

Composition and characterization of pea and chickpea starches

Composição e caracterização de amido de ervilha e grão-de-bico

Autores | Authors

Luis Fernando POLESI

Universidade de São Paulo (USP)
Escola Superior de Agricultura Luiz de
Queiroz (ESALQ)
Departamento de Agroindústria, Alimentos
e Nutrição
e-mail: lfpolesi@yahoo.com.br

✉ **Silene Bruder Silveira SARMENTO**

Universidade de São Paulo (USP)
Escola Superior de Agricultura Luiz de
Queiroz (ESALQ)
Departamento de Agroindústria, Alimentos
e Nutrição
Av. Pádua Dias, 11
CEP: 13418-900
Piracicaba/SP - Brasil
e-mail: sbssarme@usp.br

Carlota Boralli Prudente dos ANJOS

Universidade de São Paulo (USP)
Escola Superior de Agricultura Luiz de
Queiroz (ESALQ)
e-mail: cbpanjos@esalq.usp.br

✉ Autor Correspondente | Corresponding Author

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■ Summary

Non conventional starch sources have been intensively studied in recent years, searching for new properties and uses as food ingredients. The objective of this study was to evaluate the physicochemical, structural and functional characteristics of starches from two legumes: green pea (cultivar Utrillo) and chickpea (cultivar BRS Cícero). The pea starch showed high amylose (61%) and resistant starch (RS) (39.8%) contents, with 14.3% of total dietary fibre (TDF). The pea starch presented simple and composite granules with various shapes and sizes, a wide distribution in the amylose elution profile in gel permeation chromatography, B-type crystallinity pattern, gelatinization peak temperature of 74.8 °C, reduced granule swelling with increasing temperature and did not generate high viscosity in the Rapid Visco Analyzer (RVA). The chickpea starch showed large oval shaped granules and small spherical shaped ones, all with a smooth surface, C-type crystallinity pattern, gelatinization peak temperature of 64.6 °C, good granule swelling and high viscosity in the RVA. With respect to composition, the amylose content was 29.2%, the RS 31.9% and TDF 2.7%. These starches could be used in different food applications. From the nutritional point of view, pea starch is interesting due to its considerable resistant starch and total dietary fibre contents. From the technological point of view, chickpea starch is interesting for use in foods requiring hot viscosity.

Key words: *Pisum sativum*; *Cicer arietinum*; Physicochemical characterization; Structure; Resistant starch.

■ Resumo

A busca por fontes de amido não convencionais, com propriedades diferenciadas para uso na indústria alimentícia, tem sido intensa nos últimos anos. O objetivo do presente trabalho foi avaliar as características físico-químicas, estruturais e funcionais dos amidos de duas leguminosas: ervilha verde (cultivar Utrillo) e grão-de-bico (cultivar BRS Cícero). O amido de ervilha apresentou na sua composição teores altos de amilose (61%) e de amido resistente (AR) (39,8%), com 14,3% de fibra dietética total (FDT). O amido de ervilha exibiu grânulos simples e compostos, com formas e tamanhos variados, distribuição ampla no perfil de eluição da amilose em cromatografia de permeação em gel, padrão de cristalinidade tipo B, pico de temperatura de gelatinização de 74,8 °C, baixa expansão dos grânulos com o aumento da temperatura, não gerando viscosidade elevada no *Rapid Visco Analyser* (RVA). O amido de grão-de-bico apresentou grânulos grandes de formato oval e pequenos de forma esférica, com superfície lisa, padrão de cristalinidade tipo C, pico de temperatura de gelatinização de 64,6°C, boa expansão dos grânulos, com viscosidade elevada no RVA. Na composição, o teor de amilose foi de 29,2%, o de AR foi de 31,9% e o de FDT, de 2,7%. Esses amidos podem ser utilizados em diferentes aplicações alimentícias. Do ponto de vista nutricional, o amido de ervilha é interessante pelo seu considerável teor de amido resistente e de fibra dietética total. Do ponto de vista tecnológico, o amido de grão-de-bico é interessante para o uso em alimentos que necessitam de viscosidade a quente.

Palavras-chave: *Pisum sativum*; *Cicer arietinum*; Caracterização físico-química; Estrutura; Amido resistente.

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1 Introduction

The search for new starch sources is currently a subject of scientific research, mainly aimed at new physicochemical and functional properties.

Up to the 80's, all the peas (*Pisum sativum*) domestically consumed in Brazil were imported. However currently the entire demand is met by domestic production (COSTA et al., 2006) and Utrillo is one of the cultivars grown in this country.

Peas can be used for human and animal nutrition, as well as in industry. On average the grains contain 24% protein, 3% lipids, 3% ash, 12% crude fibre and 58% carbohydrates (COSTA et al., 2006), and the starch content varies between 18 and 40%. Starch, protein and fibres can be extracted from peas, but the starch can be extracted from a by-product generated from the protein extraction (RATNAYAKE et al., 2002). This is a commercial procedure in some countries such as Canada and France, using yellow peas.

Chickpea (*Cicer arietinum*), another legume of great importance, has an average composition of 16-21% protein, 3% ash, 3-7% lipids, 5-13% crude fibre and 59-67% carbohydrates, and of the total grain carbohydrates, about 40-50% is starch (SINGH et al., 2004; COSTA et al., 2006).

The starches extracted from legumes are characterized by high amylose contents. According to Ratnayake et al. (2002), the amylose content can vary from 30-40% in the starch from smooth peas to 60-76% in the starch from wrinkled peas. In chickpea, the amylose content varies from 28 to 40% (SINGH et al., 2004; HUGHES et al., 2009).

In general, starch is potentially digestible by enzymes in the gastrointestinal tract and is absorbed as glucose in the small intestine. Starches from legumes, however, have reduced digestibility, being compared to other sources such as cereals (HOOVER and ZHOU, 2003). Furthermore, a significant amount of starch can escape digestion, and this fraction is called resistant starch (RS).

Resistant starch is defined as the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals (ENGLYST et al., 1992). The RS passes through the digestive tract to the large intestine, where it serves as a substrate for microorganisms that can ferment starch, i.e. it behaves as dietary fibre, with benefits in reducing the risk of colon cancer, reducing the glycemic index, acting as a prebiotic, showing a hypocholesterolemic effect, inhibiting fat accumulation and, when compared to digestible starch, allows greater apparent absorption of calcium and iron (SAJILATA et al., 2006).

Costa et al. (2006) evaluated the RS contents of chickpea (cv. Marrocos) and pea (cv. Maria), but only in raw and cooked whole grains, finding values between 2.45 and 1.89% for peas and between 3.39 and 2.23% for chickpeas, respectively.

Although starch is the major component of pea and chickpea grains, there are few studies in the literature concerning the composition, physicochemical and functional properties of the starches extracted from these legumes. Thus the objective of this study was to evaluate the composition and physicochemical, structural and functional characteristics of the green pea and chickpea starches.

2 Material and methods

Wrinkled green peas (cultivar Utrillo) were acquired from a producer in the state of Minas Gerais, Brazil, and the chickpea cultivar BRS Cícero was acquired from EMBRAPA HORTALIÇAS in Brasília/DF, Brazil. The pea starch was extracted using the modified alkaline method of Davydova et al. (1995). The pea grains were crushed with distilled water (1:5), sieved (60 and 325 mesh) and the pH adjusted to 7.6 (0.08 M NaOH) to promote flocculation of the proteins and separation of the starch. This material was centrifuged at 1120 g for 15 min. The supernatant was discarded and the decanted starch re-suspended in distilled water and sieved (60 and 325 mesh). The starch suspension was cleaned by re-centrifugation (5 times), and the starch thus obtained dried in an oven with circulating air at 40 °C for 12 h, ground in a mortar and sieved (60 mesh). The chickpea starch was extracted according to Singh et al. (2004). The starch obtained was dried in an oven with circulating air at 40 °C for 12 h and the dried starch ground in a mortar and sieved (60 mesh).

The moisture content was determined in a Moisture Analyzer (AND - Mod MX-50), using 1 g of sample at a temperature of 105 °C. To assess the purity level of the starches extracted, the ash, protein and lipid contents were evaluated using the AOAC methods (HORWITZ; LATIMER, 2006) and the crude fibre using the AACC method (FENTON, 1995). The factor used in the conversion of the nitrogen content (micro-Kjeldahl) to crude protein was 6.25. The apparent amylose content was determined according to the ISO 6647 methodology (1987).

The appearance of the granules was evaluated using the scanning electron microscope (ZEISS, DSM 940 A) with an amperage of 80 mA and voltage of 5 kV. The samples were assembled on stubs using double-sided adhesive tape, and the starches fixed and covered with a thin layer of gold in a BAL-TEC SCD 050 apparatus for 220 s.

The molecular weight distribution profiles of the starches were determined by gel permeation chromatography using a GE XK 26/70 column (2.6 cm

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in diameter and 70 cm high), packed with Sepharose CL-2B gel. The samples were prepared according to Song and Jane (2000). Approximately 10 mL of 90% dimethyl sulphoxide (DMSO) were added to 0.1 g of starch. The mixture was heated in a boiling water bath for 1 h, and then incubated at 25 °C for 24 h with constant stirring. An aliquot of 3 mL was mixed with 10 mL of absolute ethanol to precipitate the starch, and then centrifuged for 30 min at 3000 g. The precipitated starch was re-dissolved in 9 mL of hot distilled water. Approximately 1 mL of glucose (1 mg.mL⁻¹) was added and the mixture placed in a boiling water bath for 30 min. An aliquot of 4 mL was applied to the bottom of the column and eluted in the ascending mode. A solution containing 25 mM NaCl and 1 mM NaOH was used as the eluent at a rate of 60 mL.h⁻¹. Fractions of 4 mL were collected and analyzed for their total carbohydrate content (phenol-sulphuric acid) at 490 nm (DUBOIS et al., 1956) and blue value (iodine staining) at 630 nm (JULIANO, 1971).

Resistant starch (RS) content was measured using the Goñi et al. (1996) procedure, including the following steps: removal of protein with pepsin (Sigma P-7000, 40 °C, 1 h at pH 1.5), incubation with alpha-amylase (Sigma A-3176, 37 °C, 16 h at pH 6.9) to hydrolyze the digestible starch, treatment of the precipitates with 2 M KOH to dissolve the RS, incubation with amyloglucosidase (Sigma A-1602, 60 °C, 45 min., pH 4.75), and determination of the glucose produced using the glucose oxidase assay (Sigma GAGO-20). The RS was calculated as glucose x 0.9.

The total dietary fibre content was determined using the enzymatic-gravimetric method of AOAC 985.29 (HORWITZ; LATIMER, 2006). The enzymatic hydrolysis of the starch and protein was carried out as follows: gelatinization in the presence of thermo-stable α -amylase (Termamyl 120 L, 97 °C, 15 min, pH 6.0), incubation with pepsin (Sigma P-7000, 40 °C, 30 min, pH 1.5) and incubation with amyloglucosidase (Sigma A-7255, 55 °C, 30 min, pH 4.0-4.6). The total dietary fibre was precipitated with 4 volumes of 95% ethanol and recovered by filtration in a # 2 sintered crucible with celite. The fibre values were corrected by subtracting the indigestible protein (Kjeldahl N x 6.25) and ash (incineration at 525 °C, 5 h.).

The samples were submitted to X-ray diffraction (Shimadzu - XRD 7000) with Cu_k α radiation at a speed of 2°.min⁻¹ with 2 θ diffraction angles of 4 and 50° at 40 kV and 30 mA. The X-ray diffraction profiles were classified according to the patterns described by Zobel (1964). The relative crystallinity of the starches was quantitatively estimated using the method of Nara and Komiya (1983) with the Origin - version 7.5 software (Microcal Inc., Northampton, MA, USA). The graphs were plotted between the 2 θ angles of 4 and 30 and smoothed with the 'Adjacent Averaging' tool.

The thermal properties of the starches were evaluated using a differential scanning calorimeter (DSC-Pyris 1, Perkin Elmer, USA) according to Liu et al. (2005), with modifications. The starch was weighed (6 mg) in a high-pressure stainless steel pan (PE 0319-0218) and deionized water added using a microsyringe in a starch:water ratio of 1:3. The pans were sealed in a universal press (PE B013-9005) with an adapter (PE B050-5340) and equilibrated at room temperature for 24 h before measurement in the DSC. The equipment was calibrated with indium. The scanning temperature range and the heating rate were 40-200 °C and 5 °C.min⁻¹, respectively. An empty pan was used as the reference. Based on the thermograms, the following gelatinization values were obtained: onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), temperature range ($\Delta T = T_f - T_i$) and enthalpy variation (ΔH).

The swelling factor of the starch granules was evaluated according to the direct method proposed by Tester and Morrison (1990), at temperatures from 50 to 120 °C.

The viscoamylographic properties were evaluated in a Rapid Visco Analyzer (RVA-S4A, Newport Scientific, Warriewood, NSW, Australia) using 3 g of sample (14% of moisture) in 25 g of water. The Standard 2 analysis program was used. The parameters Paste Temperature, Peak Time, Peak Viscosity, Breakdown Viscosity, Final Viscosity, and Setback Viscosity were obtained from the viscoamylogram.

3 Results and discussion

The ash, protein, lipid and fibre contents of the pea and chickpea starches (Table 1) were in agreement with values found in the literature (HOOVER and RATNAYAKE, 2002; SINGH et al., 2004) and reflect the purity of the starches extracted, allowing for further analysis. In the macromolecular composition, the amylose content was 61.0% for pea starch, similar to that found in wrinkled pea cultivars (RATNAYAKE et al., 2002), which range from 60.5 and 88.0%. For the chickpea starch, the amylose content was 29.2%, i.e. within the range of values reported in the literature, which is from 27.2 to 34.3% (SINGH, et al., 2004; HUGHES et al., 2009).

The pea starch showed 39.8% of RS. Themeier et al. (2005) studied starches from 11 pea varieties and found RS amounts from 18.0 to 19.6% in pea varieties with amylose contents between 62.5 and 70.6%. The resistant starch found in natural starches is of the RS₂ type, which is due to the granular structure of the starch. The chickpea natural starch showed 31.9% of RS, a value higher than the 27.2% found by Marconi et al. (2000) in the same botanical source.

The pea starch showed 8 percentage points more RS than the chickpea starch. The RS content may be

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Table 1. Chemical composition, resistant starch and total dietary fibre contents of the pea and chickpea starches.

Parameter	Pea	Chickpea
Ash (%)	0.08 ± 0.01	0.10 ± 0.01
Protein (%)	0.31 ± 0.02	0.10 ± 0.01
Lipid (%)	0.01 ± 0.00	0.01 ± 0.00
Crude fibre (%)	0.65 ± 0.07	0.33 ± 0.03
Apparent amylose (%)	61.00 ± 0.85	29.20 ± 0.30
Resistant starch (%)	39.85 ± 1.15	31.87 ± 1.35
Total dietary fibre (%)	14.33 ± 0.46	2.66 ± 0.51

related to the amylose content present in these starches, considerably higher in that from the pea. The positive correlation between amylose content and RS level was also confirmed in other studies found in the literature (THEMEIER *et al.*, 2005; LI *et al.*, 2009).

However, the RS levels in the starch granules can be modified if the starch is submitted to hydrothermal processing. The TDF determination is aimed at evaluating or quantifying the RS fraction that resists the boiling process, *i.e.*, the thermally stable RS (BRUMOVSKY and THOMPSON, 2001).

The pea starch had higher TDF levels (thermal stability) than the chickpea starch. When the RS content as assessed by physiological methods (GOÑI *et al.*, 2006) was compared with that assessed by the TDF method, a reduction of 63% in the pea starch resistance was observed as compared to enzymatic digestion. As for the chickpea starch, this reduction was 92%. The amylose content can also affect the TDF content of starches, and the higher the amylose content, the higher the TDF content. This effect was also observed by other authors (THEMEIER *et al.*, 2005).

Figures 1a and 1b show the differences in general appearance (size and shape) between the starch granules, according to their botanical source. The pea starch (Figure 1a) showed relatively smaller granules when compared with those of the chickpea starch. Pea starch (Figure 1a) appears to be a mixture of simple and composite granules (4-5 granules), with varied shapes and sizes. Most of the simple granules (especially small granules) showed a rounded shape, while the composite granules (larger granules) showed an irregular shape. Similar results were found by Zhou *et al.* (2004) for wrinkled pea starch. The chickpea starch (Figure 1b) showed large oval shaped granules and small spherical shaped granules. SEM showed that the starch granules had smooth surfaces with no evidence of cracks. Similar observations for chickpea starch were reported in previous studies, such as those of Miao *et al.* (2009) for the Kabuli and Desi cultivars.

Figure 2 shows the gel permeation chromatograms of the pea and chickpea starches. The first peak

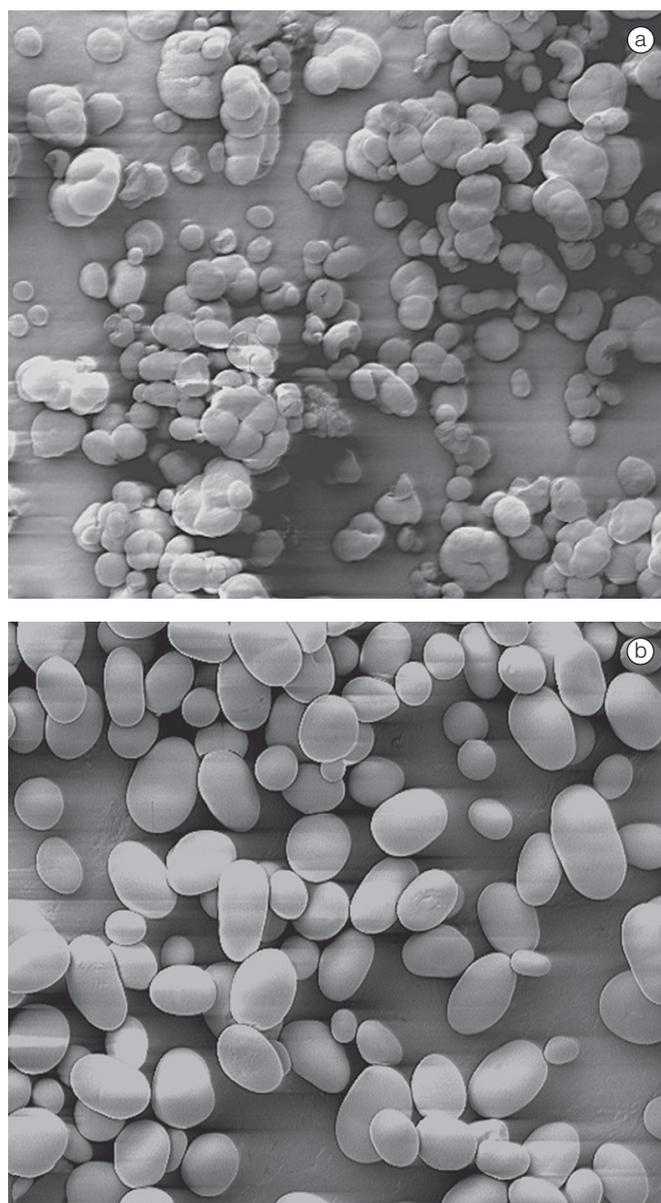


Figure 1. SEM of starches at the same magnification. a) Pea; and b) Chickpea.

corresponds to the amylopectin fraction. The second peak, which showed a significant reaction with iodine, corresponds to the amylose fraction. The last peak corresponds to glucose, which was added to mark the end of elution.

The pea starch showed a lower total carbohydrate content and slightly higher iodine reaction than the chickpea starch with respect to the first peak (amylopectin). This may be related to the length of the amylopectin side chains, which, when larger, show a greater reaction with iodine, producing a more intense color (LI *et al.*, 2008). The blue value and total carbohydrate ratio (BV/CHO) of the amylopectin peak (fraction 23) was 0.22 for the pea starch and 0.16 for the chickpea starch. This relationship

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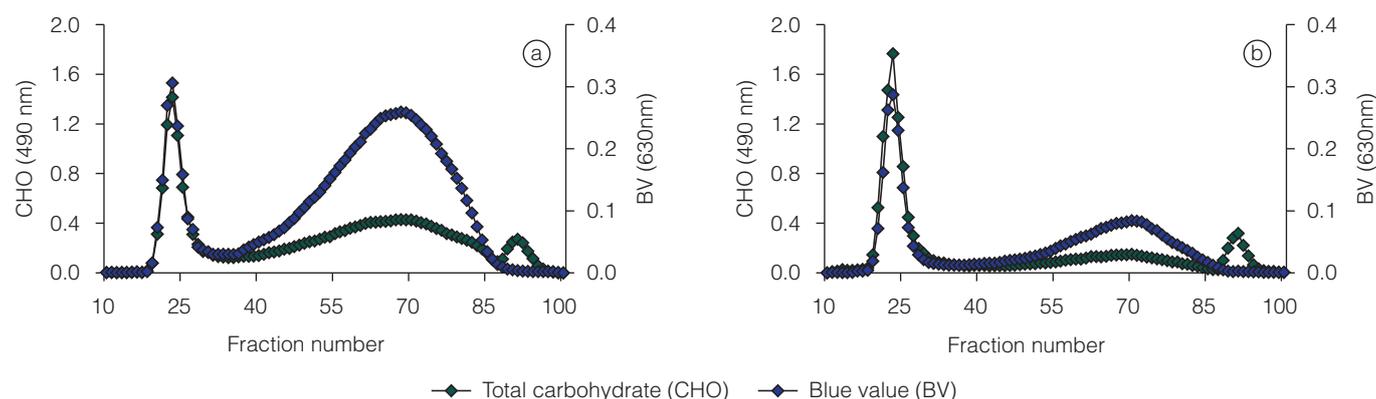


Figure 2. Sepharose CL-2B gel permeation chromatographic profile. a) pea starch and b) chickpea starch.

also indicates a greater iodine reaction for the amylopectin of the pea starch.

The second peak (amylose) was wider and more acute for the pea starch and more reduced and flat for the chickpea starch, consistent with the higher amylose content of the former. Furthermore, the amylose content of the pea starch seemed to have a higher molecular weight, since its distribution in the chromatographic profile was broader.

The pea starch showed a B-type crystallinity pattern (Figure 3), with an intermediate intensity peak at the 2θ diffraction angle of 5.5° , intermediate-strong intensity peaks at the 2θ angles of 22.2° and 24.0° and strong intensity peaks at the 2θ angle of 17.1° (ZOBEL, 1964). The B polymorph is characteristic of tuberous starch and corn with a high amylose content (ZHOU et al., 2004). Hedley et al. (2002) also found a B crystallinity pattern for the mutant pea *r* genotype.

The chickpea starch showed a C-type crystallinity pattern (Figure 3), with an intermediate intensity peak at the 2θ diffraction angles of 5.6° and 15.1° and strong intensity peaks at the angles of 17.1° and 23.1° (ZOBEL, 1964). This pattern is considered characteristic of starch from legumes and consists of a mixture of types A and B crystalline structures. Other authors also found the C pattern for natural chickpea starch (HUGHES et al., 2009).

The RS content of the natural pea starch with a B crystallinity pattern was higher than that of the natural chickpea starch with a C pattern. This confirms information found in the literature that the B crystallinity pattern of starches is more resistant to enzymatic digestion than the others (ENGLYST et al., 1992).

The pea starch showed a wide range of gelatinization temperatures (Table 2) as also cited in literature (HEDLEY et al., 2002). However, the variation in enthalpy was higher for this pea cultivar when compared with those cited by the same authors for the mutant *r* pea genotype.

When studying the starch from the mutant *r* pea, some authors (HEDLEY et al., 2002) observed that

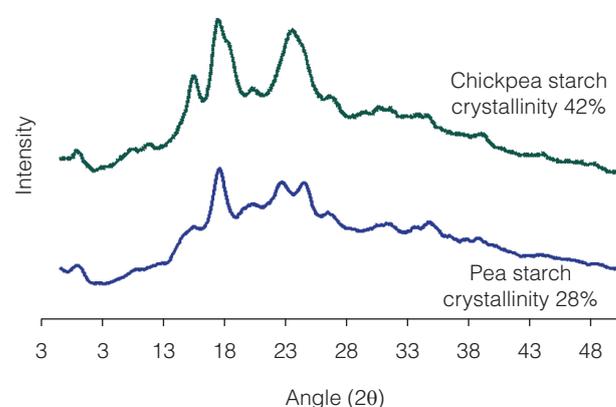


Figure 3. X-ray diffraction of pea and chickpea starches.

the change in heat capacity during gelatinization was very slow and could not be referred to as a first-order transition. According to these authors, this slow change led to a high ΔT value. The absence of drastic changes in the heat capacity during gelatinization did not allow for the definition of a peak temperature for this starch, and therefore T_p was determined as a temperature range. Moreover, the changes in the heat capacity were relatively low and resulted in low ΔH values.

The chickpea starch showed a well defined endothermic peak, characteristic of this source of starch. The gelatinization temperatures found were in agreement with values reported in the literature (HOOVER and RATNAYAKE, 2002; SINGH et al., 2004; MIAO et al., 2009), while the gelatinization enthalpy was higher.

The ΔT corresponding to the chickpea starch was lower than that of the pea starch, indicating a greater homogeneity of the crystals within the granules (HOOVER and RATNAYAKE, 2002). The values for T_o and ΔH of the chickpea starch were higher than those of the pea starch. This difference can be attributed to differences in the degree of crystallinity (28% for pea and 42% for chickpea), since high transition temperatures result from high degrees of crystallinity, which provide structural stability and make

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Table 2. Thermal properties of the pea and chickpea starches.

Starch	Thermal properties				
	T_o (°C)	T_p (°C)	T_c (°C)	ΔT (°C)	ΔH (J.g ⁻¹)
Pea	56.1 ± 1.6	74.8 ± 1.5	89.7 ± 1.5	33.6	4.2 ± 1.1
Chickpea	59.9 ± 0.2	64.6 ± 0.2	68.2 ± 0.5	8.3	14.7 ± 1.3

T_o = onset temperature, T_p = peak temperature, T_c = conclusion temperature, $\Delta T = T_c - T_o$ and ΔH = gelatinization enthalpy.

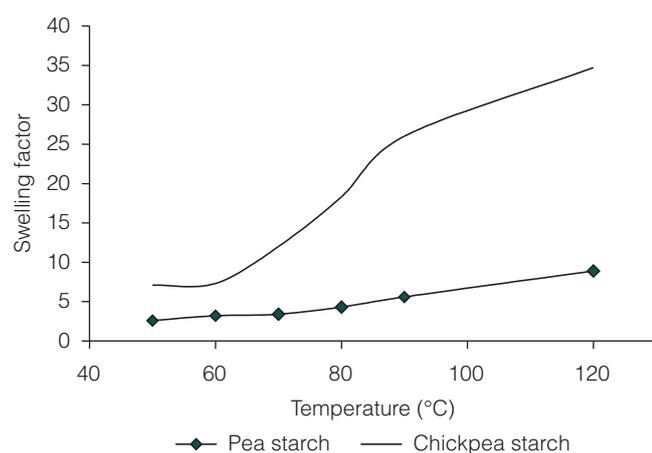


Figure 4. Swelling factors of starch granules at different temperatures.

the grain more resistant to gelatinization. The extent of crystalline perfection is reflected in the gelatinization temperature range and in the enthalpy variation obtained by DSC (SINGH et al., 2004).

The swelling factors were evaluated at temperatures from 50 to 120 °C, ranging from 2.6 to 8.9 for pea starch and from 7.1 to 34.7 for chickpea starch (Figure 4). The pea starch only showed 26% of the chickpea starch swelling at the highest temperature (120 °C). Zhou et al. (2004) examined starches from various legumes and also observed a low swelling factor (3.4) for wrinkled pea starch. This fact was attributed to the low amylopectin content of this starch and/or to strong interactions between the amylose chains. Hughes et al. (2009) found values of 3.6, 4.6, 11.0, 17.8 and 25.9 for the swelling factor of chickpea starch, cv. Desi (ICC 12512-9), at temperatures of 50, 60, 70, 80 and 90 °C, respectively.

The low swelling power of pea starch indicates that granule swelling does not play a very important role in the gelatinization of this starch. Thus crystals at different parts of the granules break independently during gelatinization. It is likely that differences in the thermal stability of the crystals within the granules of this starch result in the disruption that occurs over a wide temperature range (BOGRACHEVA et al., 2001).

Pea starch (Figure 5) showed a very low viscosity (near zero) during the time/temperature programming of the viscograph. This low viscosity was due to the reduced swelling of the granules, as shown from the swelling factor.

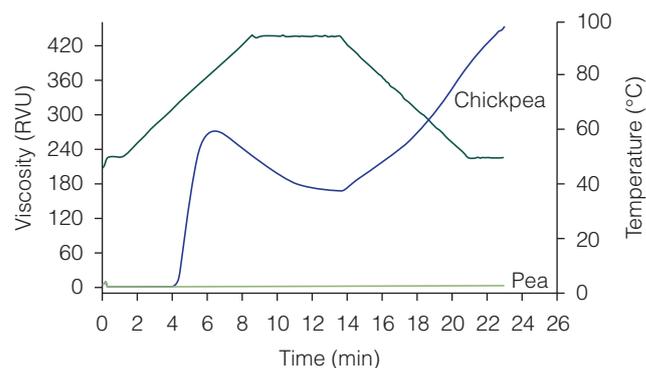


Figure 5. Pasting profiles of the starches as measured by RVA.

This association between lower viscosities and higher amounts of amylose in starch has also been reported in the literature (SONG and JANE, 2000; JUHÁSZ and SALGÓ, 2008). Hedley et al. (2002) found low viscosity values when studying the starch from the mutant *r* pea genotype.

In studies with model systems using corn starch with different amylose contents, Juhász and Salgó (2008) concluded that the amylopectin was primarily responsible for the water uptake. When the amylose content is 27% (normal corn starch), the amylose helps to maintain the integrity of the swollen granules when it interacts with the amylopectin. However a high amylose content suppresses the hot paste viscosity, due to the alignment and orientation of its molecules.

The chickpea starch showed (Figure 5) a well-defined pasting temperature and peak viscosity, some tendency to breakdown due to hot shear, and to setback with cooling. The pasting temperature of the starch was 69.3 °C. Singh et al. (2004) found values between 75.1 and 77.1 °C for starch suspensions (6%) from different chickpea cultivars. However, Miao et al. (2009) found higher values when compared to those of the present study (70.7 and 73.4 °C) for the Desi and Kabuli chickpea starches, respectively, at the same starch concentration.

4 Conclusions

These starches may be used for different food applications, depending on the particular characteristics desired. From the technological point of view, the

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chickpea starch is of interest for use in foods requiring hot viscosity, such as soups, porridges and others. From the nutritional point of view, the pea starch is interesting due to its considerable resistant starch and total dietary fibre contents, thus showing lower digestibility. The use of these starches as ingredients in frozen desserts is restricted for both of them due to their high amylose levels, which promote considerable retrogradation.

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