Effects of pressure, temperature, and treatment time on oscillatory rheological properties and non-isothermal gelatinization kinetics of chickpea (Cicer arietinum L.) flour slurry

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Abstract. The effect of high hydrostatic pressure (HHP), temperature at pressurization, and treatment time at three levels (200, 400, 600 MPa; 10, 25, 50 °C; 5, 15, 25 min) on the gelatinization of chickpea flour (CF) slurry during non-isothermal heating from 25 to 95 °C and gel rheological properties after subsequent cooling to 25 °C was investigated. 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 576.6 and −128.9 to 538.9 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 pretreatment.

1. Introduction
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 [1]. In addition to starch, CF slurries contain a relatively large amount of protein (20.64 g 100 g⁻¹ of flour), which could give a second endothermic peak on heating in water [2]. Differential scanning calorimetry (DSC) measurement could not detect gelatinization temperature for all studied specimen and the endotherm depends upon starch to water ratio and some other factors [3]. 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. A suitable selection of HHP treatment at appropriate levels can produce chickpea pastes that possess the desirable handling properties in terms of sheeting, flattening, rolling, and taking form leading to preparation of products. Differences
between pressure- and temperature-induced gelatinization offer benefits with respect to new product development [1].

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. Unpressurized and HHP-treated CF slurries 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. After heating, CF slurries were cooled to 25 °C and then evaluated for changes in their viscoelastic measurements.

2. Materials and Methods
2.1. Slurry used in the experiments
Spanish chickpea (C. arietinum ‘Castellano’) flour was a commercially available product donated by the Los Pinos flour milling company (Zamora, Spain). CF slurries were prepared at a concentration of 1:5 flour-to-water ratio.

2.2. 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). Four packed samples were inserted into the pressure unit filled with water, and treated at pressures of 200, 400, or 600 MPa. Pressure was increased at 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. Representations of the variation of pressure and temperature vs. time during HHP treatment carried out at 600 MPa and 25 °C for 15 min is shown in figure 1. Increases of up to a maximum of 8 °C ± 1 °C, 13 °C ± 2 °C, or 17 °C ± 2 °C at 200, 400, or 600 MPa, respectively, due to compressive heating, were observed in the temperature of the pressuring fluid, but they were transient and equilibrated at 10, 25, and 50 ± 2.5 °C during the holding period at those pressure levels. After HHP treatment, samples were immediately stored in a refrigerator at 4 °C for 24 h before further use.

![Figure 1](image.png)

**Figure 1.** Representation of the temperature and pressure vs. time variation during HHP treatment (600 MPa at 25 °C for 15 min).
2.3. Rheological measurements
A Kinexus pro rotational rheometer (Malvern Instruments Ltd, Worcestershire, UK) was used to conduct SAOS measurements using concentric cylinder geometry. Temperature sweeps 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 controlled shear stress at 1 Pa. Values of elastic modulus ($G'$, Pa) and viscous modulus ($G''$, Pa) were recorded. Onset elastic modulus at 25 °C ($G'_{\text{initial}}$, Pa), starting gel point temperature ($T_{\text{gel}}$, °C), considered as the temperature where there was cross-over of $G'$ and $G''$, temperature where $G'$ achieved its maximum value ($T_{G'_{\max}}$, °C), and $G'_{\max}$ (Pa) value itself were also obtained. After heating, the sample was immediately cooled down to 25 °C. Mechanical spectra at 25 °C in the linear region at variable angular frequency ($\omega$) from 1 to 100 rad s$^{-1}$ (~0.16 to 16 Hz) were also recorded.

3. Results and Discussion
3.1. Kinetic modeling of rheological data during non-isothermal heating
The non-isothermal kinetic is based on a combination of the Arrhenius equation and the time-temperature relationship [4]. The kinetic equation is converted in terms of rheological parameters ($G'$ and $dG'/dt$), as described by other authors [5-7]. However, in this study, it was observed that the structure development rate was better described as the sum of two reaction processes for the case of $n = 0$. The constants $a$, $b$, $c$, and $d$ from this sum of two reactions were determined in each process by nonlinear regressions. Therefore, the structure development rate ($dG'/dt$) is described by two exponential functions, and zero-order reaction kinetics adequately described the CF gelatinization process. A coming complete paper will illustrate the detailed description of the kinetic modeling developed to obtain the kinetic parameters. By representing both $G'$ and $dG'/dt$ vs. temperature, it was possible to appreciate a significant change in the profiles of the CF slurries as the pressure applied increased. Figure 2 shows the changes in both $G'$ and $dG'/dt$ vs. temperature (°C) for four different CF slurries: untreated (figs. 2a, 2e), pre-treated with 200 MPa at 50 °C for 5 min (figs. 2b, 2f), pre-treated with 400 MPa at 25 °C for 5 min (figs. 2c, 2g), and pre-treated with 600 MPa and 50 °C for 25 min (figs. 2d, 2h). For the samples pre-treated with 0.1, 200 and 400 MPa, a continuous increase in $G'$ was observed with increasing temperature from the $T_{\text{gel}}$ up to 95 °C. On the other hand, for the unpressurized CF slurry, $dG'/dt$ decreased with temperature, and exhibited a minimum value ($T_0$) that was followed by a continuous increase upon further heating. CF slurries pre-treated with 200 MPa at 50 °C for 5 min and with 400 MPa at 25 °C for 5 min 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. $T_0$ 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. In addition, generally $T_0$ was higher for pressurized CF slurries as compared with unpressurized sample (84.3 °C, fig. 2e). 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. Indeed, as stated by Ahmed et al. [8] 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. 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 at ambient pressure. As pre-treatment with HHP changed the granule structure and crystallinity in varying degrees, it changed the behavior of CF slurries upon subsequent pasting. In contrast, it was found that after pressurization at 600 MPa and 50 °C for 25 min, $G'$ increased markedly up to 82 °C, 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. 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 severe HHP treatment.
Figure 2. Variation of both $G'$ and $dG'/dt$ vs. temperature (from starting gel point temperature to the temperature where $G'$ achieved its maximum value) for: (a, e) unpressurized CF slurry; (b, f) CF slurry pre-treated at 200 MPa and 50 °C for 5 min; (c, g) CF slurry pre-treated at 400 MPa and 25 °C for 5 min; (d, h) CF slurry pre-treated at 600 MPa and 50 °C for 25 min. Mean values of six measurements ± error bars.
A previous study also showed that complete gelatinization (100%) was associated with CF slurries at the same concentration after HHP with 600 MPa at 25 °C for 15 min [9]. Nevertheless, likely protein denaturation, aggregation or gelation as well affected the behavior of this sample. From the sum of the two reaction processes, the corresponding activation energies for the downward ($E_{\text{adown}}$) and upward ($E_{\text{aup}}$) $dG/dt$ vs. temperature curves were obtained for all the cases. The goodness of fit for the two exponential functions 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, the worst fits of experimental data corresponded to the CF samples HHP-treated at 600 MPa and 50 °C for 25 min. The $E_{\text{adown}}$ and $E_{\text{aup}}$ values for the untreated case were 247.8 kJ mol$^{-1}$ and 173.5 kJ mol$^{-1}$, respectively, evidencing that for single temperature-induced gelatinization of unpressurized CF slurry a higher activation energy is required for granule swelling than for leaching of solubilized amylose/amylopectin. Remarkably, the lowest $E_{\text{adown}}$ and $E_{\text{aup}}$ values were obtained for CF slurries treated with 600 MPa at 50 °C for 25 min. Likewise, a significant increase in $E_{\text{adown}}$ 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. 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.

3.2. Effect of pressure, temperature, and treatment time on kinetic and gelatinization parameters of CF slurry under non-isothermal heating and on rheological properties of heat-induced CF gel after cooling to 25 °C

$G_{\text{max}}$ 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. On the other hand, after cooling down to 25 °C, pressure combined with heating resulted in reduction in CF paste rigidity. Increasing temperature during pressurization also increased the degree of gelatinization of slurries pressurized at 400 and 600 MPa, and consequently the CF gels induced subsequently had inferior mechanical strength. However, the temperature effect was more significant at constant pressure of 600 MPa for 15 min. The elasticity of CF slurry and gel treated with 200 and 400 MPa at 10, 25, and 50 °C decreased with increasing holding time during pressurization in proportion to the extent of HHP-induced gelatinization. This result corroborates that HHP-induced gelatinization is also a time-dependent process [10, 11].

In contrast, rigidity also increased at higher values of pressure, temperature and holding time. 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. 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 [12].

Between kinetic and gelatinization parameters and rheological properties, the highest positive correlation was found between $G_{\text{max}}$ and $G''$ ($r = 0.809$), evidencing that the higher the maximum value of elasticity reached during non-isothermal heating, the higher the viscosity of the gels was after cooling. However, correlations between $T_{\text{gel}}$ and $T_{\text{Gmax}}$ and $G''$ and $G''$, and $\eta^*$ were all significant but negative. The highest correlation was between $T_{\text{gel}}$ and $G''$ ($r = -0.567$), reflecting that the lower the onset temperature of gelatinization was, the higher the viscoelastic properties of the CF gels were.
4. Conclusion

A two-step mechanism for heat-induced gelatinization process of HHP-treated CF slurry was observed from the $dG'/dt$ 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. Although the effect of pressure was dependent on temperature and time, the impact of pressure on CF gelatinization was much more significant. The HHP-treated CF slurry exhibited solid-like behavior upon heating and cooling. Increasing temperature increased the degree of gelatinization of slurries pressurized at 400 and 600 MPa, and accordingly the CF gels induced subsequently had inferior mechanical strength. 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 $T_g$). An increased rapid retrogradation of leached amylose (as indicated by a higher increase in $G'$ values on cooling) due to disintegration might explain the significant increase in either $G'_{max}$ or $G'$ upon cooling for CF slurries pre-treated at 600 MPa. 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. Further research is necessary between 400 and 600 MPa, and between 25 and 50 °C. Overall, CF starch granules with different degree of gelatinization can be obtained by HHP pre-treatment with 200 and 400 MPa at 10 to 50 °C and 5 to 25 min, to obtain CF-based products with easier handling properties.

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References

[1] Vallons K J R, Ryan L A M and Arendt E K 2014 Crit. Rev. Food Sci. Nutr. 54 399-409
[2] Meares C A, Bogracheva T Y, Hill S E and Hedley C L 2004 Starch/Stärke 56 215-224
[3] Ahmed J 2012 J. Food Eng. 109 321-328
[4] Rhim J W, Nunes R V, Jones V A and Swartzel K R 1989. J. Food Sci. 54 446-450
[5] Ahmed J, Ramaswamy H S and Alli I 2008 Food Hydrocoll. 22 278-287
[6] Yoon W B, Gunasekaran S, Park J W 2004 J. Food Sci. 69 E338-E343
[7] Alvarez M D, Fuentes R, Olivares M D, Cuesta F J, Canet W 2014 J. Food Eng. 136 9-18
[8] Ahmed J, Almusallam A S, Al-Salman F, AbdulRahman M H, Al-Salem E 2013 LWT-Food Sci. Technol. 51 409-416
[9] Alvarez M D, Fuentes R, Olivares M D, Canet W 2014. Innov. Food Sci. Emerg. Technol. 21 12-23
[10] Bauer B A, Knorr D 2005 J. Food Eng. 68 329-334
[11] Stolt M, Oinonen S, Autio K 2001 Innov. Food Sci. Emerg. Technol. 1 167-175
[12] Han X Z, Campanella O H, Guan H, Keeling P L, Hamaker B 2002 Carbohydr. Polym. 49 323-330