Rheometric non-isothermal gelatinization kinetics of high hydrostatic pressure treated chickpea flour slurry

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ABSTRACT

The effect of high hydrostatic pressure (HHP) as a function of treatment pressure (200, 400, 600 MPa), temperature at pressurization (10, 25, 50 °C), and treatment time (5, 15, 25 min) on the subsequent temperature-induced gelatinization of chickpea flour (CF) slurry during non-isothermal heating from 25 to 95 °C was investigated. Temperature-induced gelatinization was sensitive to the changes in pressure, temperature, and time during HHP pre-treatment. During heating, CF gelatinization kinetics from the cross-over of elastic modulus (G') and viscous modulus (G'') to 95 °C were considered for rate estimation. Zero-order reaction kinetics adequately described the CF gelatinization process. Structure development rate (dG'/dt) is described by two exponential functions with activation energies ranging from 51.2 to 577 and –129 to 539 kJ mol⁻¹ for downward and upward gelatinization curves, respectively. Changes in dG'/dt vs. temperature seem to be closely related to the degree of gelatinization induced by HHP pre-treatment.

Keywords:

Chickpea flour

High pressure treatment

Elastic modulus

Gelatinization

Reaction kinetics

Activation energy

1. Introduction

Starch gelatinizes in a wide range of temperature in the presence of a sufficient amount of water, and thermal starch gelatinization at atmospheric pressure can start at anywhere between 55 and 80 °C (Ahmed, 2012; Stolt et al., 2001). Gelatinization – a thermal transition of hydrated starch – is an important phenomenon for cereal and legume starches and flours. This process disrupts the structure of the starch granules and causes swelling up to several times their original size. HHP treatment of starch in the presence of water can also induce its gelatinization (Bauer and Knorr, 2005; Oh et al., 2008a; Stute et al., 1996; Vallons and Arendt, 2009a, 2009b). However, when heating a barley starch suspension subjected to a pressure higher than 200 MPa, gelatinization takes place at a lower temperature range than when heating at atmospheric pressure (Stolt et al., 2001). In turn, a pressure above 400 MPa at excess water is e.g. not sufficient for potato starch gelatinization but shows effects for wheat starch (Stute et al., 1996). All the factors such as pressure, temperature at pressurization, treatment time, starch type, and other major components (protein and fiber) interact and contribute to the effect of pressure-induced gelatinization of starch. There are also significant differences in the structural and rheological properties between heated and pressurized starches (Vallons et al., 2014).

In addition to starch, CF slurries contain a relatively large amount of protein, which could give a second endothermic peak on heating in water (Meares et al., 2004). Ahmed et al. (2007) studied the effect of HHP treatment of basmati rice flour slurries and found both gelatinization of starch and denaturation of proteins. In turn, Ahmed et al. (2009) studied the thermal characteristics of HHP-treated lentil flour slurries at selected moisture levels and found no starch gelatinization peak during thermal scanning. In contrast, the protein denaturation temperature shifted with applied pressure. It was also assumed that gluten inhibited HHP-induced starch gelatinization, explaining the lower paste consistency of the wheat flour suspensions compared with the isolated starch (Vallons et al., 2010).

Gelatinization kinetics of starch has been studied extensively by different techniques. Most of the researchers have used differential scanning calorimetry (DSC) for the study where the degree of gelatinization is directly measured from gelatinization enthalpy (Ahmed et al., 2008). Other authors used either DSC or rheology to analyze the pressure-induced gelatinization process of sorghum and buckwheat starches, and to compare them to the effect of temperature (Vallons et al., 2009a,b). Poor correlation coefficients between gelatinization temperatures determined by DSC and rheology were found, which was explained by the different properties measured with the two methods. While a differential scanning calorimeter measures gelatinization as melting of

starch crystals, the rheometer measures gelatinization as an increase in viscosity. In turn, Ahmed (2012) stated that DSC measurement could not able to detect gelatinization temperature for all studied specimen and the endotherm depends upon starch to water ratio and some other factors. According to the author just cited, rheometric measurement (small amplitude oscillation shear (SAOS) measurement) has advantages over DSC and can successfully detect the gelatinization temperature in addition to the gel point during non-isothermal heating and provide reliable information on gelatinization reaction kinetics. Limited information is available on dynamic rheology to characterize the gelatinization process especially non-isothermal studies (Ahmed, 2012; Alvarez et al., 2014a; Yoon et al., 2004). In this work, rheometric measurement of pressurized CF slurries is focused to obtain the temperature induced gelatinization reaction kinetics which are limited in the literature.

Little information is in addition available concerning the impact of pressure on whole flour systems. Screening for variation in starch properties using flour, however, is difficult because of the presence of other seed components, in particular protein, lipid and fiber (Meares et al., 2004). It was observed that pressure combined with thermal treatment resulted in a significant reduction in CF paste rigidity as compared with unpressurized samples (Alvarez et al., 2014b). Elasticity of pressurized CF slurry increased significantly with increasing pressure applied in proportion with the extent of HHP-induced gelatinization of starch. Consequently, weaker pastes are formed in the subsequent heating process, because there is an increase in the amount of starch pre-gelatinized by pressure and pastes are formed solely by melting of the crystallites that still remain. The handling of CF pastes in terms of sheeting, flattening, rolling, and taking form is difficult due to the absence of gluten forming proteins. Therefore, a suitable selection of HHP treatment at appropriate levels can produce chickpea pastes that possess the desirable handling and machinability properties leading to preparation of products.

The objective of this work was to evaluate the combined effects of treatment pressure (0.1, 200, 400, and 600 MPa), temperature at pressurization (10, 25, and 50 °C), and treatment time (5, 15, and 25 min) on the subsequent temperature-induced gelatinization of CF slurry, with a view to providing information that can be translated to develop CF-based food products. Unpressurized and HHP-treated CF slurries at the different combinations were then pasted under non-isothermal (from 25 up to 95 °C) heating conditions and the kinetics of CF gelatinization were established by an oscillatory rheological approach.

2. Materials and methods

2.1. Materials

Spanish chickpea (*C. arietinum* ‘Castellano’) flour was a commercially available product donated by the Los Pisones flour milling company (Zamora, Spain). CF was supplied packed in polyethylene pouches (500 g) and was stored in watertight containers (10 °C and $73 \pm 3\%$ relative humidity) until use. Mean values for proximate analysis (g 100 g⁻¹) of CF samples (as analyzed by the AOAC method, 1984) were: moisture, 8.49 ± 0.34 , total ash, 2.77 ± 0.24 , and crude protein ($N \times 6.25$), 20.64 ± 0.05 .

2.2. Sample preparation

CF slurries were prepared at a concentration of 1:5 flour-to-water ratio. The required amounts of CF and distilled water were placed in a 250-ml beaker, hand-mixed with a glass rod, and kept for half an hour at room temperature (25 ± 1 °C) for hydration with stirring at 900 rpm before subjecting each sample to the HHP treatments.

2.3. High hydrostatic pressure treatment

CF slurries (200 ml) were vacuum packaged in a very low gas permeability bag type, Doypack® (Polyskin XL, Flexibles Hispania, S.L.). HHP treatment was performed using a Stansted Fluid Power Iso-lab 900 High Pressure Food Processor (Model: FPG7100:9/2C, Stansted Fluid Power Ltd., Harlow, Essex, UK), with 2925 ml capacity, maximum pressure of 900 MPa, and a potential maximum temperature of 100 °C. Four packed samples were inserted simultaneously into the pressure unit filled with pressure medium (water), then treated at pressures of 200, 400, or 600 MPa, and compared with untreated sample. The pressure was increased at a rate of 500 MPa min⁻¹ and maintained at the desired level for holding times of 5, 15, and 25 min; the decompression time was less than 4 s. The temperature of the pressure unit vessel was thermostatically controlled at 10, 25, and 50 °C throughout the different treatment combinations. Pressure, temperature, and time were controlled by a computer program, being constantly monitored and recorded during the process. Increases of up to a maximum of $8 \text{ °C} \pm 1 \text{ °C}$, $13 \text{ °C} \pm 2 \text{ °C}$, or $17 \text{ °C} \pm 2 \text{ °C}$ at 200, 400, or 600 MPa, respectively, due to compressive heating, were observed in the temperature of the pressuring fluid, but equilibrated at $10, 25, \text{ and } 50 \pm 2.5 \text{ °C}$ during the holding period. Average adiabatic heating during pressurization was $\sim 3.4 \text{ °C } 100 \text{ MPa}^{-1}$. After HHP treatment, samples

were immediately stored in a refrigerator at 4 °C for 24 h before further use. All the HHP treatments were performed twice (two batches).

2.4. Rheological measurements

A Kinexus pro rotational rheometer (Malvern Instruments Ltd, Worcestershire, UK) was used to conduct SAOS measurements under non-isothermal heating conditions in combination with a concentric cylinder geometry (C25 DIN SW1009 SS: PC25 DIN CO155 AL), and a solvent trap was used to minimize moisture loss during tests. Sample temperature was internally controlled by a Peltier system (−40 to 200 °C with an accuracy of ± 0.1 °C) attached to a water circulation unit. Following an initial equilibration of CF slurry for 2.5 min at 25 °C, temperature sweep pasting profiles were recorded from 25 to 95 °C at a heating rate of 2 °C min^{−1} and at a frequency (f) of 1 Hz, with the amplitude of the periodic shear stress (σ) ranging between 0.01 and 1 Pa during heating to guarantee the existence of linear viscoelastic (LVE) response. During pasting profiles, values of elastic modulus (G' , Pa) and viscous modulus (G'' , Pa) were recorded. Moreover, several parameters that provide information about gelatinization characteristic were also obtained from the temperature ramps: the onset elastic modulus at 25 °C (G'_{initial} , Pa), the starting gel point temperature (T_{gel} , °C), considered as the temperature where there was cross-over of G' and G'' , the temperature where G' achieved its maximum value ($T_{G'_{\text{max}}}$, °C), and the G'_{max} (Pa) value itself. All rheological measurements were carried out in triplicate for each batch (six replicates were measured), and rheological properties were obtained directly from the manufacturer-supplied computer software (rSpace for Kinexus v. 1.40, Malvern Instruments Ltd., Worcester, UK).

2.5. Statistical analysis

To examine the impact of pressure, temperature, and treatment time on the gelatinization kinetics of HHP-treated CF slurry during non-isothermal heating, a three-way analysis of variance (ANOVA) with interactions was used, including the unpressurized sample. Additionally, to establish the effect of pressure at constant temperature and treatment time, the effect of treatment temperature at constant pressure and treatment time, and the effect of treatment time at constant pressure and temperature, three one-factor ANOVAs were performed separately for the different combinations of factors. Minimum significant differences were calculated by Fisher's least significant difference (LSD) multiple range tests at a significance level of 0.05. Furthermore, in order to

obtain a preliminary view of the relationship between variables, Pearson product-moment correlations were determined with significance levels based on the Student's distribution. Statistical analyses were carried out using the SPSS 19.0 statistical software package (SPSS, Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Kinetic modeling of rheological data during temperature-induced gelatinization of HHP-treated CF slurry

The pasting profile for the unpressurized CF slurry is shown in Fig. 1. It can be observed that for untreated sample the starting gel point temperature (T_{gel}) was reached at ~75 °C, confirming previous findings (Alvarez et al., 2014a). The T_{gel} , considered as the temperature where there was cross-over of G' and G'' , generally occurs due to early stage of gelatinization of starchy foods (Ahmed et al., 2008). After gel point, the G' and G'' values continued to develop up to 95 °C; more extensive granule swelling and amylose solubilization is responsible for a rapid increase in consistency of the starch–water mixture (termed pasting). A maximum paste viscosity is obtained during heating when there is a majority of swollen granules (Thomas and Atwell, 1999), whereas upon further heating the granules begin to break down and amylopectin solubilization increases, resulting in a decrease in consistency.

The non-isothermal kinetic is based on a combination of the Arrhenius equation and the linear time-temperature relationship (Rhim et al., 1989). The kinetic equation can be converted to Eq. (1) in terms of rheological parameters (G' and dG'), as described by Ahmed et al. (2008) and Yoon et al. (2004):

$$\frac{dG'}{dt} = k_0 G'^n \exp\left(-\frac{E_a}{RT}\right) \quad (1)$$

where G' (Pa) and dG'/dt (Pa s⁻¹) represent the reactant concentration and the change in concentration at time t (s), k_0 the frequency factor (Pa s⁻¹), n the order of the kinetic reaction, E_a the activation energy (kJ mol⁻¹), T the absolute temperature (K), and R the universal gas constant (8.314 J mol⁻¹ K⁻¹).

Eq. (1) has been used successfully by other authors to describe the n^{th} -order reaction kinetics for starch gelatinization and protein gelation (Ahmed et al., 2008; Yoon et al., 2004), and the dG'/dt measures the structure development rate (Yoon et al., 2004).

The Appendix A. Supplementary data illustrates the detailed description of the kinetic modeling developed to obtain the kinetic parameter values shown. In this study, it was observed that the Eq. (1) was not sufficient to fully reflect the actual evolution of the gelatinization process taking place in the HHP-treated CF slurries under non-isothermal heating. In contrast, the structure development rate was better described as the sum of two reaction processes (Eq. 2), each one in the form of Eq. (1) for the case of $n = 0$. In turn, the constants a , b , c , and d from the Eq. (2) were determined in each process by nonlinear regressions, all being positive values, with one exception as explained below.

$$\frac{dG'}{dt} = \left(\frac{dG'}{dt}\right)_1 + \left(\frac{dG'}{dt}\right)_2 = ae^{bx} + ce^{-dx} \quad (2)$$

Consequently, the results led to the proposition of a multistep mechanism for the temperature-induced gelatinization of HHP-treated CF slurry, and the theoretical n value of the reaction kinetics was determined to be zero, i.e., the reaction rate is independent of the G' value. Alvarez et al. (2014a) also found that the temperature-induced gelatinization of CF slurry under non-isothermal conditions was well described by zero-order reaction kinetics when logarithmic fits were used to approximate the change of G' vs. time. In contrast, temperature-induced starch gelatinization process of mung bean starch followed first-order kinetics well, i.e. $n = 1$ (Ahmed, 2012). On the other hand, second-order reaction kinetics has been reported for protein gelation in a wider temperature range from 20 to 80 °C (Yoon et al., 2004). The differences with previous work in the n value could be explained by the combined effects of HHP and temperature treatments considered in this study.

In turn, Figs. 2 and 3 show the changes in both G' and dG'/dt vs. temperature for four different CF slurries: untreated (Figs. 2a, 2c), pre-treated with 200 MPa at 50 °C for 5 min (Figs. 2b, 2d), pre-treated with 400 MPa at 25 °C for 5 min (Figs. 3a, 3c), and pre-treated with 600 MPa and 50 °C for 25 min (Figs. 3b, 3d). For the samples pre-treated with 0.1, 200 and 400 MPa, a continuous increase in G' can be observed with increasing temperature from the T_{gel} up to 95 °C (Figs. 2a, 2b, 3a). In addition, for the unpressurized CF slurry, dG'/dt decreases with temperature (Fig. 2c), and exhibits a minimum value (T_0) that is followed by a continuous increase upon further heating. CF slurries pre-treated with 200 MPa at 50 °C for 5 min (Fig. 2d) and with 400 MPa at 25 °C for 5 min (Fig. 3c) followed the same pattern, although the increase after T_0 was smaller with increasing pressure, probably in proportion to the extent of HHP-induced starch gelatinization (Alvarez et al., 2014b). T_0 was considered as the end point for the first downward curve and the beginning of the second curve, being the inflection point of the experimental curve dG'/dt vs. temperature. Indeed, as stated by Ahmed et al.

(2013) for the case of the change of G' vs. temperature during non-isothermal heating of blended dough, the curve above the T_0 resembles the gelatinization similar to DSC curve.

On the other hand, the T_0 values increased with increasing pressure up to 400 MPa (Figs. 2c, 2d, 3c). As a result, when heating pre-treated CF slurries subjected to 200 and 400 MPa, at the various temperature and time combinations, the second phase of the gelatinization process took place in a narrower temperature range than when heating unpressurized CF slurries. As pre-treatment with HHP changed the granule structure and crystallinity in varying degrees, it changed the behavior of CF slurries upon subsequent pasting. Further evidence of this is that the ratio between the $G'_{\max}$ value and the onset elastic modulus (G'_{initial}) value also decreased with increasing pressure, being almost 10 for the untreated case (Fig. 2a), and 8.7 and 6.1, respectively, for the 200 and 400 MPa pre-treated CF slurries shown (Figs. 2b, 3a). This could enable a better control of the process, e.g. if the aim is to produce swollen starch granules possessing a certain degree of crystallinity (Stolt et al., 2001). Figs. 2 and 3 show that the extent of gelatinization achieved during the pressurization and, thereby, the rheological properties of the pressurized CF slurry upon subsequent pasting were highly dependent on the processing conditions (pressure, temperature, and holding time) used.

Rubens et al. (1999) studied the gelatinization of starches under pressure in situ with Fourier-transform infrared spectroscopy. Analysis of the spectra led to the proposition of a multistep gelatinization mechanism, similar to the temperature-induced gelatinization. A two-step mechanism of HHP-induced gelatinization was further confirmed by Blaszcak et al. (2005a), who studied the gelatinization of potato starch treated at 600 MPa. This two-step mechanism was also in agreement with the study of Ahmed et al. (2013), who observed similar two phases for the temperature-induced gelatinization of wheat flour dough incorporating water insoluble date fiber. In that study G' increased abruptly with temperature, exhibiting a peak value at $\sim 67^\circ\text{C}$, which was followed by a continuous decrease up to 95°C . Therefore the authors divided the complete gelatinization curve (G' -temperature) into two parts: up curve (pre-gelatinization, swelling dominant) and down curve (gelatinization, disruption and leaching of the amylose, as well as amylopectin breakdown). In this study, for unpressurized and 200 and 400 MPa pre-treated CF slurries (Figs. 2a, 2b, and 3a), it is possible to see an inflection point in the increase of G' vs. temperature, again indicative of the existence of two phases.

In contrast, it was found that after pressurization at 600 MPa and 50°C for 25 min, G' increased markedly up to 82°C (Fig. 3b), followed by a drop in magnitude. As a consequence, for this CF slurry, the dG'/dt was positive up to $T_{G'_{\max}}$ (82°C), where it became negative (Fig. 3d). In addition, dG'/dt increased only slightly after reaching the inflection point, and then dropped continuously in the temperature range considered for the

adjustment. Note that, for this exceptional case, only data from the cross-over of G' and G'' up to 90 °C were considered to model the change of the derivative of G' with respect to time, because between 90 and 95 °C the G' values initially increased slightly and then continued decreasing (Fig. 3b). At first, this change in the behavior of CF slurry pre-treated with 600 MPa and 50 °C for 25 min may possibly indicate that complete starch gelatinization was induced by this more severe HHP treatment. A previous study also showed that complete starch gelatinization (100%) was associated with CF slurries at the same concentration after HHP with 600 MPa at 25 °C for 15 min (Alvarez et al., 2014b). Nevertheless, likely protein denaturation, aggregation or gelation during pressurization as well affected the posterior behavior of this sample. It has been stated that HHP treatment changes the conformation and coagulation of proteins by opening the native structures, resulting in denaturation and aggregation (Ahmed et al., 2009). According to those authors is possible to denature the protein components of lentils completely by a combination of pressure and elevated temperature. Analysis of proteins extracted in different buffers also revealed that pressures higher than 200 MPa induced the formation of urea-insoluble complexes, disulphide bonds and/or other strong protein aggregates in chickpea batters, although the extent of protein modification was dependent on the applied pressure (Angioloni and Collar, 2013).

It seems obvious that for CF slurry pre-treated with 600 MPa and 50 °C for 25 min, the fit approximating the change in dG'/dt vs. temperature could be reduced to a single exponential (Fig. 3d). This result can be interpreted as indicating that only one reaction occurred, in response to a cumulative effect of higher pressure, temperature, and treatment time on starch gelatinization, unfolding of proteins, and from the combination of both components. However, for purposes of comparison, it was also decided to represent this case by a sum of two exponential functions. From the sum of the two reaction processes, the corresponding activation energies for the downward (E_{down}) and upward (E_{aup}) dG'/dt vs. temperature curves were obtained for all the cases (see Appendix A. Supplementary data).

The goodness of fit for Eq. (2) was very high, with the coefficients of determination (R^2) and standard errors (SE) ranging between 0.985 and 1.000, and between 0.017 and 0.126, respectively. As could be expected from the shape of the curves (Figs. 2, 3), the worst fits of experimental data for Eq. (2) corresponded to the CF samples HHP-treated at 600 MPa and 50 °C for 25 min. The values obtained for constants a , b , c , and d have been omitted for the sake of brevity. Nevertheless, the a and c values ranged from 0.021 to 0.920 and from – 0.122 to 0.285, whereas the b and d values varied from 24.8 to 192.1 and from 20.8 to 180.4, respectively.

3.2. Effect of pressure, temperature, and treatment time on kinetic and gelatinization parameters of HHP-treated CF slurry under non-isothermal heating

The three-way mixed-model ANOVA showed significant ($P < 0.05$) pressure and temperature main effects for the three derived kinetic parameters (T_0 , E_{adown} , E_{aup}), as well as for the total activation energy of the gelatinization process ($E_{\text{adown}} + E_{\text{aup}}$) (Table 1). Treatment time was not significant for E_{adown} and E_{aup} values. In addition, between kinetic parameters, pressure, temperature, and time had a more significant effect on the T_0 values. ANOVA also showed that both pressure and treatment time main effects were a significant ($P < 0.05$) source of variation for all gelatinization parameters derived from the pasting profiles (G'_{initial} , T_{gel} , $T_{G'_{\text{max}}}$, and G'_{max}). Temperature at pressurization had a non-significant effect for the temperature where G' achieved its maximum value ($T_{G'_{\text{max}}}$). Nevertheless, except for the onset elastic modulus at 25 °C (G'_{initial}), pressure had a much more significant effect on both kinetic and gelatinization parameters (higher F values), followed by temperature and treatment time (in that order). With regard to interactions, pressure $\times$ temperature, temperature $\times$ time, and pressure $\times$ temperature $\times$ time interactions were also significant for all kinetic and gelatinization parameters. Consequently, the effect of pressure was dependent on both temperature at pressurization and treatment time, corroborating previous findings. Stolt et al. (2001) showed that in barley starch the same degree of gelatinization can be brought about by different pressure-time combinations, i.e. lower pressures can, to a certain extent, be compensated for by longer treatment times. According to Bauer and Knorr (2005), at constant temperature and time, the degree of gelatinization increased with increasing pressure. Besides, the higher the temperature, the lower the pressure of complete gelatinization was in the temperature range investigated (29 - 57 °C), and at constant temperature and pressure the degree of gelatinization of potato, wheat, and tapioca starch suspensions increased with increasing treatment time between 5 and 60 min.

Tables 2 and 3 show the mean values of kinetic and gelatinization parameters, respectively, for the CF slurries HHP- treated at the various combinations of the three factors, where values for unpressurized sample are given as a reference, but they were not included for statistical analyses. The E_{adown} and E_{aup} values for the untreated case were 248 kJ mol⁻¹ and 174 kJ mol⁻¹, respectively (Table 2), evidencing that for temperature-induced gelatinization of unpressurized CF slurry a higher activation energy is required for granule swelling than for leaching of solubilized amylose/amylopectin. In turn, for pressurized samples the magnitude of the activation energy varied from 51.2 to 577 kJ mol⁻¹ and -129 to 539 kJ mol⁻¹ for downward (E_{adown}) and upward (E_{aup}) gelatinization curves, respectively. The lowest E_{adown} and E_{aup} values were obtained for CF slurries treated with

600 MPa at 50 °C for 25 min. For temperature-induced gelatinization and from experimental data of G' vs. temperature, the magnitude of E_a varied from 129 to 175 kJ mol⁻¹ and 169 to 194 kJ mol⁻¹ for upward and downward curves, respectively (Ahmed et al., 2013). As stated by Alvarez et al. (2014a), the different methods used by authors for calculating derivatives of experimental data probably influence the E_a values reported in the literature, and therefore comparing different studies is difficult.

In addition, generally T_0 was higher for pressurized CF slurries as compared with unpressurized sample (Table 2). Except for HHP treatments at 600 MPa and 50 °C for 15 and 25 min, at constant temperature and treatment time, T_0 values tended to increase with increasing pressure. Both pressure and temperature pre-treatments made buckwheat starch granules more resistant to destruction by further heating, as indicated by a higher temperature where the complex modulus (G^*) reached a maximum (Vallons & Arendt, 2009b). However, in whole CF slurry, protein may contribute to the pressure effect on the T_0 values. HHP promoted batter structure rearrangements in chickpea and green pea by altering the folding/unfolding and aggregation/disaggregation equilibrium of proteins (Angioloni and Collar, 2013). The author just cited showed that pressures higher than 350 MPa downward shifts gelatinization temperatures in chickpea and green pea batters regardless the hydration level. Likewise, a significant increase in E_{adown} was observed with increasing pressure for CF slurries pre-treated at 10 °C for 5 min and at 25 °C for 5, 15, and 25 min. Gelatinization enthalpies of HHP-treated CF slurries reflected progressive gelatinization as the pressure level increased from 150 to 600 MPa at 25 °C for 15 min, i.e., the enthalpy decreased with increasing pressure applied (Alvarez et al., 2014b). Consequently, it seems logical that higher minimum energy is required to start the swelling (pre-gelatinization) of an increasingly smaller amount of remaining ungelatinized starch granules with increasing pressure. For the remaining temperature and treatment time combinations, the E_{adown} values also increased between 200 and 400 MPa. However, E_{adown} decreased significantly after treatment with 600 MPa at 10 and 50 °C for both 15 and 25 min, the decrease being more significant at the higher temperature. This result might reflect the presence of an appreciable number of swollen starch granules in the CF slurries after these HHP treatments at 600 MPa, which prevent further swelling.

According to Oh et al. (2008a), in normal rice starch the degree of swelling did not change until the treatment pressure was greater than 300 MPa, and then increased rapidly as the treatment pressure increased up to 500 MPa. The maximum degree of swelling was approximately 50%. Waxy rice starch showed a minor increase in the degree of swelling at treatment pressures below 300 MPa. The degree of swelling then increased very sharply between 300 and 400 MPa and reached 100% at 400 MPa. Pasting profiles of sorghum flour-water

suspensions showed complete pressure-induced gelatinization of starch after treatment with 600 MPa, as no increase in consistency was observed for these samples upon subsequent pasting (Vallons et al., 2010). Moreover, after 600 MPa at 10 and 25 °C for 5, 15 and 25 min, as well as at 50 °C for 15 min, CF slurries had a significantly higher E_{aup} than after 200 and 400 MPa. In turn, this result would reflect a higher energy requirement with increasing pressure for the disruption and release of the amylose of more resistant granules. In contrast, E_{aup} decreased significantly after 600 MPa at 50 °C for 25 min as compared with 200 and 400 MPa, exhibiting a negative value. Therefore, it is possible that this severe HHP treatment would result in complete breakdown of the granules in CF slurry. In addition, this result could be due to the aggregation and precipitation of the protein caused by extreme HHP conditions. A chickpea protein isolate was HHP pre-treated before hydrolysis at increased pressure (100–600 MPa), and the hydrolysis rate increased significantly with pressure above 300 MPa (Zhang et al., 2012).

On the other hand, at constant pressure and holding time the T_0 values increased and decreased significantly with increasing temperature from 10 to 50 °C for samples HHP-treated at 400 MPa for 5 min and at 600 MPa for 15 and 25 min, respectively. However, the effect of treatment temperature on the E_{adown} and E_{aup} values was much more pressure- and time-dependent. In turn, at constant pressure and temperature, samples HHP-treated at 200 MPa and 10, 25, and 50 °C and at 400 MPa and 10 °C showed a significant increase in E_{adown} values with increasing holding time from 5 up to 25 min, whereas the contrary was true for the samples HHP-treated at 600 MPa and 10 and 50 °C. For the CF slurries HHP-treated at 200 MPa and 10 °C, an increase in time also significantly reduced the E_{aup} values, reflecting a decrease in the energy required for the melting of the amylopectin crystals accompanying the increase in energy required by swelling. The different E_{adown} and E_{aup} values obtained for the HHP-treated CF slurries would appear to be due to a shift in the balance between swelling and amylose leaching accompanied by amylopectin breakdown. Similarly, for the samples HHP-treated at 600 MPa and 25 °C the E_{aup} values decreased with increasing treatment time, again indicating that pressurization at 600 MPa and 25 °C, even at the shortest holding time, could be associated with a high amount of gelatinized granules.

However, by considering the E_a of the complete gelatinization process, i.e., by adding the E_a corresponding to both downward and upward curves, it can be observed that the total E_a increased with increasing holding time after pre-treatment with 200 and 400 MPa at 10 and 25 °C, while for 600 MPa at the same temperatures the total E_a was significantly lower when the samples were pressurized for 25 min. At 200 MPa and 50 °C, the total E_a also increased with treatment time, whereas the contrary was true after pressurization at both 400 and 600 MPa

and 50 °C. The highest E_a values of the complete gelatinization process corresponded to CF slurries pre-treated with 600 MPa at 25 °C for 5, 15 and 25 min, and, except for the samples pressurized at the highest levels of the three factors (600 MPa and 50 °C for 25 min), in all cases they were higher than the total E_a obtained for the untreated case. Clearly, other additional structural features seem to be responsible for the altered shape of the curves and activation energy values obtained for samples pre-treated with 600 MPa and 50 °C for 25 min.

Buckwheat starch–water suspensions were pre-treated with either pressure or temperature and subsequently analyzed for changes in their properties produced by a heating process, using either calorimetric measurements or rheology (Vallons and Arendt, 2009b). The melting enthalpy, the number of no-birefringence-showing granules, and the onset G^* all gave information on the amount of gelatinized granules due to the pre-treatment. Similarly, in this study, all the pressurized CF slurries were subsequently subjected to a heating process up to 95 °C, which caused an additional temperature-induced gelatinization of the remaining ungelatinized starch granules.

It is thought that both the E_{adown} and the E_{aup} values obtained for the various pressurized samples could similarly be related to the degree of gelatinization induced by HHP pre-treatment (as also indicated by the shape of the curves of structure development rate; Figs. 2, 3). Pressure has been shown to induce starch gelatinization with preservation of granular structure, restrained amylose release from starch granules, and limited retrogradation (Douzals et al., 1998; Stolt et al., 2001; Vallons and Arendt, 2009a, 2009b). This statement is corroborated by a higher total E_a requirement for the subsequent temperature-induced gelatinization of pressurized samples.

Clearly, other additional structural features seem to be responsible for the altered activation energy values obtained for samples pre-treated with 600 MPa and 50 °C for 25 min. Using DSC, both starch and protein peaks could be distinguished clearly, when a sample size of 4.5 mg of CF (~10% moisture) in 23 mg of water was used (Meares et al., 2004). Temperatures of starch peak gelatinization and protein denaturation were found to be in the ranges 75–90 °C and 100–120 °C, respectively. It can be expected, because of the temperature difference in starch and protein conformation disruption, that the developing elasticity (G') after HHP-treatments will firstly be based on starch gelatinization and that protein denaturation will have an input later and at higher pressures, temperatures, and times, explaining the different shape of the structure development rate curves, as well as the lower kinetic parameter values obtained for the CF slurries HHP-treated at 600 MPa and 50 °C for both 15 and 25 min. Meares et al. (2004) reported that, within CF, there is also a small amount of lipid in the flour, which can interact with amylose in starch to give a reversible endothermic peak on heating.

In turn, the $G'_{\max}$ obtained at 95 °C for the untreated case was 566 Pa (Table 3). For CF slurries HHP-treated with 200 MPa at 10 °C for 5 min, with 600 MPa at 10 °C for 15 and 25 min, and with 600 MPa at 50 °C for 25 min, the $G'_{\max}$ value was higher than that of the untreated case. For starches that were fully gelatinized when pressure treatment was applied (waxy rice, waxy corn, and tapioca starches), peak viscosity was not notably different from that of the untreated starch (Oh et al., 2008b). This is contrary to the findings of Katopo et al. (2002), who reported that the peak viscosity of pressure-treated waxy corn starch (with 690 MPa at 25 °C for 5 min) was lower than that of the untreated sample, as was also observed generally in this study. In addition, the author just cited also showed that the pasting properties of different pressurized starches changed depending on the structure of the starch.

According to Katopo et al. (2002) and Oh et al. (2008a), under pressure reactions are favored that involve a volume reduction. At pressures over 300 MPa, the reduction in volume is greater for a wheat starch suspension than for pure water at the same pressure (Douzals et al., 1998). This indicates that the water molecules linked with starch occupy a smaller volume than the molecules in pure water. After pre-treatment with 50 °C for 5, 15, and 25 min, the G'_{initial} values increased significantly with pressure (Table 3). Vallons and Arendt (2009a, 2009b) also observed an increase in onset complex viscosity (η^*) and onset complex modulus (G^*) with increasing amounts of pre-gelatinized granules from the pasting profiles of HHP-treated sorghum and buckwheat starch suspensions, respectively. By using initial viscosity (η_{initial}) as an indicator for the degree of gelatinization, Oh et al. (2008a) also showed that pressure treatments gelatinized normal rice starch and waxy rice starch to different extents depending on treatment pressure, duration, and temperature at treatment. The authors just cited observed that HHP treatment at 350 MPa was enough to increase the η_{initial} value of the waxy rice starch suspension approximately tenfold, from 0.007 (untreated) to 0.078 Pa s, followed by a further approximately tenfold increase after treatment with 375 MPa.

In this study, a much higher increase with increasing pressure occurred for the G'_{initial} value at the highest temperature and longest time (approximately sixty-fivefold as compared with the untreated case). At 600 MPa and 50 °C for 25 min, the protein denaturation input into the significant increase observed in G'_{initial} (up to 0.833 Pa) may be very high.

According to Oh et al. (2008b), partial gelatinization is observed when the η_{initial} of the treated starch suspension is higher than that of the untreated case, but under non-isothermal heating the suspension still exhibits an increase in viscosity when temperature increases above T_{gel} . However, the elastic modulus of pre-treated CF slurry after 600 MPa and 50 °C for 25 min subsequently increased with temperature up to 655 Pa

(Fig. 3b). At first, this result would appear to indicate that the starch had not been completely gelatinized by the HHP treatment at 600 MPa and 50 °C for 25 min. In contrast, samples of sorghum starch pre-treated at 500 and 600 MPa at 20 °C for 10 min showed no increase in viscosity, and therefore a complete gelatinization induced by HHP treatment was assumed (Vallons and Arendt, 2009a). Likewise, also after pressurization with 500 MPa at 20 °C for 10 min, gelatinization of buckwheat starch was nearly complete and only a very small increase in complex modulus occurred upon pasting (Vallons and Arendt, 2009b). Moreover, the onset complex modulus increased sharply between 300 and 500 MPa and exhibited a sigmoidal-shaped curve. In this study, denatured protein may interact with intact protein, or even with the starch, which also could result in increasing G'_{initial} .

Furthermore, at 10 °C for 5 min, at 25 °C for 15 and 25 min, and at 50 °C for 15 min, T_{gel} increased significantly with pressure (Table 3). However, at 10 °C for 15 and 25 min, at 25 °C for 5 min, and at 50 °C for 5 and 25 min, T_{gel} increased between 200 and 400 MPa, but decreased to a minimum after pre-treatment with 600 MPa. According to the rheological measurements, the onset gelatinization temperature also decreased with increasing degree of pre-gelatinized sorghum starch (Vallons and Arendt, 2009a). There is not really a noticeable trend regarding the change in T_{gel} with pressure, temperature, and treatment time. In turn, CF slurries HHP-treated with 600 MPa had lower $T_{G'_{\text{max}}}$ values. In view of the fact that 600 MPa is high enough to complete denaturation, it should be taken into consideration that denatured protein may interact with the starch, which also could result in decreased T_{gel} and $T_{G'_{\text{max}}}$ values. According to Marques et al. (2006), by increasing the volume fraction and thus reducing the inter-particle distance the rheological signature is more rapidly detected once the swelling of the granules has started.

At 10 °C for 5 min and at 50 °C for both 5 and 15 min, G'_{max} decreased with increasing pressure. However, for the other combinations the values of G'_{max} only decreased when the pressure increased from 200 to 400 MPa, but increased when the pressure increased to 600 MPa, and the maximum elasticity values corresponding to the highest pressure were significantly greater than those corresponding to 200 MPa in the case of the treatments applied at 10 and 25 °C for 15 and 25 min. In contrast, the pasting curves for waxy rice, waxy corn, and tapioca starches that were pressure treated at 600 MPa did not show a peak viscosity because the initial viscosity was already equal to the peak viscosity (Oh et al., 2008b). A lower maximum complex modulus was observed for HHP pre-treated buckwheat starch suspensions compared with the control samples (Vallons and Arendt, 2009b). In this study, G'_{max} values decreased sharply between 200 and 400 MPa and increased between 400 and 600 MPa, indicating that there might be a critical pressure level between 400 and 600 MPa for the HHP-treated CF

slurries. In CF slurry, the formation of protein networks induced by HHP might in part explain the increase of the solid character observed in the samples pressurized at the highest pressure level.

With regard to treatment time effect, at constant pressure and temperature, samples HHP-treated at 200 MPa and 10, 25, and 50 °C showed a decrease in $G'_{\max}$ (Table 3) with increasing treatment time from 5 up to 25 min, resulting in a reduction in CF paste, confirming that HHP-induced gelatinization is also a time-dependent process (Bauer and Knorr, 2005, Stolt et al., 2001). However, as mentioned previously, for the slurries pre-treated at 200 MPa and 10 °C for 5 min, the $G'_{\max}$ value (749 Pa) was noticeably higher than the value achieved by the unpressurized sample, indicative of its higher ability for temperature-induced gelatinization even after pressurization at the lowest level. Similarly, CF samples containing a flour-to-water ratio of 1:5 that were pre-treated at 150 MPa (at 25 °C for 15 min) and then heated under isothermal conditions up to 75 and 90 °C had significantly higher G' and G'' values than the unpressurized case, suggesting that HHP-induced melting of granules started at pressures > 150 MPa (Alvarez et al., 2014b). According to Oey et al. (2008), the increase in elasticity of the samples treated at the lowest pressure and temperature and for the shortest time might be attributable to an increase in the linearity of the cell walls and volumes of particles owing to the permeability of the cell walls. A sigmoidally shaped gelatinization curve means that pressure-induced gelatinization occurs within a HHP range (Vallons et al., 2014), and that pressure has to be above a critical level for gelatinization to occur effectively (Oh et al., 2008a). In this study, the $G'_{\max}$ value would appear to indicate that at 10 °C the 200 MPa-induced melting of granules started at holding times > 5 min, even though the G'_{initial} values for the three times were higher than those obtained for the untreated sample.

Samples treated at 400 MPa and 10 °C showed a significant increase in T_{gel} and decrease in G' and G'' values with increasing treatment time, although without significant differences between starting gel point temperatures corresponding to 15 and 25 min. Similarly, using DSC to characterize gelatinization temperatures, an increase in the onset gelatinization temperature was observed with increasing degree of gelatinization (Vallons and Arendt, 2009a). In addition, a significantly lower $G'_{\max}$ value was observed after HHP treatment at 400 MPa for 15 min as compared with a sample pressurized for 5 min, although the $G'_{\max}$ value increased after 25 min, without significant differences between the parameter values obtained between 5 and 25 min.

In turn, treatment time had a significant effect on the T_{gel} and $G'_{\max}$ values of CF slurries treated at 600 MPa and 10 °C (Table 3). However, in this case, T_{gel} decreased significantly from 78.1 to 73.4 °C for 5 and 25 min, respectively. In turn, $G'_{\max}$ value increased significantly. This result could indicate an association between amylose and amylopectin induced by pressurization at 600 MPa and 10 °C, which produced a cross-linking-like

network that displayed resistance to shear force and increased viscosity (Katopo et al., 2002). For this treatment combination, the starch seemed to be partially gelatinized after the initial phase of pressure build-up, and increases in G' values detected during prolonged pressurization might be attributable to loss of crystallinity and loss of helicity of starch during or immediately after the HHP treatment at 600 MPa.

However, the extent of the pressure effect on the crystalline structure depends on the type of crystallinity. A- and C-type (as in the case of chickpea) starches are more sensitive to pressure than B-type starches (Rubens et al., 1999), but there seems to be a clear overlapping in the pressure range above 600 MPa (Stute et al., 1996). The sensitivity of the C-type starches is located in between B- and A-type starches: Bauer and Knorr (2005) showed that the phase gelatinization of tapioca starch (C-type) occurred at higher pressures than the phase change of wheat starch (A-type) but below the melting of potato starch. In addition, Hibi et al. (1993) reported a destruction of the A-type crystalline structure of corn starch during pressure treatment. The A-type X-ray diffraction pattern tended to change into a faint B-type pattern. Katopo et al. (2002) attributed the differences in pressure sensitivity between A- and B-type starches to their crystal structure.

In turn at 25 and 50 °C, when pressures of 200 and 400 MPa were applied to the slurries, only the $G'_{\max}$ values decreased significantly as treatment time increased. For 400 MPa and 50 °C, and for 600 MPa and 25 °C, the T_{gel} values also rose as the duration of pressurization increased, reflecting a delay in gelatinization of the starch as the volume fraction of the slurries increased. Presence of significant amount of proteins with sharp endothermic peak could hinder traceability of gelatinization chickpea starch peak. This observation is duly supported by previous studies on pressure treated rice and lentil flour dispersions (Ahmed et al., 2007, 2009).

As expected, after pressurization with 600 MPa at 50 °C, treatment time had a significant effect on the values of all the gelatinization parameters of the HHP-treated CF slurries. The lowest T_{gel} and $T_{G'_{\max}}$ and the highest G'_{initial} values were obtained for the samples HHP-treated at the longest time. According to Oh et al. (2008a), this result might reflect that a breakdown of granules caused an increase in elasticity and viscosity of samples HHP-treated at 600 MPa and 50 °C for 25 min with significant leaching of amylose, and therefore a higher extent of recrystallization occurring outside the starch granules. Similarly, pressure treatment at 650 MPa for 6 min resulted in a complete breakdown of granules in waxy corn starch, whereas high-amylose corn starch retained a granular structure (Błaszczak et al., 2005b).

Nevertheless, a number of factors could influence and alter the gelatinization and rheological parameters of CF slurries HHP-treated at 600 MPa and 50 °C for 25 min. The formation of protein networks induced by HHP might in part explain the increase of the solid character observed in this sample. Disulfide cross-linking of

proteins within rice granules has also been linked with altered pasting properties and waxy corn starches with elevated internal protein content (Hamaker and Griffin, 1993; Han et al., 2002). In that respect, HHP also provides the conditions that can modify the structural properties of proteins and contribute to their functionality. According to Vallons et al. (2014), difference in granule structure preservation between pressure- and temperature-induced gelatinization might be due to a shift in the balance between swelling and polymer cross-linking. On the other hand, we thought that CF slurries HHP-treated at 600 MPa and 50 °C for 25 min contained starch ghosts after heating, as a sticky surface film or coat enveloping this slurry was clearly perceptible by eye after pressurization under these conditions followed by temperature treatment. The surface regions of chickpea granules contain proteins and lipids providing potential for film formation. According to Debet and Gidley (2007), effective cross-linking of starch polymers by proteins present inside the granule takes place. Proteins have been visualized within ghosts from some starches, and there is evidence that they are involved in granule swelling and ghost integrity in mung bean starch (Oates, 1991). In this study, interestingly, CF slurry HHP-treated at 600 MPa and 50 °C for 25 min had a $G'_{\max}$ (655 Pa) value very similar to that obtained for the unpressurized case (Table 3).

3.3. Correlations between kinetic and gelatinization parameters of HHP-treated CF slurry under non-isothermal heating

For kinetic parameters, there was only a significant and positive correlation between the T_0 and E_{adown} values (Table 4). In the data corresponding to gelatinization parameters, the highest positive correlation was found between the two temperatures T_{gel} and $T_{G'_{\max}}$ ($r = 0.789$), whereas the highest negative correlation was found between G'_{initial} and $G'_{\max}$ ($r = -0.790$) in response to a higher degree of HHP-induced starch gelatinization. Among the kinetic parameters, both T_0 and E_{adown} showed a significant correlation with all the gelatinization parameters of the CF slurries. The highest negative correlations were found between T_0 and G'_{initial} ($r = -0.776$), and between E_{adown} and $G'_{\max}$ ($r = -0.485$), showing that the higher the energy requirement was for partial swelling of the remaining starch granules in the CF slurries, the lower the elasticity of the CF pastes was after heating. A high positive correlation was also found between the two temperatures T_0 and T_{gel} ($r = 0.653$). E_{aup} was less correlated with the gelatinization parameters. However, a negative correlation was also found between E_{aup} and $G'_{\max}$, reflecting that a higher energy requirement for breaking down of amylopectin crystallites was

accompanied by a decrease of the elasticity of the CF pastes upon further heating, owing to a higher degree of gelatinization induced by increasing pressure level.

4. Conclusions

A two-step mechanism for the heat-induced gelatinization process of HHP-treated CF slurry was observed from the change of the derivative of elastic modulus with respect to t (dG'/dt) vs. temperature. Activation energy values (E_{adown} and E_{aup}) derived for the two phases seem to be related to the degree of swelling (E_{adown}) and amylose leaching together with amylopectin breakdown (E_{aup}), both induced by the HHP treatment. The effect of treatment pressure, temperature at pressurization, and treatment time was detectable upon further heating. Although the effect of pressure was dependent on temperature and treatment time, the impact of pressure on CF gelatinization was much more significant. Increasing temperature at pressurization increased the degree of gelatinization of CF slurries pressurized at 400 and 600 MPa. In turn, the elasticity of CF slurries treated at 200 and 400 MPa and 10, 25, and 50 °C decreased with increasing holding time in proportion to the extent of HHP-induced gelatinization. Pre-treatment with 200 and 400 MPa, at constant temperature and treatment time, caused restricted swelling (as indicated by an increase in E_{adown} and decrease in G'_{max} values) and restricted granule disintegration (as indicated by a higher T_{gel}). In contrast, the interaction between higher HHP-induced starch gelatinization and protein denaturation might explain the significant increase in G'_{max} upon heating for CF slurries pre-treated at 600 MPa. Mainly, the results obtained for CF slurries pre-treated with 600 MPa and 50 °C for 25 min could be explained by various mechanisms and probably one mechanism is not the sole origin for the altered rheological properties. However, to obtain a more detailed understanding of this difference in behavior, further research is necessary between 400 and 600 MPa, as well as between 25 and 50 °C. The information gathered from rheological measurements needs to be related to other analyses to characterize the HHP-induced gelatinization of CF slurry bearing in mind the alterations caused in the conformation of other components such as protein and fiber. Overall, the results showed that CF starch granules with different degree of gelatinization can be obtained by HHP pre-treatment with 200 and 400 MPa at treatment temperature and time ranging from 10 to 50 °C and 5 to 25 min, respectively, to obtain CF-based products with easier handling properties (lower consistency).

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617

618 **Nomenclature**

619 *Symbols and abbreviations*

620 ANOVA analysis of variance

621 CF chickpea flour

622 DSC differential scanning calorimetry

623 dG' change of elastic modulus, Pa

624 dG'/dt derivative of elastic modulus with respect to time, Pa s⁻¹

625 E_a activation energy, kJ mol⁻¹

626 E_{adown} process activation energy for downward dG'/dt vs. temperature curve, kJ mol⁻¹

627 E_{aup} process activation energy for upward dG'/dt vs. temperature curve, kJ mol⁻¹

628 $\eta_{initial}$ initial apparent viscosity, Pa s

629 f frequency, Hz

630 $G'_{initial}$ onset elastic modulus at 25 °C before non-isothermal heating, Pa

631 G'_{max} maximum value of elastic modulus, Pa

632 G^* complex modulus, Pa

633 HHP high hydrostatic pressure treatment

634 k_0 frequency factor, Pa s⁻¹

635 LSD least significant difference

636 LVE linear viscoelastic range

637 n order of the kinetic reaction

638 ω angular frequency, rad s⁻¹

639 R universal gas constant, J mol⁻¹ K⁻¹

640 r correlation coefficient

641 SAOS small amplitude oscillation shear

642 σ shear stress, Pa

643 T absolute temperature, K

644	T_{gel}	starting gel point temperature in the rheogram (G' -temperature-time), °C
645	$T_{G'_{\text{max}}}$	temperature corresponding to the maximum value of elastic modulus G'_{max} in the rheogram (G' -
646		temperature-time), °C
647	T_0	temperature where dG'/dt achieved its minimum value, °C
648	t	time, s

649

650 **Appendix A. Supplementary data**

651

652 **References**

653

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