Original article

Effect of jet-cooking and hydrolys with amylases on the physicochemical and *in vitro* digestion performance of whole chickpea flours

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(Received 9 April 2019; Accepted in revised form 5 August 2019)

Summary Whole chickpea flours were subjected to a pilot plant process aimed to understand the effects of jet-cooking followed by α-amylase or isoamylase hydrolyses in terms of physicochemical and the *in vitro* digestion performance of starch and proteins. Jet-cooked flours generated lower viscosities and had lower gelatinisation temperatures when compared with their raw counterparts; furthermore, the amylolytic enzymes improved both starch and protein *in vitro* digestion rates (HI of 85.33 and relative digestion of 88.92%, respectively) that were strongly correlated with the amylose content (*P* < 0.05). By means of principal component analyses (PCA) is concluded that the changes in granular architecture, reflected by lower Δ*H* values and new linear structures after isoamylase hydrolysis (treated = 44.36% vs. raw = 26.43%) as well as protein denaturation promoted similar glycemic responses in raw flours compared with jet-cooked counterparts (83.12 and 84.77, respectively). The combination of a thermal-enzymatic method could be a useful alternative to produce novel pulse flours.

Keywords Chickpea flour, functional properties, glycemic index, isoamylase, jet cooker, α-amylase.

Introduction Nowadays, pulse seeds are on the spotlight of food industry, mostly due to its functional and nutritional properties. Among them, chickpea (*Cicer arietinum*) has received special attention because the crop is high yielding and is considered as staple food in many Eastern countries (Du et al., 2014; de la Rosa-Millán et al., 2017; Chávez-Murillo et al., 2018; Milán-Noris et al., 2019). This pulse crop often presents higher protein content when compared with others, as well as a relative higher proportion of carbohydrates, in which starch and β-glucans play a significant role in their functional and nutritional properties; particularly their low glycemic response (Chung et al., 2008; Rachwa-Rosiak et al., 2015). Previous studies have shown that chickpea flours impart higher viscosities when compared with other pulses and cereals (Kaur & Singh, 2005; Ma et al., 2011). This has been related with its protein (de la Rosa-Millán et al., 2018), soluble fibre (Rachwa-Rosiak et al., 2015) and amylose content (Kaur & Singh, 2005; Milán-Noris et al., 2017). Furthermore, many of the functional and nutritional properties of pulse flours can be influenced by their molecular organisation within the cotyledon, where interactions among proteins, fibre and starch molecules occur. These relevant interactions commonly prevail after cooking (Clemente et al., 1998; Chávez-Murillo et al., 2018); nevertheless, other investigations have suggested that new molecular interactions can be promoted after processing under high temperature and pressure conditions, which in the case of legumes partially or totally gelatinise starch granules and denature proteins (Ma et al., 2011; Felker et al., 2018), resulting in differentiated ingredients (Shogren et al., 2006; Kenar et al., 2012). The incurred changes during these processes often reduce water solubility and affect other relevant functional properties (de la Rosa-Millán et al., 2017). In this sense, the combination of thermal processing and biocatalysis with amylolytic enzymes has the potential to produce an array of differentiated ingredients, free of antinutritional factors and with diverse functionalities
especially in terms of water solubility and protein and starch availability (Ma et al., 2011). For this, the selective starch hydrolysis with amylolytic enzymes and its combination with a rapid and controlled thermal process like jet-cooking may promote increased solubility and a reduced amount of trypsin inhibitors in end products, and also expand the food and technological applications (Fanta et al., 2008; Felker et al., 2018). For this, the aim of this investigation was to analyse the impact at pilot plant scale of the combination of jet-cooked thermal processes and selective starch hydrolyses using α-amylase or isoamylase in whole chickpea flours.

**Materials and methods**

**Whole flour preparation**

Food-grade chickpea seeds (*Cicer arietinum*) were acquired with a distributor located in Los Mochis, Sinaloa, Mexico. The cleaned seeds were ground in a Wiley mill (Arthur Thomas, Philadelphia, PA, USA) equipped with a 2-mm diameter screen to produce whole flours. All flours were sieved to pass the US 80 mesh. The overs or coarse particles were further milled to pass this sieve. Afterwards, the resulting whole flours were stored in air-tight containers at −20 °C until analysis.

**Jet-cooking of flours**

Jet-cooking of whole flours was performed according to the procedure described by Fanta et al. (2008) with some modifications. Briefly, a homogeneous dispersion of 4 kg of chickpea flour in 40 L of distilled water at 35 °C was passed through a Hydrothermal M-130 steam jet cooker (Waukesha, WI, USA), operating under excess steam conditions. Cooking temperature was 140 °C, 2 min hold at 90 °C and a 2 min cooling cycle to reach 35 °C (Fig. 1). After this hydrothermal process, each experiment was accurately weighed to determine the process yield (eqn 1).

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\text{Yield} = \left( \frac{\text{Treated flour weight}}{\text{Initial flour weight}} \right) \times 100.
\]  

**α-amylase and isoamylase hydrolysates**

The enzymes used herein were selected to test the effects of their different hydrolyses patterns to generate hydrolysates with different carbohydrate profiles. α-amylase was chosen in order to generate maltose from linear segments of both amylose and amylpectin and limit dextrins from the branching points of the latter (Ao et al., 2007) whilst isoamylase to selectively cleave 1–6 linkages of amylpectin without affecting the amylose structure (Fanta et al., 2013). For this, both enzymes were tested to improve starch hydrolysis and broaden applications of resulting flours. For the enzymatic hydrolysis, 15 L of the chickpea slurry (with or without jet-cooking) were transferred into a stainless steel reactor and maintained to 35 °C with a constant mixing speed (120 r.p.m.) and mixed with either 40 mL of an enzyme dispersion of 13 U mL⁻¹ of Pancreatic porcine amylase (E-PAANA), or 40 mL of a 13 U mL⁻¹ of isoamylase (E-ISAMY) solution (Megazyme ltd., Wicklow, Ireland). Each enzyme process was carried out during 12 h before stopping the enzymatic reaction performing a second jet-cooking step under the aforementioned conditions. Then, the slurries were vacuum-dried and then milled to pass a US 80 mesh in preparation for storage at −20 °C until analysis.

**Chemical composition**

The moisture, protein, fat and ash contents of all flours were assayed according to approved AACC methods 44-01.01, 46-13.01, 30-20.01 and 08-01.01, respectively (AACC, 2000). Total starch (K-TSTA), amylose (K-AMYL) and dietary fibre (K-TDFR) contents were determined using Megazyme kits (Megazyme).

**Starch granules morphology**

The starch morphology and birefringence patterns were viewed with a Motic BA-210 digital microscope (Hong Kong, China). The images were acquired at 40× magnification (40×) under normal and polarised light.

**Rapid viscosity analysis**

The pasting profiles of flours were acquired with a Rapid Visco Analyzer (RVA Model 1170, Newport Scientific, Warriewood, NSW, Australia). For each treatment, a 25.5 g suspension with 12% solids was prepared and then subjected to a 2 min heating profile where temperature first increased from 50 to 90 °C, 4-min hold at 90 °C and a 2 min cooling cycle to reach 50 °C. Both heating and cooling rates were 15 and −15 °C min⁻¹, respectively.

**Thermal properties**

For this analysis, the procedure described by de la Rosa-Millán et al. (2017) was used. Briefly, 2 mg
(based on total starch content) of each sample was placed in hermetic anodised aluminium capsules (Perkin Elmer, B02190062, Norfolk, VA, USA), hydrated with the appropriate amount of distilled water (three volumes, based on total sample weight) and containers carefully sealed. The starch gelatinisation parameters, onset \( (T_o) \), peak \( (T_p) \) and conclusion \( (T_c) \) temperatures of gelatinisation as well as the endothermic enthalpy \( (\Delta H) \) were calculated using the Pyris manager software (Perkin Elmer). Furthermore, the analysed capsules were stored at 4°C for 7 days and rescanned under the above conditions with the aim to understand changes due to starch retrogradation.

**In vitro starch and protein digestions**

The *in vitro* starch digestion fractions of the different pulse flours were determined according the Englyst *et al.* (1992) protocol with slight modifications, as reported by Chávez-Murillo *et al.* (2018). Samples were digested using a mixture of enzymes (pancreatin, amyloglucosidase and invertase) throughout 120 min reaction time. Aliquots (1 mL) were withdrawn at 20 and 120 min of reaction, and samples immediately mixed with 4 mL of absolute ethanol (200 proof). The glucose content was quantified with the glucose oxidase-peroxidase reagent. Starch classifications based on the rate of hydrolysis were as follows: rapidly digestible starch (RDS) (digested within 20 min), slowly digestible starch (SDS) (digested between 20 and 120 min) and resistant starch (RS) (undigested after 120 min). With the aim to study their molecular differences by FTIR due to the applied process, an additional 5 mL aliquot was taken before and after 120 min of the *in vitro* digestion; then mixed with 20 mL of absolute ethanol and tube contents centrifuged at 7000 g for 30 min. The supernatant was discarded, and the resulting pellet was freeze-dried, milled and sieved to pass a 100 US mesh. Additionally, with the aim to estimate the extent of hydrolysis and predicted glycemic performance of the treated flours, the protocol of Granfeldt *et al.* (1992) was employed in cooked flours dispersions, and these were incubated in a boiling water bath for 30 min with constant magnetic stirring (300 r.p.m.). The percentage of hydrolysed starch by Porcine pancreatic \( \alpha \)-amylase at 30, 60, 90, 120 and 180 min was estimated. The hydrolysis index (HI) was calculated from the ratio between the area under the hydrolysis curve compared with a reference sample (white bread). The predicted GI (pGI) was estimated from the HI and relative values calculated using the equation established by Goñi *et al.* (1997), with a reported correlation coefficient of \( R = 0.89, P < 0.05 \) (eqn 2).

\[
pGI = 39.71 + 0.549 (HI).
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To estimate the protein digestibility of flours, the protocol of Hsu *et al.* (1977) was employed. Samples, adjusted to a pH of 8, were hydrolysed with a multienzyme solution of 1.6 mg of trypsin (15 units mg\(^{-1}\)), 3.1 mg of chymotrypsin (60 units mg\(^{-1}\)) and 1.3 mg of peptidase (40 units g\(^{-1}\)) per mL. The pH drop after 10 min of hydrolysis was recorded to estimate the *in vitro* protein digestibility. Bovine casein was employed as control to estimate the efficacy and accuracy of the assay.

**Molecular characteristics of flours by Fourier transformed infrared spectroscopy**

Native chickpea flours, as well as their treated counterparts were recovered, dried, ground to pass a No. 80 US Mesh and analysed in a ATR-FTIR apparatus (Spectrum 1, Perkin Elmer). The molecular spectral data of whole and enzyme-treated flours were collected.
and corrected with the air background and analysed with the Spectrum software (ver. 5.3.0). The spectra were generated in absorption mode with mid-IR (ca. 4000–800 cm\(^{-1}\)) with a resolution of 4 cm\(^{-1}\) by fifty scans. A half-band width of 15 cm\(^{-1}\) and a resolution enhancement factor of 1.5 with Bessel apodization were employed. Intensity measurements were applied in the deconvoluted spectra by calculating the height of the absorbance bands from their baseline. The regions of interest were the carbohydrate zones related to starch crystalline and amorphous structures (1047 and 1022 cm\(^{-1}\), respectively), the secondary protein structures, \(\alpha\)-helix, \(\beta\)-sheet and the ratio of intramolecular to intramolecular associations (1635, 1650 and 1615:19/C19, respectively). All chemical functional groups were identified according to previous reports (Chávez-Murillo et al., 2018; de la Rosa-Millán et al., 2018).

**Statistical analyses**

A one-way variance analysis was performed, and when differences were found at a significance level of 0.05, a Tukey’s test of multiple comparisons was used. With the aim to understand the possible interactions among the flour components as consequence of the thermal and enzyme treatment, a Pearson correlation analysis was performed at both \(P < 0.05\) and \(P < 0.01\) levels of significance. Additionally, to evaluate the influences of composition and treatment, a principal component analysis (PCA) was conducted. All statistical analyses were performed using the (Minitab software 17 (version 17.3), State College, PA, USA). All experiments and procedures described in this research were performed in triplicate unless otherwise specified.

**Results and discussion**

**Chemical composition**

The raw chickpea flour contained a relatively high protein (23.14%) with low amounts of lipids (4.32%) and ash (3.61%) (Table 1). The major fraction was carbohydrates constituted by starch and insoluble and soluble fibres; these values are online with previous investigations that assayed the same chickpea Kabuli variety (Milán-Norris et al., 2017; de la Rosa-Millán et al., 2018). The proposed jet-cooking procedure did not significantly affect the chemical composition \((P > 0.05)\). Despite this, there were significant differences in the yield of the different treatments \((P < 0.05)\).

**Starch and fibre characteristics**

The major carbohydrate fractions associated to the chickpea flours are depicted in Table 2. Raw flour, as well as jet-cooked and/or hydrolysed samples showed similar TS values of \(\approx 59\%\), which in the case of raw flour has been reported previously (Kaur & Singh, 2005; Milán-Norris et al., 2017). Despite this, in raw flour, the use of \(\alpha\)-amylase decreased the amount of assayable amylose from 26.43% to 14.36%, whilst the use of isoamylase increased values up to 36.32%. Regarding jet-cooked flours, they showed significant differences in amylose contents compared with their raw counterparts \((P < 0.05)\). The use of \(\alpha\)-amylase resulted in lower amylose content, whilst isoamylase treatment increased its content to a level of 44.36%. This behaviour could be related with the specific hydrolytic patterns of both enzymes, and amylases are known to randomly hydrolyse the \(\alpha\) 1–4 glycosidic bonds, with no specificity for amylose or amyllopectin molecules. As a result, these enzymes reduce the size and MW of both amylose and amyllopectin (Englyst et al., 1992; Goni et al., 1997). Whilst isoamylase selectively cleaves the \(\alpha\) 1–6 glycosidic linkages, which imply the release of linear glucan chains from amyllopectin molecules, thus making them more available for further hydrolysis into glucose during the enzymatic protocol (McCleary et al., 1997). These differences showed that jet-cooking promoted granular disruption which increased substrate availability for both enzymes. The utilisation of a debranching enzyme could be useful to produce unique ingredients rich in linear chains (Byars, 2003). Compared with the fibre

| Material                  | Moisture (%) | Protein (%) | Lipids (%) | Ash (%) | Carbohydrates (%)\(^1\) | Yield (%) |
|---------------------------|--------------|-------------|------------|---------|--------------------------|-----------|
| Raw                       | 10.67 ± 0.67 | 23.14 ± 0.14| 4.32 ± 0.70| 3.61 ± 0.39| 68.93 ± 0.20 | 93.36 ± 1.14 |
| Raw + \(\alpha\)-amylase  | 9.10 ± 0.10  | 23.19 ± 0.35| 3.98 ± 0.46| 3.66 ± 0.77| 68.56 ± 0.70 | 45.33 ± 1.16 |
| Raw + isoamylase          | 10.78 ± 0.78 | 23.33 ± 0.34| 4.07 ± 0.59| 3.64 ± 0.85| 68.97 ± 0.90 | 80.26 ± 1.19 |
| Jet cooked                | 11.50 ± 0.50 | 23.75 ± 1.00| 4.13 ± 0.33| 3.17 ± 0.85| 69.32 ± 0.15 | 90.33 ± 2.32 |
| Jet cooked + \(\alpha\)-amylase | 9.18 ± 0.18 | 23.87 ± 0.11| 4.17 ± 0.62| 3.26 ± 0.48| 69.02 ± 0.18 | 70.25 ± 2.41 |
| Jet cooked + isoamylase   | 10.53 ± 0.53 | 23.67 ± 0.34| 4.32 ± 0.66| 3.43 ± 0.65| 69.01 ± 0.72 | 75.43 ± 2.67 |

Results are the average of three replicates ± standard deviation. Different letters in the same column indicate significant differences \((P < 0.05)\).

\(^1\)Calculated by difference.
of raw flours, the fibre fractions showed slight but significant differences due to the proposed jet-cooking (Table 2). Other studies which studied jet-cooking have reported significant changes in the ratio of soluble:insoluble fibre fractions, mainly because this hydrothermal process generates high shear forces that disrupt the internal structural matrix of fibre-rich cell walls, which usually increases the amount of assayable soluble fibre (Ma et al., 2011; Felker et al., 2018). Additionally, in recent years, there has been an increased interest in β-glucans associated to chickpeas because they exert several functional and nutritional properties (Milán-Noris et al., 2019). Results herein showed that the amounts of these molecules were in the range of 0.79–0.85%, with no significant differences due to thermal or enzymatic treatments. These values are also on line with previous reports with similar chickpea cultivars. The high prebiotic values are also in line with previous reports with similarities due to thermal or enzymatic treatments. These rates of both starch and protein fractions (Ma et al., 2011; Jukanti et al., 2012).

Microscopy analysis of chickpea flours

In a general way, pulse flours are known for its low digestion rates, that prevail even after cooking. Such resistance has been related with their protein and fibre compositions, and more specifically to the starch molecular arrangement, in which amylase plays a significant and key role (Chung et al., 2008; Du et al., 2014). For this reason, the information about their starch granular features and overall flour organisation is fundamental to understand the behaviour in food systems (Kadam et al., 1989; Clemente et al., 1998). When observing the raw flour, the presence of apparently intact starch granules with well-defined birefringence was evident (Fig. 2a, b), alongside with the companionship of fibre and protein structures from the cotyledon matrix. However, such starch granular arrangement was lost after jet-cooking, evidencing by the presence of shapeless aggregated particles or clusters, which in some sections still showed birefringence (Fig. 2c, d). When combining jet-cooking and α-amylase treatment, smaller aggregates were observed, but with apparently larger proportions of birefringent areas, that could be related with the amylolysis of amorphous zones of the starch granules (Fig. 2e, f). In the same sense, isoamylase hydrolysis in jet-cooked flours resulted in evidently larger structures with diffused birefringence (Fig. 2g, h). We hypothesize that both morphological and birefringent characteristics of samples were related with the catalytic mechanisms of enzymes. On one hand, α-amylases degrade in a random way, leaving aside maltose form the linear segments of starch molecules or limit dextrins from the branched structures, hence promoting a rapid decrease in the Mw of starch molecules. On the other hand, the isoamylase selectively hydrolyses the α 1–6 linkages from starch molecules, leaving aside larger amounts of linear glucan structures with higher Mw (Ao et al., 2007). Such structural and conformational characteristics of these molecules are known to enhance inter-entanglement or complexing with protein and fibres, thus exerting significant changes of physicochemical and functional properties (Ma et al., 2011; Felker et al., 2018).

Rapid viscosity analysis

In eastern countries, chickpea flours are widely used either as food ingredients or for direct consumption (FAOSTAT, 2016, Gupta et al., 2017). In this regard, processed pulses are well known to provide high viscosities and thick textures, and to exert good emulsifying properties, which has been associated to the amount and molecular characteristics of both soluble and insoluble fibres, as well as to the starch molecules (Kaur & Singh, 2005, Iqbal et al., 2006). The use of amylolytic enzymes promoted significant differences on the pasting characteristics of jet-cooked flours when compared with their raw counterpart (Table S1). In raw samples, the lowest viscosity profiles were obtained after treatment with α-amylase (Fig. 3a). This is related with the action of this enzyme which

| Material          | TS (%)  | Amylose (%) | TDF (%)  | IDF (%)  | SDF (%)  | β-Glucans (%) |
|-------------------|---------|-------------|----------|----------|----------|--------------|
| Raw               | 59.42±0.47 | 26.43±0.76  | 10.54±0.67 | 7.61±0.91 | 2.92±0.12 | 0.85±0.51    |
| Raw + α-amylase   | 59.56±0.40 | 18.43±0.62  | 10.00±0.23 | 7.67±0.33 | 2.33±0.78 | 0.83±0.54    |
| Raw + isoamylase  | 59.36±0.26 | 36.32±0.50  | 10.61±0.18 | 7.77±0.74 | 2.84±0.57 | 0.80±0.33    |
| Jet cooked        | 59.26±0.44 | 27.71±0.84  | 10.06±0.59 | 5.45±0.51 | 4.61±0.30 | 0.81±0.53    |
| Jet cooked + α-amylase | 59.32±0.32 | 14.36±0.75  | 10.71±0.84 | 6.56±0.55 | 4.15±0.64 | 0.82±0.39    |
| Jet cooked + isoamylase | 59.22±0.39 | 44.36±0.24  | 10.79±0.75 | 6.77±0.65 | 4.02±0.73 | 0.79±0.37    |

Results are the average of three replicates ± standard deviation. Different letters in the same column indicate significant differences (P < 0.05). IDF, insoluble dietary fibre; SDF, soluble dietary fibre; TDF, total dietary fibre; TS, total starch.
hydrolyses randomly starch molecules into linear and branched dextrins and maltose. Furthermore, the enzyme is known to disrupt the structural granule order which significantly reduces peak viscosity values (Ao et al., 2007; Zhang & Hamaker, 2009). Additionally, after jet-cooking (Fig. 3b), the viscosity of chickpea flour greatly decreased due to the loss of internal granular structure as a result of the harsh jet-cooking processing conditions (Byars, 2003; Felker et al., 2018). Regarding the enzymatic treatments, the α-amylase promoted the lowest viscosity values in both raw and jet-cooked flours. There are few studies that deal with the enzymatic treatment of whole flours. However, several investigations have attributed low paste viscosities to remaining granular structures composed by different chain length molecules either from amyllose or amylopectin (Kaur & Singh, 2005; Zhang & Hamaker, 2009).

Figure 2 Normal (left) and polarised light (right) microscopy images of chickpea flours. a and b: Raw flour, c and d: jet-cooked flour, e and f: jet-cooked flour + α-amylase, g and h: jet-cooked flour + isoamylase. [Colour figure can be viewed at wileyonlinelibrary.com]
Gelatinisation and retrogradation properties

Flours are complex systems in which the molecular interaction of their components may influence their thermal properties that could drive the energy consumption and overall behaviour of the materials (Kaur & Singh, 2005; Ma et al., 2011). In raw pulse flours, thermal performance relies on their cotyledon composition, in which starch granules are imbedded in a protein matrix along with both soluble and insoluble fibres that may difficult heat transfer during cooking, decreasing starch gelatinisation and promoting partial/total protein denaturation (Rachwa-Rosiak et al., 2015; Felker et al., 2018). In this study, raw chickpea flour showed a $T_p$ of 72.15 °C and a $\Delta H = 9.26 \text{ J g}^{-1}$, which is online with previous reports (Du et al., 2014). The hydrolysis with either $\alpha$-amylase or isoamylase significantly decreased $T_p$ (to 69.41 °C) and $\Delta H$ (3.44 and 5.57 J g$^{-1}$, respectively) ($P < 0.05$) due to structural damage in the crystalline zones of starch granules (de la Rosa-Millan, 2017). Although, jet-cooked flours still presented significantly lower transition gelatinisation temperatures and enthalpies ($T_p = 63.26$ °C and a $\Delta H = 1.20 \text{ J g}^{-1}$). When $\alpha$-amylase and isoamylase were used, there were significant differences on the gelatinisation parameters ($T_p = 56.15$ and 60.13 °C, $\Delta H = 0.26$ and 1.15 J g$^{-1}$, respectively). The existence of a remaining thermal transition in these materials evidenced the residual organisation of gelatinised and hydrolysed starch fragments, which was likely related with the protective thermal effect of the other cotyledon components (Kaur & Singh, 2005; Du et al., 2014). With the aim to further comprehend the behaviour of the obtained flours after storage, the retrogradation parameters were acquired. In raw flours, similar $T_{pr}$ values for $\alpha$-amylase and isoamylase-treated flours (64.19–65.55 °C, respectively) were found (Table S2); however, they significantly differed in $\Delta H_R$ values that were 2.36–4.17 J g$^{-1}$ for isoamylase-treated and raw flours, respectively. Likewise, lower $T_{pr}$ were observed for jet-cooked flours. However, $\Delta H_R$ values indicated a possible reorganisation of the linear structures. For the specific case of $\alpha$-amylase-treated flours, they almost matched their $\Delta H$ and in the case of isoamylase-treated flours they greatly surpassed the same parameter implying that the linear structures generated during this process enhanced the reorganisation (Ao et al., 2007; Zhang & Hamaker, 2009; Table 3).

Starch and protein in vitro digestibilities

Cooked chickpeas are known for their low digestion rates, which are mainly related to their fibre fractions and amylose contents (Chung et al., 2008; Du et al., 2014). However, when cooked, the digestion of both starch and proteins increases, enhancing the overall

![Figure 3 RVA profiles of $\alpha$-amylase and isoamylase-treated chickpea flours. (a) Raw flours, (b) jet-cooked flours.](image-url)
nutritional value (Gupta et al., 2017). Other studies in which starches or proteins were jet-cooked have shown that the digestion rate of both molecules greatly increased due to mechanisms of starch gelatinisation and protein denaturation (Ma et al., 2011). Raw chickpea flours contained 46.26% and 42.11% of SDS and RS, respectively, which is on line with previous reports (de la Rosa-Millán et al., 2017; Milán-Norís et al., 2019). When either α-amylase or isoamylase were used, they resulted in significantly higher RDS and SDS fractions (P < 0.05). Furthermore, jet-cooking significantly increased the RDS to 51.63% (P < 0.05) due to the disruption of starch granules (Table 4). The usage of both enzymes in jet-cooked flours resulted in significantly higher RDS fractions (65.53% for α-amylase treatment), with lower amounts of SDS (11.21% and 22.17%, α-amylase and isoamylase, respectively). Similarly, the RS contents of jet-cooked samples were comparatively higher, compared with the raw counterpart likely due to the higher rate of starch retrogradation. The flours treated with the two different enzymes presented significant differences in RS contents. The flour with the highest content was the one produced with jet-cooking + isoamylase. Other studies have shown that the starch digestion fractions may be modulated by both the amount and molecular characteristics of their constituents. In this case, the linear chains complexed with other molecules hindering their digestion rate (Bryars et al., 2008, Zhang & Hamaker, 2009). These results were complementary when calculating the pGI, that fluctuated between 83.81 (for jet-cooking + α-amylase) and 85.34 (for jet-cooking). These values are classified as high GI. In this regard, slow hydrolysis rates in starchy ingredients have been attributed to a large proportion of dextrins and/or a high amount of densely branched structures (Ao et al., 2007; Zhang & Hamaker, 2009). The hypothesis of this work was that on one hand, the α-amylase-treated flours were comprised by high amounts of low Mw molecules along with branched dextrins, which led to a rapid glucose release during the first 20 min of the Englyst protocol. However, after this initial stage, the remnant starch structures may have interacted with proteins and fibres decreasing digestion rates (de la Rosa-Millán et al., 2018). On the other hand, isoamylase could have promoted an increase in the amount of linear glucans, which may be inter-entangled as well as with proteins and fibres, therefore lowering even more digestion rates (Fanta et al., 2008; Du et al., 2014); thus, the behaviour of the enzyme-treated flours was comparable with their raw counterparts. Regarding proteins, the use of amylolytic enzymes improved their digestion likely due to the hydrolysis of starch granules that are associated with proteins stored in the cotyledon matrix; in this case, the in vitro protein digestibility of taw chickpea was 23.46% whereas the counterpart treated with α-amylase 38.55%. Jet-cooking greatly improved in vitro protein digestibilities to the greatest extent. The sample treated with jet-cooking + α-amylase showed a value of 88.92%. The hydrothermal treatment denatured proteins exposing amino acid residues to proteases, whereas the α-amylase hydrolysed starch granules that interfere with proteins (Hansen, 1986; Clemente et al., 1998). These results are useful to modulate flour properties and produce new ingredients with contrasting characteristics. For instance, a flour with high rates of both protein and starch digestibilities or with low glycemic index can be purposely produced. These pretreatments can also be used to improve extraction and yields of protein isolates or concentrates (de la Rosa-Millán et al., 2017; de la Rosa-Millán et al., 2018).

### Molecular structure analysis by FTIR

Flours were analysed by ATR-FTIR with the aim of further inquire into their molecular characteristics. The chickpea flours showed evident changes in their molecular composition after the applied enzyme treatments (Fig. 4a). Results indicate that the α-amylase

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**Table 3** Thermal parameters of chickpea flours under Jet cooker and amylolytic enzymes processes

| Material           | Tg (°C) | Tc (°C) | Tp (°C) | ΔH (J g⁻¹) | Tc − Tg (°C) |
|--------------------|---------|---------|---------|------------|--------------|
| Raw                | 68.22±0.35 | 72.19±0.47 | 76.33±0.38 | 9.26±0.32 | 8.11±0.36 |
| Raw + α-amylase    | 65.33±0.30 | 69.41±0.30 | 71.17±0.24 | 3.44±0.20 | 5.84±0.26 |
| Raw + isoamylase   | 67.11±0.66 | 69.41±0.59 | 73.16±0.53 | 5.57±0.64 | 6.09±0.64 |
| Jet cooked         | 56.43±0.52 | 63.26±0.46 | 65.15±0.30 | 1.20±0.36 | 8.72±0.46 |
| Jet cooked + α-amylase | 51.26±0.21 | 56.15±0.65 | 57.22±0.76 | 0.26±0.19 | 5.96±0.34 |
| Jet cooked + isoamylase | 55.36±0.42 | 60.13±0.51 | 62.31±0.49 | 1.15±0.43 | 6.95±0.49 |

Results are the average of three replicates ± standard deviation. Different letters in the same column indicate significant differences (P < 0.05). ΔH, gelatinisation enthalpy; Tg, conclusion gelatinisation temperature; Tc, Tg, gelatinisation temperature interval; Tg, onset gelatinisation temperature; Tp, peak gelatinisation temperature. © 2019 The Authors. International Journal of Food Science & Technology published by John Wiley & Sons Ltd on behalf of Institute of Food, Science and Technology (IFSTTF).
promoted greater disruption as reflected by the lower spectra intensities in both carbohydrates and protein zones. Furthermore, the deconvoluted spectra shows differences in the amorphous (1022 cm$^{-1}$) and crystalline (1047 cm$^{-1}$) starch structures (Fig. 4b). Other studies have found that the granular integrity of starch plays a significant role in the material functional properties and that when a disruption occurs in the crystalline component it may result in lower water uptakes and viscosities (Shogren et al., 2006; de la Rosa-Millán, 2017). Regarding proteins, there were substantial differences in their interaction with other chemical components. These interactions were observed at 1616 and 1625 cm$^{-1}$ wavelengths, which are related with the inter and intramolecular associations, respectively. These kind of interactions are known to occur among proteins and other macromolecules such as soluble and insoluble fibres (Himmelsbach et al., 1998).

Results are the average of three replicates ± standard deviation. Different letters in the same column indicate significant differences ($P < 0.05$).

| Material                        | RDS (%) | SDS (%) | RS (%) | HI       | pGI       | Protein digestion (%) |
|---------------------------------|---------|---------|--------|----------|-----------|-----------------------|
| Raw                             | 11.83$^d$ ± 0.56 | 46.26$^f$ ± 0.66 | 42.11$^c$ ± 0.16 | 71.76$^a$ ± 0.75 | 79.11$^a$ ± 0.47 | 23.46$^f$ ± 1.12 |
| Raw + α-amylase                 | 22.43$^d$ ± 0.11 | 51.17$^b$ ± 0.33 | 26.41$^b$ ± 0.03 | 78.90$^a$ ± 0.21 | 83.03$^a$ ± 0.96 | 38.55$^b$ ± 1.19 |
| Raw + isoamylase                | 18.63$^c$ ± 0.20 | 55.33$^c$ ± 0.69 | 26.01$^f$ ± 0.93 | 79.07$^a$ ± 0.85 | 83.12$^a$ ± 0.79 | 30.19$^c$ ± 1.23 |
| Jet cooked                      | 51.63$^c$ ± 0.01 | 31.24$^d$ ± 0.45 | 17.13$^c$ ± 0.05 | 83.12$^a$ ± 0.49 | 85.34$^a$ ± 0.02 | 67.25$^a$ ± 1.45 |
| Jet cooked + α-amylase          | 65.53$^c$ ± 0.43 | 11.21$^d$ ± 0.58 | 23.26$^c$ ± 0.58 | 80.33$^a$ ± 0.19 | 83.81$^a$ ± 0.29 | 88.92$^c$ ± 1.55 |
| Jet cooked + isoamylase         | 58.43$^b$ ± 0.48 | 22.17$^c$ ± 0.16 | 19.40$^d$ ± 0.75 | 82.09$^b$ ± 0.25 | 84.77$^c$ ± 0.24 | 80.06$^b$ ± 1.98 |

Figure 4 Effect of jet cooking and amylase treatment on whole chickpea flours. (a) Normalised spectra; (b) deconvoluted spectra of carbohydrates zone; (c) deconvoluted spectra of protein zone.
case of jet-cooked flour, the intermolecular associations prevailed, featuring a single (broad) peak, that may indicate strong and complex molecular associations with fibre’s and starch, that were the major components in chickpea flour (Fig. 3c). However, when a combination with enzymes was used, the signal split in two peaks of different intensities, but in the same wavelength range (from ≈ 1616 to 1622 cm\(^{-1}\)). This particular characteristic has been observed in other studies where thermal treatments and \textit{in vitro} digested food samples were analysed and reflect the loss of structural organisation by the harsh thermal and/or digestive processes (Chávez-Murillo et al., 2018). Similarly, the intramolecular associations signal (≈ 1625 cm\(^{-1}\)) of both thermal-enzymatic treatments showed both amplitude and intensity differences when compared with the non-treated counterpart. The logical explanation for this difference is protein denaturation. This pattern could also be related with the differences in protein secondary structures at 1635 and 1650 cm\(^{-1}\), which corresponded to \(\alpha\)-helix and \(\beta\)-sheet, which showed the highest and lowest intensities in the jet-cooked-treated flour as well as in the counterparts obtained by mingling with amylases (Himmelsbach et al., 1998). The observed spectra patterns showed that the use of amylolytic enzymes decreased the overall organisation of the flour components and that the linear glucan chains generated after the isoamylase treatment may have helped to stabilise protein structures. These phenomena influenced viscosity properties, promoted thermal resistance and modified the \textit{in vitro} digestion profiles compared with their non-enzyme-treated counterpart (de la Rosa-Millán et al., 2017; Chávez-Murillo et al., 2018).

**Figure 5** Principal component analysis of processed chickpea flours. (a) Loading plot, (b) cluster analysis. [Colour figure can be viewed at wileyonlinelibrary.com]
Principal component analysis (PCA)

The use of PCA analyses helped to elucidate the effects of the different variables and their interactions within chickpea flour as a consequence of the applied thermal or enzymatic treatments. The first and second components (PC1 and PC2) accounted for an accumulative variance of 86.7%. The loading plot (Fig. 5a) depicts the correlations among some physicochemical, thermal, digestion and molecular characteristics of samples. This analysis showed that PC1 accounted 74.2% of the accumulative variance, in which the main contributor factors were the crystalline structure of starch granules as well as the protein content and both SDF and IDF fractions of flours. Furthermore, the PC2 accounted for a 12.5% of the variance, in which the main contributors were the amylose followed by the β-glucans contents. These results showed that such physiochemical characteristic were driven by the resulting molecular composition of the jet-cooked and hydrolysed materials. Cluster analyses of PC1 and PC2 formed two groups (Fig. 5b), which reflected the differences between raw and jet-cooked samples, and beyond that the profound molecular differences between α-amylase and isoamylase hydrolysis treatments. Results obtained herein may significantly influence flour characteristics for different food systems (Zuclo et al., 2011; Rachwa-Rosiai et al., 2015). For this reason, further studies aimed to understand better the molecular and structural composition of these materials are ongoing.

Conclusions

The use of amyloytic enzymes improved starch and protein digestion rates that were related with the disruption of granular architecture and the improvement of protein accessibility by digestive enzymes. Such increase was further enhanced by jet-cooking which in this case proved to be an effective method of improving the protein digestion whilst maintaining similar pGI values compared with its raw counterpart. Overall, the applied combination of the thermal and enzymatic method was effective and could be a useful alternative to produce novel pulse flours.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Pasting properties of chickpea flours.

Table S2. Retrogradation parameters of chickpea flours under Jet cooker and amylolytic enzymes processes.