AGRICULTURAL AND FOOD SCIENCE

Agricultural and Food Science (2024) 33: xxx–xxx

https://doi.org/10.23986/afsci.145290

Effects of Spray Drying and Lactic Acid Fermentation on the Technological Characteristics of Yellow and Green Pea (*Pisum sativum* L.) Protein Products

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This research evaluates a pH-shifting and drying process for producing powdered protein products from yellow and green peas (*Pisum sativum L.*), aiming to enhance their technological characteristics. In the precipitation stage, lactic acid bacteria (*Lactobacillus Plantarum* and *Lactobacillus Lactis*) served as a generally recognized safe precipitant agent. The fine fraction (<150 µm) from yellow and green peas milling acted as an encapsulant/adjuvant agent in the drying stage. Results showed high process productivity (0.41–0.51 kg protein product/kg pea flour) and low specific water consumption (52.58–62.39 kg water/kg protein product) with this approach. Variations in processing parameters affected protein content, yield, and specific water consumption. Significant differences were observed in wetting time, water activity, flowability (Carr index), cohesiveness (Hausner index), density, particle size, and colour of the protein products, depending on the processing alternatives. This flexibility allows tailoring the properties of the powdered protein product for various food technology applications.

Key words: yellow pea, green pea, pH-shifting process, protein content, extraction yield, lactic acid bacteria, spray drying, encapsulant/ adjuvant agent

Introduction

Yellow and green peas (*Pisum sativum* L.) show low allergenicity, high nutritional value and availability (Gao et al. 2020, Hertzler et al. 2020). Consequently, their protein concentrates and isolates are important as raw materials to produce plant-based food products (García Arteaga et al. 2021). Additionally, legumes are a rich source of calories, protein, carbohydrates, fiber, vitamins and minerals for a large proportion of the world's population (García Arteaga et al. 2021, Emkani et al. 2022), where peas stand out as one of the legumes with the highest protein content (20–25 %), while also contributing with a low fat content (1.5–2.0 %). Peas are also a good source of essential amino acids, such as lysine, leucine, isoleucine, and phenylalanine (Boye et al. 2010, Shanthakumar et al. 2022). Nonetheless, in contrast to animal proteins, pea proteins typically exhibit a diminished biological value owing to their reduced content of sulfur-containing amino acids. The carbohydrate composition for these legumes is approximately 70% on dry base (db) (Pulivarthi et al. 2021) with the main component being starch, which varies according to species between 30–48% (García Arteaga et al. 2021). Additionally, they contain dietary fiber, comprising 10–15% insoluble fiber and 2–9% soluble fiber, along with non-starch carbohydrates like sucrose, oligosaccharides, and cellulose (Tulbek et al. 2017, Lam et al. 2018, Shanthakumar et al. 2022).

Peas are one of the major food legumes grown in different parts of the world, and peas rank fourth in world production of food legumes next to soybeans, peanuts and dry beans. World production has increased in the last few decades, especially in Europe (Vidal-Valverde et al. 2003). In Argentina, peas showed an increase in the cultivated area since this crop offers potential environmental and economic benefits based on its ability to fix nitrogen, thus reducing production costs by not requiring commercial fertilizers, which also brings about a reduction in greenhouse gas emissions (Khazaei et al. 2019, Vita Larrieu and Prieto 2021). These factors encouraged the area planted

Received 25 April 2024 / Accepted 9 December 2024
The Scientific Agricultural Society of Finland

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with legumes in the Pampean region to increase to 225 000 tons, a new historical production record (Di Yenno et al. 2022). In this context, there is a growing interest in adding value to the peas industrialization chain, while also focusing on expanding the social economy of the sector.

Several studies have documented progress in the extraction of proteins from legumes using alkaline extraction and isoelectric precipitation, commonly referred to as pH-shifting (Accoroni et al. 2020, Gao et al. 2020, Tanger et al. 2020, Kolpakova et al. 2021). This method involves solubilizing proteins at a pH range of 8–11, followed by acidification to reach the isoelectric point, that results in the insolubilization of approximately 90% of globular proteins. In this context, the usage of a food-grade generally recognized as safe (GRAS) precipitant agent in the precipitation stage needs to be thoroughly tested for the process to be easily implemented in the food industry, while also aiming to enhance the accessibility and availability of nutrients (Emkani et al. 2021, Pei et al. 2022).

For the drying step of recovered protein products, spray drying is often used in the industry, while freeze drying is mainly used for scientific purposes on a laboratory scale. Then, the effects of spray drying on the protein products need to be studied, as it may affect the aroma, structure of proteins, protein profile, particle size and other technological characteristics (García Arteaga et al. 2021). Incorporating encapsulants/ adjuvants into the protein drying process stands as a highly advantageous strategy for preserving the integrity and functionality of these essential components. Primarily, encapsulants play a pivotal role by serving as a protective barrier, shielding proteins from direct exposure to external factors such as oxygen and moisture, and aiding in the preservation of their three-dimensional structure, thus maintaining their nutritional and functional properties (McClements 2018). Moreover, the encapsulation technique affords precise control over the release of flavors and nutrients during rehydration or consumption, making it particularly valuable in the food industry, and contributes to enhancing the thermal stability of proteins, safeguarding them against potential denaturation caused by high temperatures, thereby ensuring the overall quality of the end product (Sarabandi et al. 2020).

Encapsulating materials must possess effective emulsification properties and act as reliable protective agents during storage, while these adjuvants are often used to increase the yield of spray drying processes (Kurek and Pratap-Singh 2020, Vassaux et al. 2021). Therefore, a common approach involves combining polysaccharides and proteins, or using a mixture of wall materials with similar origins but different properties (Madene et al. 2006, Tamm et al. 2016, Coutinho et al. 2022). As adjuvants, proteins and starch are natural components used in many spray drying processes in the food industry to create a matrix that entraps and encapsulates the core material (Eghbal and Choudhary 2018, Furuta and Neoh 2021).

This study aims to assess the drying process of yellow and green pea protein products in a spray dryer, using the fine fraction of the pea flours obtained from their milling process as an encapsulant, as it was evaluated to be a sustainable and adequate adjuvant to increase the drying yield considering its two main components, carbohydrates and proteins, that can act synergically to increase product processing yields and to enhance the technological characteristics of the protein powders. In addition, the usage of lactic acid bacteria as a generally recognized as safe (GRAS) precipitant agent in the precipitation stage of the pH-shifting is also evaluated as an alternative to further improve the process performance and enhance the properties of the resulting protein products.

Materials and methods

Experimental design

The design of experiments included 8 experimental runs, using the yellow and green peas with 1:10 w/v solid-liquid ratios in each of the 3 cycles of the alkaline extraction stage with a pH value of 8.5, as detailed in the section titled Alkaline extraction, and the usage of two precipitating agents, hydrochloric acid, and lactic acid bacteria, in the isoelectric precipitation stage, as detailed in the section titled Isoelectric precipitation. The drying stage was carried out without encapsulant agent, as well as using the green and yellow pea fine fractions as encapsulant agents, as detailed in the section entitled Decantation, neutralization and spray drying. Each experimental run followed the processing steps shown in Figure 1 and further detailed in the following sections.

Therefore, this proposed experimental methodology was designed to evaluate the technological characteristics of the spray dried protein products and the performance of the protein recovery process when incorporating an encapsulating agent in the drying stage and using different precipitant agents in the isoelectric precipitation stage.

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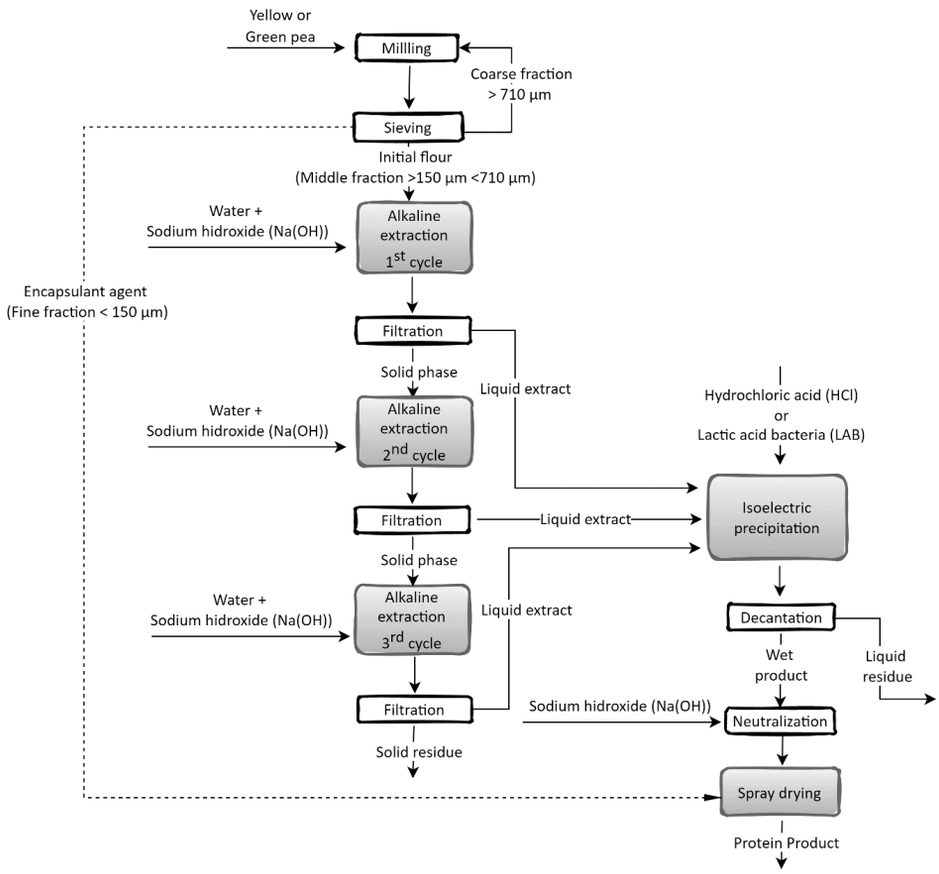

Fig. 1. Experimental methodology for the recovery of proteins from yellow and green peas, using different precipitant and drying agents

Materials

Dried yellow and green peas (*Pisum sativum L.*) were purchased from commercial vendors (Buenos Aires, Argentina, and São Paulo, Brazil, respectively). Samples were stored in sealed bags at freezer temperatures (–18 °C) until processing.

Analytical grade chemicals (Britania, Argentina, and Anidrol, Brazil) and commercial freeze-dried lactic acid bacteria starters (*Lactobacillus plantarum* SR3.54, *Lactobacillus lactis* CH6072) (CHRHansen, Denmark) were used.

Methods

Pre-processing and characterization of yellow and green peas

Yellow and green peas were ground at room temperature using a laboratory blade mill (SP-31, SP Labor, Brazil), and then sieved through ASTM-standard sieves to achieve a particle size range that passed through a 25-mesh (710 µm) sieve and was retained by a 100-mesh (150 µm) sieve. The yellow and green pea fine fractions, i.e., the particles that passed through a 100-mesh sieve (150 µm), were also separated and recovered to be later used as an encapsulating agent in the spray drying stage.

The nitrogen content of the yellow and green peas was assessed through AOAC method 2001.11 (AOAC 2005), where the total protein content was derived by multiplying the total nitrogen content by 6.25 and subsequently reported on a moisture-free basis. The protein solubility in NaOH at pH 8.5 was determined following the procedure described by Stone et al. (2015) with slight modifications. The lipid content of the yellow and green peas was determined according to Bligh and Dyer (1959). The carbohydrate content was determined by component difference (i.e. carbohydrate percentage content = 100% – protein, lipid, water and ash percentage contents). The total starch content was derived by multiplying the reducing sugar content by 0.9 and subsequently reported on a moisture-free basis, where the reducing sugar content ofhe green and yellow peas was determined according

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to Bernfeld, (1955) with slight modifications, using the technique with 3,5-dinitrisalicylic acid (DNS). The ash content was determined using AOAC method 942.05 (AOAC 2005). The moisture content of the yellow and green peas was determined using AOAC method 925.10 (AOAC 2005).

Alkaline extraction

The extraction was performed in a batch extractor with continuous stirring (RW20D S032, Ika, China) consisting of three 15-minute extraction cycles using water as the solvent. The pH was adjusted to 8.5 with 1N NaOH at a constant temperature of 60 °C. A solid: liquid ratio of 1:10 (w/v) was used in each extraction cycle.

At the end of each extraction cycle, the remaining solid was separated from the protein solution, and fresh water at 60 °C was added at the beginning of the second and third cycles to adjust the solid-liquid ratio to the required value. The three protein solutions obtained from each extraction cycle were combined into a liquid pool and transferred to a beaker for subsequent precipitation.

Isoelectric precipitation

The isoelectric precipitation stage was performed using two alternative precipitants: 0.1 N hydrochloric acid, and freeze-dried lactic acid bacteria starters (GRAS), *Lactobacillus plantarum* and *Lactobacillus lactis*.

For the first alternative, coded as HCl, 0.1 N hydrochloric acid was used to acidify the liquid pool at a temperature of 20 °C until the pH reached a value of 4.5. The resulting mixture was allowed to settle in a refrigerator until it reached a temperature of 4 °C, which facilitated the decantation of the protein product.

As a second alternative, coded as LAB, acidification was performed with two lactic acid bacteria, *Lactobacillus plantarum and Lactobacillus lactis*. These bacteria were added at a ratio of 0.003 g l^{-1} in the liquid pool at a temperature of 35–37 °C for approximately 18 hours in a laboratory oven (002CB, Fanem LTDA, Brazil), where the pH value reached the isoelectric point. The resulting mixture was allowed to settle in a refrigerator until it reached a temperature of 4 °C, which facilitated the decantation of the protein product.

Decantation, neutralization and spray drying

The liquid supernatant of the mixture obtained at the isoelectric precipitation stage was partially separated by decantation, where some supernatant was not removed in order to obtain a pumpable slurry that could be directly fed to the spray dryer. Then, the pH of the precipitated suspension was adjusted to a value of 7, by adding 5 N NaOH at ambient temperature with agitation for 1 hour. According to the total solids content of the wet product, calculated with an accuracy of 0.0001 g using a moisture analyzer (MB-45, OHAUS, Switzerland), the yellow and green pea fine fractions were added as encapsulant agents in a solid content of the wet product: encapsulant ratio (w/w) of 1:1. The mixture was thoroughly agitated before proceeding to its drying.

Note that no washing step was applied to the wet product, as previously proposed in several studies, including (L'hocine et al. 2006, Stone et al. 2015, Chao and Aluko 2018, Alonso-Miravalles et al. 2019a, Vogelsang-O'Dwyer et al. 2020, Sajib et al. 2023a). The washing step was omitted based on previous results reported by various authors (Owusu-Ansah and McCurdy 1991, Qi 2004, Peter 2018, Paleologo 2021), who found that solubilization and functionalization of sodium proteinates are improved in comparison to protein products produced with an additional washing stage before the neutralization step. Moreover, the omission of the washing step supports sustainable processing by reducing water consumption.

Spray drying was performed in a laboratory spray dryer (MSD 1.0, Labmaq, Brazil) with co-current flow and nozzle diameter of 0.5 mm. The suspension was fed into the main chamber with a peristaltic pump at 15% of the maximum flow (2 l h^{-1}). The dry air temperature was set at 160 °C, and the resulting outlet air temperature was measured at an average value of 54–60 °C. After spray drying, the powder was collected from the cyclone and cylindrical parts of the dryer chamber and stored in sterilized flasks for further analysis. In addition, extra low-quality powder was collected from the walls of the main chamber and stored in sterilized flasks for further analysis if necessary.

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LAB cells count

The lactic acid bacteria concentration was evaluated for the experiences where they were used as precipitant agent. Representative samples were grown on MSR agar plates, by incubation at 37 °C for 72 hours in microaerophilic conditions. Viable cell counts were determined by visual inspection and expressed as colony forming units per gram of sample (CFU g^{-1}).

Performance of the protein recovery process

The nitrogen content of the protein products was assessed through AOAC method 2001.11 (AOAC 2005), where the total protein content was derived by multiplying the total nitrogen by 6.25 and subsequently reported as % db (i.e. on a dry basis or moisture-free basis). The moisture content of the protein products was determined using AOAC method 925.10 (AOAC 2005) and reported as % wb (i.e. on a wet basis). Sodium content was analyzed according to the analytical standards of the (Instituto Adolfo Lutz—IAF 2008) with slight modifications, using flame photometry at a wavelength of 589 nm after ashing the sample at 550 °C, and quantification was performed using standard curves of sodium chloride.

The protein recovery yield Y_T (% db) was computed as the quantity of protein in the powdered product collected from the cyclone and cylindrical parts of the dryer chamber, per kilogram of protein in the flour, as defined in Eq. (1).

$$Y_T = \frac{mass\ of\ protein\ in\ the\ powdered\ protein\ product\ (kg\ db)}{mass\ of\ protein\ in\ the\ initial\ flour\ (kg\ db)\ +\ mass\ of\ protein\ in\ the\ encapsulant\ agent\ (kg\ db)} \quad (1)$$

The productivity of each protein recovery process P_T (kg product db/kg flour db) was computed as the quantity of powdered product collected from the cyclone, cylindrical parts of the dryer chamber and main chamber, per kilogram of pea flour and encapsulant agent, as defined in Eq. (2).

$$P_T = \frac{mass\ of\ powdered\ protein\ product\ (kg\ db)}{mass\ of\ initial\ flour\ (kg\ db)\ +\ mass\ of\ encapsulant\ agent\ (kg\ db)} \quad (2)$$

The specific water consumption G_W (kg water/kg final product db) was computed as the water consumed for obtaining each kilogram of the powdered product collected from the cyclone, cylindrical parts of the dryer chamber and main chamber, as defined in Eq. (3).

$$G_W = \frac{mass\ of\ consumed\ water\ (kg\ water)}{mass\ of\ powdered\ protein\ product\ \ (kg\ product\ db)} \quad (3)$$

The drying yield Y_D (% db) was computed as the quantity of powdered product collected from the cyclone, cylindrical parts of the dryer chamber and main chamber, per kilogram of wet protein product and encapsulant agent, as defined in Eq. (4).

$$Y_D = \frac{mass\ of\ powdered\ protein\ product\ (kg\ db)}{mass\ of\ wet\ product\ (kg\ db) +\ mass\ of\ encapsulant\ agent\ (kg\ db)} \quad (4)$$

Technological characteristics

Color

The color of the powdered protein products was measured with a colorimeter (ColorQuest XE, HunterLab, USA) calibrated with a white standard in the form of a plate (L* = 97.79, a* = -0.53 and b* = +2.28), reading with a C-type illuminant, obtaining the L*, a*, and b* factors of the Hunter system.

Wetting time

The wetting time is the time necessary to complete wetting and immersion of a powder on a liquid surface and was determined according to (Hogekamp and Schubert 2003) with slight modifications. The wetting time was measured as the time required for 3 g of powder to disappear from the surface of water (70 ml at 25 °C) when the slider that separates the powder and liquid parts were removed.

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Water activity (a_w)

The water activity of the powdered protein products was measured by direct reading of the samples with a water activity meter (4TE, Aqualab, USA) calibrated from 0.11 to 0.75 with standard solutions.

Flowability and cohesiveness

The flowability of powdered protein products was quantified using the Carr index (*CI*), with reference to tapped density (ρ_t) and bulk density (ρ_b), following the methodology outlined by Jinapong et al. (2008).

$$CI = \frac{\rho_t \left(\frac{g}{ml}\right) - \rho_b \left(\frac{g}{ml}\right)}{\rho_t \left(\frac{g}{ml}\right)} . 100 \quad (5)$$

CI values below 15% indicate very good flow capacity; those ranging from 15 to 20 % indicate good flow capacity; those between 20 and 35 % exhibit fair flow capacity; those between 35 and 45 % demonstrate poor flow capacity; and those surpassing 45% are characterized by very poor flow capacity (Santhalakshmy et al. 2015).

The cohesiveness of the powdered protein products was assessed using the Hausner index (*HI*), derived from the bulk density (ρ_b) and tapped density (ρ_t) calculations, following the methodology outlined by Jinapong et al. (2008).

$$HI = \frac{\rho_t \left(\frac{g}{ml}\right)}{\rho_b \left(\frac{g}{ml}\right)} \quad (6)$$

Powders exhibiting an HI below 1.2 are categorized as having low cohesion; those with an HI falling between 1.2 and 1.4 demonstrate intermediate cohesion; and those with an HI exceeding 1.4 are considered to have high cohesion (Santhalakshmy et al. 2015).

The bulk density (ρ_b), defined as the weight per unit volume, was determined by loading 40 g of powdered protein products into a 100 ml graduated cylinder. The occupied volume, encompassing the contribution of the void space between particles, was recorded and utilized in the calculation of bulk density. The tapped density (ρ_t) was calculated by tapping the cylinder for 5 min (250 taps per minute) using a tap density tester (Tap-2S, Logan, China) and following the method II detailed in US pharmacopeia (2012). Subsequently, the final volume was measured and employed in the computation of tapped density.

Granulometry

The particle size distribution of the powdered protein products (i.e., the 10, 50, and 90% percentiles, hereafter identified as D10, D50, and D90, and reported in μm) was measured using a particle size analyzer (S3 Plus BT-803, Bettersize Instruments, China), with ethanol as a dispersion medium at 25 °C.

Particle density

The real density of the powdered protein products was measured at 25 °C by means of a nitrogen density pycnometer (AD200, ACP Instruments, Brazil), by determining the pressure change of nitrogen in a calibrated volume. The mass of the protein product is measured with a balance. The value of the mass is entered so the density can be derived automatically.

Statistical analysis

Results were analyzed using multi-factor ANOVA assuming normal distribution with a two-sided confidence level of 95%. Each experimental run and measurement were performed in duplicate, and the results were presented as the mean value with its corresponding standard deviation. For a given variable, significant differences ($p < 0.05$) were found among the experimental values when different letters are shown next to them, according to post-hoc Tukey tests. Statistical analyses were done in R-3.6.0 software.

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Results and discussion

Characterization of the fine and middle fractions of the yellow and green peas

During the milling process, the coarse fraction is repeatedly milled until only the middle and fine fractions remain. The middle fraction represents 95 % of the total milled peas, while the fine fraction constitutes the remaining 5 %.

These middle and fine fractions of the yellow and green peas were characterized by determining their protein, carbohydrate, starch, lipid, ash and moisture contents, and their protein solubility in sodium hydroxide (NaOH), as shown in Table 1. These values will be used as reference to evaluate the performance of the process for obtaining powdered protein products from yellow and green peas as proposed in Figure 1, and to compare with the properties of the respective powdered protein products.

Table 1. Chemical composition and technological characteristics of fine and middle fractions of yellow and green peas

	Yellow pea middle fraction	Green pea middle fraction	Yellow pea fine fraction	Green pea fine fraction
Protein (% db)	24.92 ± 0.09 b	26.49 ± 0.14 a	19.15 ± 0.75 d	22.59 ± 0.27 c
Protein solubility (%)	71.88 ± 8.01 a	74.13 ± 12.29 a	–	–
Carbohydrates (% db)	69.36 ± 0.04 c	67.94 ± 0.33 d	75.29 ± 0.12 a	72.07 ± 0.53 b
Starch (% db)	39.19 ± 1.05 b	39.42 ± 2.67 b	55.23 ± 0.09 a	54.06 ± 3.07 a
Lipids (% db)	2.16 ± 0.02 b	2.53 ± 0.06 a	2.06 ± 0.07 b	2.58 ± 0.02 a
Ash (% db)	3.61 ± 0.00 a	3.05 ± 0.07 b	3.06 ± 0.02 b	2.71 ± 0.05 c
Moisture (% wb)	12.60 ± 0.00 a	10.49 ± 0.00 b	12.46 ± 0.28 a	10.98 ± 0.28 b

One-way ANOVA for each variable with respect to pea variety and fraction. Different letters represent significant differences between experimental results according to post-hoc Tukey tests.

Significant differences ($p < 0.05$, one-way ANOVA) were found for the protein content between both varieties and fractions. The middle fractions presented higher protein contents, being the protein content of the green pea the highest. Significant differences ($p < 0.05$, one-way ANOVA) were also found in the carbohydrate content, being higher for the fine fractions of both varieties due to their lower protein contents. Some significant differences ($p < 0.05$, one-way ANOVA) were found in lipid, ash, and moisture contents between samples.

It is observed that the fine fraction primarily consists of carbohydrates and proteins. Proteins are generally considered as suitable wall materials and emulsifiers due to their amphiphilic nature (Jarzębski et al. 2019). Pea protein presents several advantages, such as cost-effectiveness, hypoallergenic properties, and positive consumer perception (Lam et al. 2018). In addition, legume starch and soluble polysaccharides can effectively be used as encapsulating agents due to their low viscosities at high solid content and excellent solubilization characteristics. However, they may lack the necessary interfacial properties for achieving high microencapsulation efficiency on their own, and are typically combined with other encapsulating materials, such as proteins or gums (Gharsallaoui et al. 2007). Therefore, the fine fraction of the yellow and green peas was selected to be used as encapsulating agent in the spray drying step, as it includes proteins, starch and soluble polysaccharides, which can act synergistically.

The technological characteristics of the fine and middle fractions of the yellow and green peas, including their density, water activity, wetting time, flowability, cohesiveness, granulometry, and color (L*, a*, b*) were determined, as shown in Table 2. The yellow and green pea fine fractions showed similar values of density, moisture and water activity than the middle fractions. As expected, both middle fractions were made of larger particles, as expressed by the 10, 50, and 90 percentiles for the size distribution (Pulivarthi et al. 2021, Nkurikiye et al. 2023). This also resulted in lower wetting times, as the penetration of water should be facilitated by larger interparticle spaces (Silva and O'Mahony 2017). On the other hand, flowability and cohesiveness did not vary between the

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middle and fine fractions for both pea varieties. It was also observed that both fine fractions were lighter than the respective middle fractions, as indicated by higher values of L*, while no trend was found in terms of a* and b*. This difference in color may be explained by the protein content, presence of colored components, occurrence of browning reactions, among others (Sharan et al. 2021).

Table 2. Technological characteristics of fine and middle fractions of yellow and green peas

	Yellow pea middle fraction	Green pea middle fraction	Yellow pea fine fraction	Green pea fine fraction
Color - L*	84.33 ± 0.41 c	75.80 ± 0.45 d	89.96 ± 0.18 a	86.89 ± 0.22 b
Color - a*	3.84 ± 0.12 a	5.07 ± 0.09 c	0.86 ± 0.01 b	-4.97 ± 0.01 c
Color - b*	17.84 ± 10.37 a	22.43 ± 0.25 a	15.44 ± 0.15 a	18.87 ± 0.10 a
Wetting time (min)	0.13 ± 0.00 c	0.03 ± 0.00 c	39.50 ± 1.55 a	36.12 ± 1.13 b
Water Activity (-)	0.58 ± 0.00 a	0.51 ± 0.00 c	0.56 ± 0.00 b	0.52 ± 0.00 c
Flowability - CI (%)	16.87 ± 0.88 ab	9.88 ± 3.71 b	17.29 ± 0.29 ab	28.90 ± 6.26 a
Cohesiveness - HI (-)	1.20 ± 0.01 ab	1.11 ± 0.04 b	1.21 ± 0.00 ab	1.41 ± 0.12 a
Granulometry - D10 (μm)	163.560 ± 0.919 b	181.885 ± 0.749 a	5.22 ± 0.01 c	4.21 ± 0.02 c
Granulometry - D50 (μm)	336.47 ± 1.41 b	414.49 ± 0.02 a	45.87 ± 0.01 d	65.35 ± 0.57 c
Granulometry - D90 (μm)	554.49 ± 0.45 b	686.56 ± 0.58 a	108.74 ± 0.51 c	128.96 ± 0.37 d
Density (g ml^{-1})	1.18 ± 0.00 d	1.77 ± 0.00 a	1.50 ± 0.00 c	1.54 ± 0.00 b

One-way ANOVA for each variable with respect to pea variety and fraction. Different letters represent significant differences between experimental results according to post-hoc Tukey tests.

Evaluation of the performance of the protein recovery process from yellow and green peas

Table 3 presents the protein content, protein recovery yield, productivity, specific water consumption, and drying yield for the protein products obtained from both pea varieties, both precipitant agents, and the usage of encapsulant agent; where some significant differences ($p < 0.05$, three-way ANOVA for each response) were found with respect to pea variety, precipitant agent, and usage of encapsulant agent. The protein product with the highest protein content ($p < 0.05$, three-way ANOVA) was obtained using green peas as the raw material, HCl as the precipitant, and without the addition of the green pea fine fraction as encapsulant agent. However, other performance indicators were not favorable for these processing conditions.

As a general trend according to the ANOVA and Tukey test, the protein content of the protein products obtained using encapsulant agent were not significantly different, or slightly decreased ($p < 0.05$, three-way ANOVA), in comparison to the protein content of the protein products obtained without encapsulant agent. A reason for this trend is that the fine fraction added as encapsulant agent contained more carbohydrates and less proteins than the respective wet products.

On the other hand, an unexpected result was obtained for the protein product obtained from yellow peas with HCl as the precipitant, where the protein content increased when using the encapsulant agent. During the experimental runs it was noted a high variability on the performance of the laboratory spray dryer, reflected for example in the amount of low-quality powder adhered to the walls of the main chamber. Although not reported

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here, this low-quality powder had a low protein content and high carbohydrates content. This may constitute another factor contributing to the previously discussed increase in the protein content. Sodium content of the obtained protein products ranged from 0.88 to 1.90% db for both pea varieties. These values were in the same order of magnitude as previously reported in the literature for comparable processing conditions: 1.1 % for lentil protein isolates obtained by isoelectric precipitation (Alonso-Miravalles et al. 2019b), approximately 2–3% for pea protein isolates (Sajib et al. 2023b), 0.5% for faba bean protein isolate (Vogelsang-O'Dwyer et al. 2020), and 1.5% for commercially available pea protein isolate (McCarthy et al. 2016).

Table 3. Performance of the protein recovery process from yellow and green peas for different drying conditions

		Without encapsulant agent		With encapsulant agent	
	Precipitant agent	Yellow pea protein product	Green pea protein product	Yellow pea protein product	Green pea protein product
Protein content (% db)	HCL	41.41 ± 0.74 d	59.11 ± 0.72 a	51.02 ± 1.44 b	45.81 ± 0.29 c
	LAB	38.88 ± 0.76 de	50.58 ± 0.60 b	36.39 ± 0.74 e	48.06 ± 2.36 bc
Sodium content (% db)	HCL	1.35 ± 0.02 c	1.90 ± 0.03 a	1.00 ± 0.04 e	1.11 ± 0.00 d
	LAB	1.65 ± 0.00 b	1.90 ± 0.02 a	0.88 ± 0.02 f	1.18 ± 0.00 d
Moisture (% wb)	HCL	9.01 ± 0.93 a	7.23 ± 0.09 ab	4.40 ± 0.07 de	6.67 ± 1.08 bc
	LAB	6.34 ± 0.19 bcd	4.60 ± 0.17 cde	3.60 ± 0.27 e	4.21 ± 0.12 e
Protein recovery yield, Y_T (% db)	HCL	26.53 ± 0.08 bc	14.99 ± 0.19 f	31.19 ± 1.02 a	28.83 ± 0.33 ab
	LAB	19.83 ± 0.58 e	22.45 ± 0.04 de	24.07 ± 0.46 cd	25.98 ± 1.73 bc
Productivity, P_T (kg product db/kg flour db)	HCL	0.25 ± 0.00 g	0.27 ± 0.00 f	0.48 ± 0.00 b	0.46 ± 0.00 c
	LAB	0.23 ± 0.00 h	0.30 ± 0.00 e	0.51 ± 0.00 a	0.41 ± 0.00 d
Specific water consumption, G_W (kg water/kg final product db)	HCL	122.38 ± 0.92 b	112.75 ± 0.08 c	52.96 ± 0.06 g	56.67 ± 0.45 f
	LAB	134.22 ± 0.00 a	102.48 ± 0.10 d	52.58 ± 0.14 g	62.39 ± 0.01 e
Drying Yield, Y_D (% db)	HCL	51.05 ± 0.38 f	68.82 ± 0.05 d	62.89 ± 0.07 e	72.44 ± 0.58 b
	LAB	62.69 ± 0.00 e	71.30 ± 0.07 c	85.34 ± 0.24 a	61.95 ± 0.01 e

Three-way ANOVA for each variable with respect to pea variety, precipitant agent, and usage of encapsulant agent. Different letters represent significant differences between experimental results according to post-hoc Tukey tests.

The performance of the protein recovery process from different legumes was previously reviewed by Sari et al. (2015), where it was observed that yield and protein content in the final product depended on the biomass to solvent ratio, type of solvent, extraction pH, temperature, and time, among other processing variables. They also concluded that protein recovery from certain matrices, such as soybeans and other cereals, is easier compared to that from other novel sources. This is presumably because the proteins are more readily available, while the processing conditions have been better adjusted. For pea flour products, (Tanger et al. 2020) obtained protein contents above 70%, by means of alkali extraction at a pH value of 9.5 and freeze-drying, with extraction yields of 46–50%. For three pea cultivars, Stone et al. (2015) obtained isolates with a protein content of 83.3–86.9% through an alkaline extraction at a pH value of 9.5, with values of the protein yield of 62.6–76.7% and productivities of 15.3–16.0% (based on wt of raw material).

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Regarding the alkaline extraction stage, the implemented extraction strategy with the addition of fresh water in each cycle intended to simulate a continuous counter-current extraction effect, aiming to improve the protein recovery yield with respect to the standard process with 1 or 2 extraction cycles (Accoroni et al. 2020). Sunley (1995) explained that the addition of fresh solvent enhances the driving force to further extract soluble proteins that are still tightly bound. Shen et al. (2008) and Sunley (1995) reported high recovery yields for protein recovery using solid:liquid ratios above 1:35 or 1:40, which would imply a larger specific water consumption. In addition, Cui et al. (2020) found no effect of the pH value set at the alkaline extraction step on the final protein content of products obtained from four different yellow pea cultivars.

Regarding the isoelectric precipitation stage, the usage of precipitant agents other than HCl, such as lactic acid and lactic acid bacteria, was reviewed by Alhamad et al. (2020), as natural sourced and more environmentally friendly alternatives. Comparative values of protein recovery yield, protein recovery in the final product, and process productivity were here found when using lactic acid bacteria and lactic acid in the precipitation stage. Emkani et al. (2021) previously found that a set of different bacterial strains (*Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacterium lactis*) may increase the solubility of the extracted pea proteins presumably due to their proteolytic activity, while also enhancing the organoleptic properties of the obtained protein products.

Regarding the spray drying stage, Akbarbaglu et al. (2021) reviewed the spray drying encapsulation of bioactive compounds within protein-based carriers and found that optimization of processing variables is necessary to achieve a practical, effective, and applicable method on an industrial scale. Similarly, García Arteaga et al. (2021) observed that laboratory-scale spray drying may negatively impact the process yield, as consequence of the high losses of the implementation of this technique. On the other hand, Kurek and Pratap-Singh (2020) found that a combination of plant-based protein and polysaccharide-based carriers could improve the structure, functional properties, and nutritional value of the powdered products.

Table 4 presents the LAB cells count for the protein recovery processes where LAB were used as precipitant agent. Three samples were evaluated: the original commercial freeze-dried LAB starter, the wet product obtained from the isoelectric precipitation stage, and the protein product obtained from the spray drying stage.

Table 4. LAB cells count

Sample	Viable cell count (CFU g^{-1})	Total mass (g)	Total cell count (CFU)
LAB starter	$6.30\ 10^{13}$	$8.00\ 10^{-3}$	$5.04\ 10^{11}$
Wet product	$2.57\ 10^{12}$	$1.18\ 10^{3}$	$3.03\ 10^{15}$
Protein product	$1.60\ 10^{12}$	$1.87\ 10^{1}$	$2.99\ 10^{13}$

As expected, a substantial increase in viable bacterial mass occurs during the isoelectric precipitation stage. In a study involving five different Lactobacilli species under similar conditions, Rezvani et al. (2017) observed that these bacteria were in the exponential growth phase after 10 hours of fermentation (which aligns with the time frame used here), even though the lactic acid production rate was already decreasing. In addition, the final pH value has been shown to depend on the bacterial strain and the specific fermentation conditions (Engels et al. 2022). In that time frame, a pH value near 4.5 was achieved, which is the desired value for the isoelectric precipitation of the pH-shifting process. As stated in the section titled Isoelectric precipitation, lactic acid was used for the final pH adjustment if necessary. Alternatively, it may be feasible to adjust the solution's pH to the isoelectric point by slightly extending the fermentation time.

On the other hand, the viable bacterial mass in the protein product is reduced during the spray drying stage, though this reduction is less substantial due to the low efficiency of the laboratory spray dryer employed (Moreira et al., 2021). It has been previously reported thar the remaining bacterial cells may have an impact on the properties and probiotic potential of the protein products (Mora-Villalobos et al. 2020).

Evaluation of technological characteristics of yellow and green pea protein products

Table 5 presents the technological characteristics of the yellow and green pea protein products, including their density, water activity, wetting time, flowability, cohesiveness, granulometry, and color (L*, a*, b*).

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Table 5. Technological characteristics of powdered protein products obtained from yellow and green peas

		Without encapsulant agent		With encapsulant agent	
	Precipitant agent	Yellow pea protein product	Green pea protein product	Yellow pea protein product	Green pea protein product fine fraction
Color L*	HCL	90.03 ± 0.11 a	12.31 ± 0.06 d	11.39 ± 0.11 e	85.31 ± 0.04 c
	LAB	88.76 ± 0.17 b	85.15 ± 0.71 c	88.67 ± 0.14 b	85.75 ± 0.26 c
Color a*	HCL	1.03 ± 0.02 b	-2.19 ± 0.01 d	1.06 ± 0.07 b	-3.34 ± 0.0 f
	LAB	1.98 ± 0.06 a	-1.22 ± 0.05 c	1.98 ± 0.04 a	-2.5 ± 0.00 e
Color b*	HCL	11.3 ± 0.11 e	18.17 ± 0.24 b	12.94 ± 0.24 c	19.14 ± 0.06 a
	LAB	12.74 ± 0.12 c	18.94 ± 0.07 a	12.31 ± 0.06 d	18.44 ± 0.07 b
Wetting time (min)	HCL	8.18 ± 0.08 d	30.18 ± 0.02 a	14.29 ± 0.12 c	29.36 ± 0.09 b
	LAB	2.32 ± 0.04 f	2.19 ± 0.03 f	2.29 ± 0.08 f	3.49 ± 0.06 e
Water Activity (-)	HCL	0.41 ± 0.00 a	0.35 ± 0.00 b	0.33 ± 0.00 c	0.31 ± 0.00 e
	LAB	0.33 ± 0.00 c	0.21 ± 0.00 f	0.32 ± 0.00 d	0.21 ± 0.00 f
Flowability - CI (%)	HCL	28.34 ± 2.02 a	25.14 ± 0.20 ab	14.32 ± 0.43 d	23.34 ± 5.24 abc
	LAB	18.18 ± 1.11 bcd	28.52 ± 1.25 a	15.24 ± 0.86 cd	18.79 ± 1.48 bcd
Cohesiveness - HI (-)	HCL	1.39 ± 0.03 a	1.33 ± 0.00 ab	1.16 ± 0.00 c	1.30 ± 0.00 abc
	LAB	1.22 ± 0.01 bc	1.40 ± 0.02 a	1.18 ± 0.01 c	1.23 ± 0.02 bc
Granulometry D10 (µm)	HCL	2.49 ± 0.00 a	1.66 ± 0.00 h	2.22 ± 0.00 c	2.37 ± 0.00 b
	LAB	1.82 ± 0.00 f	2.09 ± 0.00 e	1.76 ± 0.00 g	2.20 ± 0.00 d
Granulometry D50 (µm)	HCL	10.58 ± 0.01 b	5.86 ± 0.05 g	9.37 ± 0.02 c	7.75 ± 0.04 e
	LAB	7.42 ± 0.02 f	8.73 ± 0.01 d	8.73 ± 0.01 d	10.72 ± 0.04 a
Granulometry D90 (µm)	HCL	24.83 ± 0.06 e	20.64 ± 0.86 g	27.56 ± 0.20 d	87.15 ± 0.50 b
	LAB	74.38 ± 0.13 c	22.98 ± 0.07 f	24.45 ± 0.16 e	90.75 ± 0.34 a
Density (g ml^{-1})	HCL	1.35 ± 0.00 a	1.00 ± 0.00 f	1.19 ± 0.00 e	1.25 ± 0.00 c
	LAB	1.23 ± 0.00 d	1.27 ± 0.00 c	1.28 ± 0.00 b	1.25 ± 0.00 c

Three-way ANOVA for each variable with respect to pea variety, precipitant agent, and usage of encapsulant agent. Different letters represent significant differences between experimental results according to post-hoc Tukey tests.

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Color

As a general trend, high values of the lightness parameter L* were obtained for most protein products. In some instances, lower L* values were observed, presumably not inherent to the proposed processing methodology but probably due to the inefficiencies of the implementation of spray drying at laboratory scale (García Arteaga et al. 2021). Protein products obtained from yellow peas showed positive a* and b* values, corresponding to a yellowish tinted powder; while protein products obtained from green peas showed negative a* and positive b* values, corresponding to a greenish tinted powder. The Hunter whiteness index with CIELAB coordinates, computed according to Shevkani et al. (2015), gave values of 27–56, which implies that the obtained protein products would be perceived as lightly colored powders, similar to commercially available ones (Zhao et al. 2020).

Wetting time

Shorter wetting times were observed for protein products precipitated with lactic acid bacteria, with an average value of 2.19–3.49 minutes. In contrast, all products obtained with HCl as the precipitant agent exhibited significantly larger wetting times of 8.18–30.18 minutes ($p < 0.05$, three-way ANOVA).

Rashwan et al. (2023) and Emkani et al. (2022) reviewed several case studies of the modification of the functional and technological properties of plant based proteins by means of lactic acid fermentation. Particularly, Yadav et al. (2022) postulated that lower wetting times of biologically precipitated soy protein isolates may be due to the lower particle size and higher degree of protein hydrolysis in the protein product, which may result in a higher exposure of hydrophilic groups, and therefore, an increment of interactions with water molecules.

Water activity

As a general trend, it is observed that the water activity of protein products obtained using the yellow and green peas fine fractions as encapsulant agent in the spray drying stage is lower (0.21–0.33) when compared to the water activity of protein products obtained without the use of such encapsulant agent (0.31–0.41). There may be many factors that influence the water activity of powered products obtained using a mixture of proteins and carbohydrates as encapsulant agent, such as the degree of water binding by surface proteins, the availability, distribution, and arrangement of polar groups in the polysaccharide, the occurrence of inter-chain bonds, the resulting particle size, among others (Locali Pereira et al. 2019).

It is also noted that all water activity values were below the threshold value of 0.6, which should ensure no microbial proliferation occurs in the powdered products under standard storage conditions (Beuchat et al. 2013).

Flowability and cohesiveness

Flow and cohesion of powders were evaluated using the Carr index (CI) and the Hausner index (HI), respectively (Turchiuli et al. 2005). As a general trend, all obtained protein products presented good or fair flow capacity, given by CI values below 30%. Additionally, protein products obtained using encapsulant agent in the drying stage presented better flowability values than those obtained without encapsulant agent, with some significant differences ($p < 0.05$, three-way ANOVA).

Regarding their cohesion, all obtained protein products exhibited HI values below 1.4, thus implying an intermediate or low cohesiveness. Moreover, the usage of the fine fraction of the yellow and green peas as encapsulant agent during the spray drying stage resulted in protein products with lower HI values. When used to anticipate the flow characteristics of powdered materials, lower HI values signify powders that exhibit low cohesion, and therefore, excellent flow capacity (Pulivarthi et al. 2021).

Granulometry

Most particles obtained from the spray drier measured below around 25 μm, as deduced from the 10th, 50th, and 90th percentiles for particle size. Nevertheless, some agglomeration was observed due to the inefficiencies of the laboratory scale spray drier, as captured by larger values of the 90th percentile for some protein products, where the particle size was around 90 μm.

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Several case studies have been reported in the literature regarding encapsulation and spray drying of different matrices, where the particle sizes were in the same order of magnitude. For the microencapsulation of flaxseed oil with hydrolyzed pea protein isolate, Bajaj et al. (2017) obtained particles sizes of 9, 25, and 50 μm for the 10th, 50th, and 90th percentiles. For the De Broucker (D 4,3) mean diameter of hempseed oil microcapsules encapsulated with a combination of proteins and maltodextrin, Kurek & Pratap-Singh (2020) reported values between around 34 and 56 μm, depending on the initial oil content. Locali Pereira et al. (2019) obtained microcapsules of pink pepper essential oil with soy protein isolate, high methoxyl pectin and maltodextrin as wall materials, where the particle sizes were around 4, 15, and 45 μm for the 10th, 50th, and 90th percentiles. The particle size distribution plays an important role in many functional and technological properties of protein powders, such as oil and water retention capacity, texture, apparent density, color, among others (Rashwan et al. 2023).

Particle density

As a general trend, bigger values for the particle density of the protein powders (1.19–1.28 g ml^{-1}) were obtained when using the fine fraction of the yellow and green peas as encapsulant drying the spray drying stage, than when no encapsulant was used (1.00–1.27 g ml^{-1}).

Similar particle densities have been reported in the literature for the capsules of different compounds obtained through spray drying. Locali Pereira et al. (2019) reported values around 1 g ml^{-1} for the particle density of microcapsules of pink pepper essential oil produced by single-layer and double-layer emulsions, with a porosity of around 56–61%. From the bulk density and porosity values reported by García-Segovia et al. (2021) for beetroot microencapsulation with pea protein using spray drying, the particle density can be estimated to be around 1.475 g ml^{-1}, with little variation as function of the protein content and drying air inlet temperature. According to Düsenberg et al. (2023), larger values of the particle density of a powdered product imply a lower compactness and consequently a better flowability behavior.

Conclusions

This study delved into the effects of a pH-shifting process combined with spray drying on the technological characteristics of protein products derived from yellow and green peas. Utilizing lactic acid bacteria as a GRAS precipitant agent and the fine fraction of milled peas as a drying encapsulant/ adjuvant resulted in a highly efficient process with notable advantages, including a doubling of process productivity and a 50% reduction in specific water consumption. Protein content was significantly influenced by processing parameters, reaching 59.11% for the protein product obtained from green peas without using encapsulant, while the process productivity reached values of 0.41–0.51 kg protein product/kg pea flour.

The assessment of technological characteristics of the protein products, including color, wetting time, water activity, flowability, cohesiveness, granulometry, and particle density, provided a comprehensive understanding of the impact of processing parameters on the final products. The L* color parameter reached 85–90 for several protein products, indicating high lightness values. Wetting time varied significantly, with lactic acid bacteria-precipitated products having much shorter wetting times (2.19 to 3.49 minutes) compared to HCl-precipitated products (8.18 to 30.18 minutes). Water activity was lower in products obtained using the pea fine fraction, with values between 0.21 and 0.33, compared to values between 0.31 and 0.41 for those obtained without encapsulant. Flowability and cohesiveness were also affected by the operating conditions, with Carr index (CI) values ranging from 14.32% to 28.52%, and Hausner index (HI) values between 1.16 and 1.40, indicating good flowability and low to intermediate cohesiveness. Granulometry percentiles (D50) ranged from 5.86 μm to 10.72 μm, while particle density varied between 1.00 g ml^{-1} and 1.35 g ml^{-1}. The use of lactic acid bacteria and the incorporation of the pea fine fraction during spray drying positively influenced these characteristics, demonstrating the potential for tailoring powdered protein products for diverse food technology applications.

These findings pave the way for further exploration of these techniques in the development of plant-based food products, offering sustainable alternatives with enhanced technological characteristics. Future research endeavors could focus on scaling up these processes for industrial applications and investigating the functional, technological and probiotic properties of the obtained protein products for application in diverse food formulations.

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https://doi.org/10.23986/afsci.145290

Acknowledgements

The authors appreciate the ongoing support of Instituto de Tecnología Mauá, Universidad del Centro Educativo Latinoamericano (UCEL), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Nacional del Litoral (UNL), and Universidad Tecnológica Nacional (UTN). Also, we are grateful to laboratory technicians Douglas Dalla Justina, Sandra Maria do Nascimento, Daniela Cristina do Couto, Marcia Coutinho da Silva and Lucas Felix de Arruda for assisting us in the use of the facilities and equipment.

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