022) (16.3 %) in native starches of pea seeds and lower than that found by Aggarwal *et al.* (2004) (26.9–61.5 %) in pea seed starch of different Indian cultivars. Salgado-Ordosgoitia *et al.* (2019) reported that the different contents of amylose and amylopectin are attributed to environmental conditions, age, and site of the crops. The amylose quantified in PS was lower than that reported in starch isolated in other legumes such as chickpea (33.47 %), lentil (37.24 %), and black bean (34.98 %) (Ma *et al.*, 2017). The low-amylose starches, such as PS, can present greater digestibility (Wang *et al.*, 2022). Currently, starches have various applications in food products such as sauces, soups, and meat products, and the starch from pea seeds can be used for this purpose.

Starch granule size and morphology

Light microscopy of PF and PS showed starch granules with an elliptical morphology and a central hilum (Figure 1). The shape of the starch granules was similar to that reported in other publications (Wang *et al.*, 2018). In PF, the starch granules were observed to be surrounded by protein and other flour components (Figures 1A and 1C). In PS, the presence of these is minimal, which could indicate the purity of its extraction (Figures 1B and 1D). In both preparations, granules of different sizes are observed. The average size of the starch granules was 57.2 ± 11.0 mm. According to Aggarwal *et al.* (2004) and Alcázar-Alay and Meireles (2015) the variation in the size and shape of starch granules depends on the biological origin, the biochemistry of the chloroplast or amyloplast, and the physiology of the plant.

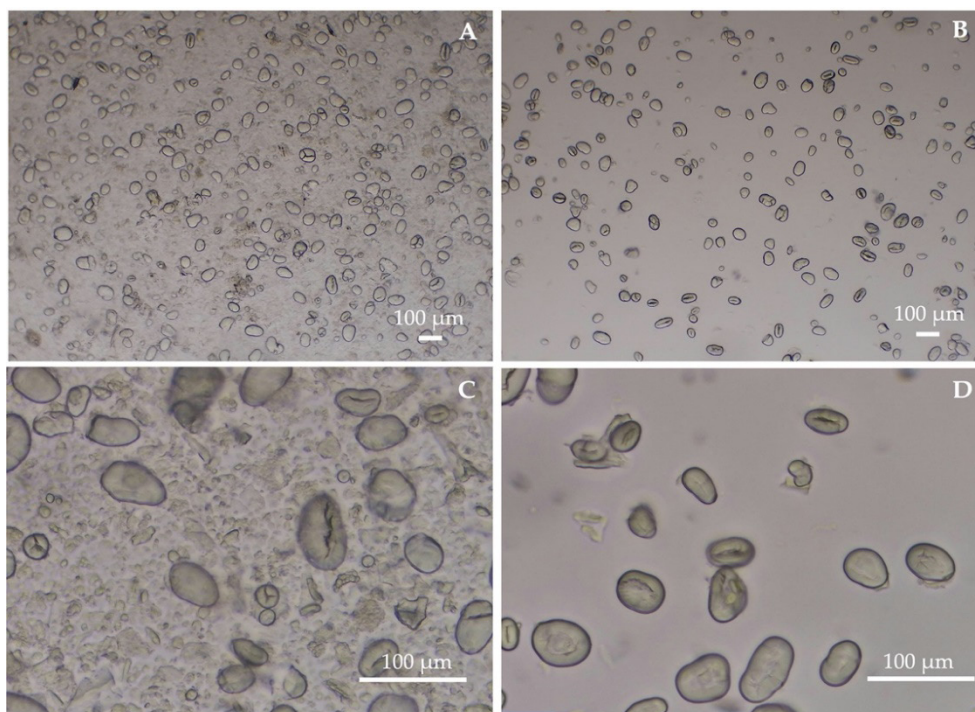


Figure 1. Starch granule morphology of dry creole pea seeds. A, C: flour (PF); B, D: starch (PS).

Color

Visually, PF, PS, and PPI presented differences in color (Figure 2). In PPI, low values of L^* were obtained; on the contrary, PF presented greater luminosity (Table 2). PS luminosity values were higher than those reported by Pietrasik *et al.* (2020) in pea starch. The differences may be due to the purity of the isolates. PS showed less lightness than potato starch (Akhila *et al.*, 2022). PPI had higher reddish values (a^*), while PF had higher yellowness (b^*).



Figure 2. Photography of A: flour (PF); B: starch (PS); C: protein isolate (PPI) of dry creole pea seeds.

Table 2. Color parameters evaluated in flour (PF), starch (PS) and protein isolate (PPI) of dry creole pea seeds.

Sample	L*	a*	b*	Chroma	Hue°
PF	63.2 ± 2.5a	3.4 ± 0.1a	16.0 ± 1.0a	16.3 ± 1.0a	77.9 ± 0.4a
PS	57.8 ± 1.5b	1.8 ± 0.2b	8.9 ± 0.3b	9.1 ± 0.3b	78.3 ± 1.1a
PPI	19.7 ± 1.8c	7.8 ± 0.2c	12.0 ± 1.7c	14.4 ± 1.5c	56.7 ± 2.8b

Values show mean ± standard deviation. a,b,c Mean values per column with a different letter are different ($p \leq 0.05$).

The values of L*, a*, and b* of PPI were different from those reported in pea protein isolates (García-Arteaga *et al.*, 2021a). In this study, higher values of L* and b* were found. It has been reported that protein extraction and drying methods, as well as the presence of other compounds such as carbohydrates and phenolic compounds, can cause the protein isolate to darken due to Maillard reactions (Guler-Akin *et al.*, 2021). The Hue angle values were between 56.76 and 78.38°. The highest Chroma values were observed in PF. Starch and proteins have many applications in food, industrial, and pharmaceutical products. In the food industry, starch is used as a texture and viscosity modifier to retain moisture and create gels and films. Seed protein is generally applied as a substitute for cereal flours, fats, and animal protein, as well as for the encapsulation of bioactive compounds, extruded foods, and edible films (Shanthakumar *et al.*, 2022). Color plays an important role in their incorporation into products. Guler-Akin *et al.* (2021) reported that the addition of pea proteins to low-fat ice cream improved its texture; however, it should be used at low concentrations because its addition generates a darker product. On the other hand, Pietrasik and Janz (2010) found no significant differences in the color of low-fat sausages added with pea seed starch and control sausages.

Structural analysis by FTIR

The FTIR spectra of PF, PS, and PPI showed differences in their composition (Figure 3). The bands in the PF spectrum between 800 and 1200 cm^{-1} were caused by starch, which is the main component of pea flour. Related bands located at wavenumbers between 1100 and 1150 cm^{-1} are attributed to C-O, C-, and C-O-H stretching bonds, whereas the absorption bands at 900–1100 cm^{-1} were attributed to C-O-H bending and glycosidic linkages of starch. The bands at 1240–1280 cm^{-1} also represent CH_2OH - related mode, which is a typical spectrum for the V form of amylose (Kizil *et al.*, 2002). The band from 1500 to 1700 cm^{-1} was associated with protein amide groups. In particular, the band located between 1500 and 1650 cm^{-1} was because of amide I (C-O stretching), amide II (C-N stretching) group, and N-H bending modes.

Lipids were detected at 1740 cm^{-1} , which corresponds to the C=O bond of the carbonyl group. On the other hand, the FTIR spectrum of PPI presents absorption bands mainly associated with amide groups, with a decrease in absorbance in the carbohydrate region. On the contrary, in the FTIR spectrum of PS, bands of greater intensity are observed in the carbohydrate region associated with the presence of starch, while bands with lower intensities are observed in the protein amide groups.

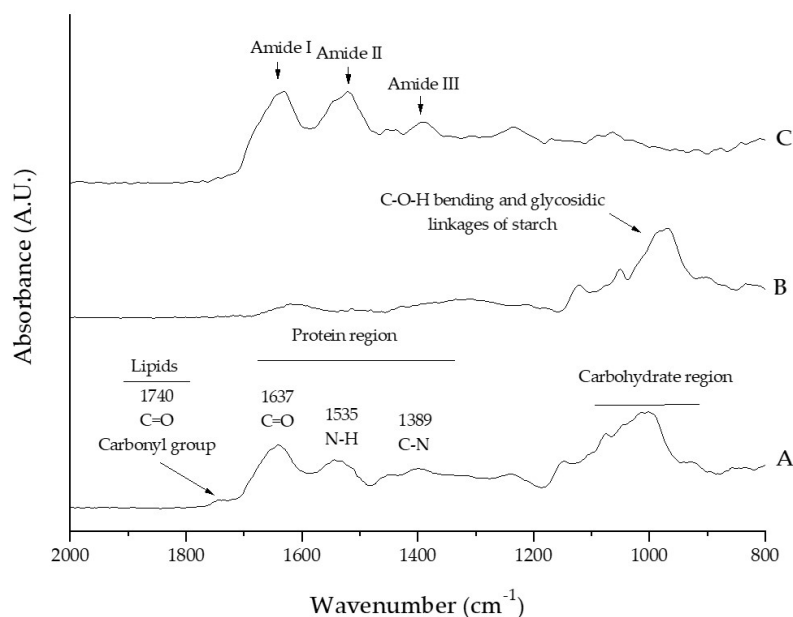


Figure 3. FTIR spectra of dry creole pea seeds. A: flour (PF); B: starch (PS); C: protein isolate (PPI).

Techno-functional properties

LBD and PDB

In the food industry, determining the LBD and PDB values of powders provides information about their packaging and handling characteristics. The LBD value in PF (Table 3) was similar to that reported by other authors in pea seed flours from different cultivars (Kaur *et al.*, 2007). According to Amandikwa *et al.* (2015), these parameters are affected by the seed variety, growing season, cultivar type, harvest maturity, storage duration, processing, and particle size at the end of processing.

Table 3. Techno-functional properties of the flour (PF), starch (PS), and protein isolate (PPI) of dry creole pea seeds.

Property	PF	PS	PPI
LBD (g mL ⁻¹)	0.4 ± 0.0a	0.4 ± 0.0a	0.6 ± 0.0b
PBD (g mL ⁻¹)	0.6 ± 0.0a	0.5 ± 0.0a	0.6 ± 0.0a
TD (g mL ⁻¹)	1.2 ± 0.0a	1.1 ± 0.1a	1.2 ± 0.0a
Porosity (%)	48.0 ± 0.3a	41.0 ± 0.6a	67.0 ± 0.6b
WAC (g water g ⁻¹ sample)	1.8 ± 0.0a	2.0 ± 0.1a	2.2 ± 0.0b
OAC (g oil g ⁻¹ sample)	0.6 ± 0.0a	0.8 ± 0.0b	0.7 ± 0.0c
FC (%)	25.1 ± 3.9a	11.6 ± 2.8b	53.7 ± 0.7c
EC (%)	9.1 ± 0.4a	6.4 ± 0.5b	16.7 ± 1.0c
SP (g g ⁻¹)	2.7 ± 0.1a	4.0 ± 0.3b	4.6 ± 0.3b
S (%)	17.0 ± 0.1a	2.4 ± 0.2b	34.1 ± 0.5c

Values show mean ± standard deviation. a,b,c Mean values per row with a different letter are different ($p \leq 0.05$). LBD: loose bulk density; PBD: packed bulk density; TD: true density; WAC: water absorption capacity; OAC: oil absorption capacity; FC: foaming capacity; EC: emulsifying properties, SP: swelling power; S: solubility.

The LBD values of PF were lower than those found in lentil flour (0.91 g mL⁻¹), chickpea (0.71 g mL⁻¹), and bean flour (0.62 g mL⁻¹) (Pedrosa *et al.*, 2020), but similar to those reported in wheat flour, which is one of the most used flours in the food industry (Amandikwa *et al.*, 2015). PPI presented higher LBD values (Table 3), which means that it compacts better than PF. This is possibly due to the fact that a smaller particle size produced during extraction and processing.

There were no significant differences found between the LBD of PF and PS. In both samples, its main component is carbohydrates, which may suggest that the particle density and distribution are similar. On the other hand, there were no significant differences in the PBD of the samples. The LBD and PBD values of PPI did not vary, indicating that this powder does not require force to compact. This occurs because the particle size improves powder distribution on the surface, resulting in a better particle-

volume relationship (Meena *et al.*, 2021), and thus no external force is necessary to achieve good compaction.

TD and porosity

TD and porosity are important parameters for creating new products with specific properties and improving the quality of existing ones. In this study, there were no significant differences in TD between PF, PS, and PPI (Table 3). The TD of the PF was lower than that reported for chickpea and bean flour, at 1.44 and 1.42 g mL⁻¹, respectively (Boucheham *et al.*, 2019). The values are higher than those obtained in potato flour and starch and wheat flour, the most commonly used flours in the industry (Akhila *et al.*, 2022). The differences found may be due to the proximal composition of the different flours, the extraction methodologies used, and the particle size generated. Regarding porosity, PF porosity was higher than that reported for wheat flour (Raihan and Saini, 2017), but lower than the porosity of potato flour (Akhila *et al.*, 2022). PPI was more porous than PF and PS. Porosity is related to the nature of the compounds, their structure, the free spaces they have, and their density. In the case of proteins, during their denaturation, free spaces are generated, and hydrophilic groups that can retain water are exposed. Porous materials can retain water, oil, or air in their pores, which may be useful for some foods.

WAC and OAC

The WAC of the PF (Table 3) was higher than that of pea flour from different Indian cultivars (Kaur *et al.*, 2007). At the time of this study, there was no research on the WAC of Mexican pea seed flour. Some publications use the entire pod and report only the water absorption index (González-Montemayor *et al.*, 2021). WAC is determined by the composition of its constituents as well as the structure acquired during processing. Kaur *et al.* (2007) explain that the presence of proteins with different structures and hydrophilic carbohydrates generates changes in the WAC of flours. Pea flour, as reported in the proximal analysis, contains a high amount of carbohydrates and protein.

The WAC of PF determined in this study was higher than those reported in whole kidney beans and soybeans, at 1.16 and 1.19 g g⁻¹, respectively (Jamalullail *et al.*, 2022). On the other hand, PF retains more water than wheat flour (0.66 g g⁻¹) (Raihan and Saini, 2017). The pea flour could replace wheat flour in some applications, such as bakery products. The highest WAC was determined in PPI, and there were no statistically significant differences between PF and PS (Table 3). The WAC of PPI coincides with that reported in pea protein isolates (Stone *et al.*, 2015). The elevated WAC of proteins is generated by hydrophobic interactions, Van der Waals forces, and hydrogen bonds. In addition, there is a relationship between protein structure and water, as well as porosity (Shanthakumar *et al.*, 2022).

As explained in the previous section, PPI had greater porosity, which could lead to a higher WAC. The WAC of PS was similar to that reported by Sun and Xiong (2014)

in pea starch and higher than potato starch (Akhila *et al.*, 2022). WAC is related to the texture, color, and sensory properties of food, mainly in minced meat products such as sausages and baked doughs. Given their WAC, PF, PS, and PPI from pea seeds can be used as water-retaining additives in various foods. PF had a lower OAC than pea flour grown in various regions of India ($1.06\text{--}1.17\text{ g g}^{-1}$) (Kaur *et al.*, 2007). The differences in this parameter may be explained by variations in lipid content, protein structure, sample processing methods, and environmental conditions. The OAC values of PF were lower than those reported in other legumes such as whole kidney bean and soybean (Jamalullail *et al.*, 2022).

OAC values in PF, PS, and PPI were statistically different (Table 3). PS retained more oil than the other samples. The presence of lipids in its composition favored the interaction with the hydrocarbon side chain of the oil. The values were higher than those reported in potato starch (Akhila *et al.*, 2022). However, they were lower than those reported in wheat flour (1.10 g g^{-1}) (Raihan and Saini, 2017). The OAC is an important functional property in foods such as pancakes, baked goods, donuts, sweets, and dressings. This property influences the taste, texture, and performance of the products.

FC and EC

FC values of PF were lower than those reported by Kaur *et al.* (2007). The differences may be due to the methodologies, concentrations, and pH used for their evaluation, as well as the different compositions of the flour. The FC of PF, PS, and PPI was statistically different (Table 3). Differences in the composition of the samples generate changes in this functional property. PPI presented the highest values. Opposite results were found by García-Arteaga *et al.* (2021b) in a pea protein isolate, where no foaming was observed. The foaming properties are dependent on the pH and the concentrations, so in order to make a better comparison, the same conditions must be used. Furthermore, protein composition, structure, and extraction method are also important factors influencing FC (Shanthakumar *et al.*, 2022).

On the other hand, PS had poor foaming ability compared to PF. The formation of foam contributes to the texture and visual appearance of some products, such as ice cream, bread, mousse, meringues, whipped cream, and milk shakes, among others. Protein isolate and pea flour from the Mixteca Alta region of Oaxaca can be valued by taking advantage of their foaming properties. The emulsifying capacity of PF (Table 3) was lower than that reported in other legumes such as soybeans, beans, and chickpeas (Jamalullail *et al.*, 2022). The variances in the protein and carbohydrate content present in these legumes make the EC different. Proteins act as emulsifiers and form a film around the oil and carbohydrates can change the viscosity of the solution, favoring emulsification. Significant differences were observed in the EC of the samples (Table 3). As in the foaming capacity, PPI presented the highest emulsifying capacity.

The values found in the EC of the protein isolate were lower than those reported in other studies (García-Arteaga *et al.*, 2021b). The emulsifying capacity is influenced by

the structure of the proteins, their function, temperature, pH, and the contact time of the oil with the protein (Shanthakumar *et al.*, 2022). The lowest EC was obtained in PS; however, it was higher compared to the EC of potato starch (4.76 %) (Akhila *et al.*, 2022). The results indicate that the starch isolated from pea seeds in this region does not have good emulsifying and foaming properties when evaluated using these methodologies. In future research, the effect of different pH levels on these properties could be evaluated. In the food industry, EC is important for the preparation of a variety of products such as drinks, milk, creams, dressings, sauces, desserts, mayonnaise, margarine, and butter. The flour and proteins from pea seeds can be used for this purpose.

SP and S

SP is a property that indicates the tendency of a substance to hydrate. This parameter is mainly applied to confectionery and baked goods (Falade *et al.*, 2019). The pea samples presented SP values between 2.73 and 4.68 g g⁻¹ at 25 °C (Table 3). These values are similar to those found in lentil (2.30 g g⁻¹) and chickpea (1.70 g g⁻¹) flour and lower than those reported in soybean (5.78 g g⁻¹) (Pedrosa *et al.*, 2020). No differences were found between the SP values of PS and PPI. It has been reported that proteins and starches have a great swelling capacity (Pedrosa *et al.*, 2020). This is related to the hydrophilic groups that constitute them, which can retain a greater amount of water. The SP of PS was similar to that reported in pea starch (Sun and Xiong, 2014) and potato starch (Akhila *et al.*, 2022). On the other hand, the solubility of the samples in water at 25 °C was statistically different (Table 3). The highest values were found in PPI, and the lowest in PS. The solubility of PS was similar to that found in native pea starches (Sun and Xiong, 2014) and higher than potato starch (Akhila *et al.*, 2022). Potato starch is used in a variety of food applications, including thickening, stabilizing, and improving the texture of desserts, soups, and sauces. Pea seeds can be an unconventional source of starch.

CONCLUSIONS

The proximal composition and techno-functional properties of flour, starch, and protein isolate from dry creole pea seeds grown in the Mixteca Alta region of Oaxaca, Mexico, were determined. The flour is composed mainly of carbohydrates (68.3 ± 0.9 %) and proteins (23.4 ± 0.8 %). Starch contains 79.6 ± 0.9 % carbohydrates with 19.6 ± 1.39 % amylose. In addition, their granules presented an elliptical shape with an average size of 57.2 ± 11.0 μm. In the protein isolate, 85.4 ± 0.0 % protein was quantified. The flour had a greater luminosity, whereas the protein isolate was more reddish. The FTIR structural analysis showed characteristic functional groups of flour, starch, and protein isolate.

The protein isolates from pea seeds had better functional properties than flour and starch. Their loose apparent density, water absorption capacity, emulsifying capacity,

foaming capacity, solubility, and swelling power values were all higher. The starch presented higher values for oil absorption capacity. The results show that the flour, starch, and protein isolate from dried creole pea seeds grown in the Mixteca Alta region of Oaxaca have good functional properties, thus representing an alternative with high potential as a source of non-conventional functional ingredients for the development of food products with improved nutritional properties and health benefits. Its application will allow the cultivation of peas to be valued for the benefit of the inhabitants of the region.

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