QUINOA WET-MILLING: EFFECT OF STEEPING CONDITIONS ON STARCH RECOVERY AND QUALITY

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Abstract

Cereal starches play an important role in the food and non-food industries because of their low cost, availability, and ability to impart a wide range of techno-functional properties. The main objective of this research was to isolate starch, germ, protein, and fiber components from quinoa by a wet-milling procedure. The effect of steeping time and temperature on starch recovery and its quality was investigated. The quinoa steeping conditions, such as time (1, 5, and 9 hours) and temperature (30, 40, and 50 °C), in SO_2 solution with lactic acid were investigated using a 3^2 factorial design in order to optimize the starch separation and its quality. The effect of steeping conditions on starch was evaluated in terms of whiteness, protein, lipid, amylase, and damaged starch contents, as well as thermal and pasting properties. Results showed how the different steeping times and temperatures affected the fraction yields and starch recovery and its quality. Optimization of the wet-milling process used in this study produced the highest starch recovery level and best starch quality after 6.5 hours of steeping at 30 °C. Experimental values were close to the predicted ones, with an error below 2% for all attributes tested.

Keywords: quinoa; wet-milling; steeping; starch recovery; starch quality; thermal and pasting properties

Abbreviations:

ΔH_G, enthalpy of gelatinization; ΔH_R, enthalpy of retrogradation; a^*, redness to greenness; a_1 is the main effect if x_1; a_{12} is the mixed coefficient that represents the interactions between factors; a_2 is the main effect of x_2; a_{ii} are the square coefficients that indicate if any of the variables has a maximum or minimum in the experimental domain; b^*, yellowness to blueness; CPV, final or cool paste viscosity; DSC, Differential Scanning Calorimetry; g, grams; h, hour, HPV, hot paste viscosity; L^*, lightness; P_{temp}, pasting temperature; P_{time}, peak time; PV, peak viscosity; rpm, revolutions per minute; RS-Q, adjusted square coefficient of the fitting model; RVA, Rapid Visco Analyser; SW, leached solids in steepwater; T_c, conclusion temperature; T_o, onset temperature; T_p, peak temperature; WI, whiteness index; WQF: whole quinoa flour; WW, solids in washing water; x_1 is the design factor steeping time; x_2 is the design factor steeping temperature; Y_{calc}, data from the model; Y_{obs}, the experimental data; ε is the difference between the experimental data and the model, the residual.

1. Introduction

The primary sources of carbohydrates for the global population are cereals and pseudocereals. Pseudocereals are essentially starch crops; however, they may contain significant quantities of protein and oil, and these constituents frequently determine their suitability for a specific end use. In particular, quinoa is a pseudocereal native to South America, mainly from Peru, Bolivia, Argentina, Colombia, Ecuador, and Chile, but in recent decades other countries such as the United States, Canada, Italy, France, Spain, England, and Sweden have also become producers (Bazile and Baudron, 2015). Structurally, quinoa is composed of three main parts: the perisperm, the embryo or germ, and the pericarp or seed hull (Reguera and Haros, 2017). The perisperm is the primary starch storage portion, the germ is the lipid storage portion, and finally the hull, also called bran, consists mainly of cellulose and hemicellulose. The physicochemical and functional properties of the main components of quinoa, starch, fiber, and protein, are widely described in the literature (Koizol, 1992; Schoenlechner et al., 2010; Kurek, et al., 2018). The objective of milling is to obtain intermediate products that can be used subsequently in the manufacture of other products. Normally, milling schemes are classified as dry- or wet-milling. In dry-milling the aim is to separate the anatomical part of the grain to produce mainly flour, whereas the purpose of wet-milling is to separate the chemical components of the grain, such as starch, proteins, fiber, and lipids, to obtain the purest possible fraction of each component (Haros and Wronkowska, 2017). The main cereal used in wet-milling is corn (maize). In conventional wet-milling, corn is steeped in an aqueous solution containing sulfur dioxide (0.1–0.3%), an antimicrobial reducing agent, which solubilizes and disperses the proteinaceous matrix that envelops and binds the starch granules (Eckhoff and Tso, 1991; Calzetta-Resio et al., 2006). Modification of the structural characteristics, and the physicochemical and functional properties of starch owing to steeping

and milling conditions has been reported in corn (Perez et al., 2001; 2003), wheat (Lorenz and Kulp, 1978), and rice (Lee et al., 2004). Wet-milling is a more complex process than dry-milling, and it is a source of a great variety of products. Although starch is the main product of wet-milling, there are other subproducts that are used for technological and food purposes, such as the fiber-rich and protein-rich fractions. The wet-milling of quinoa has not been widely studied yet, especially the optimum parameters and the steeping conditions such as time, temperature, pH, and stirring, among others. The steeping temperature is usually between 28 and 55 °C, because it must be below gelatinization temperature. The steeping time is conditioned by the type of grain, its morphology, and its size (Haros and Wronkowska, 2017). Changes in these parameters are important in starch isolation and its properties, determining its use (Haros and Wronkowska, 2017). Wright et al. (2002) used steeping with sodium hydroxide for 12 h at room temperature to isolate starch from varieties of sweet and bitter quinoa. Steffolani et al. (2013) and Jan et al. (2017a) isolated starch from the flour of several varieties of quinoa by steeping with NaOH.

The main objective of this research was to develop and optimize a quinoa wet-milling procedure for isolating the fractions of starch, proteins, and fiber at laboratory scale. The effect of steeping time and temperature on starch recovery and its quality was also investigated.

2. Materials and methods

2.1. Raw materials

Commercial Bolivian seeds of quinoa (*Chenopodium quinoa),* Organic red Quinoa Real© were purchased from ANAPQUI (La Paz, Bolivia).

2.2. Wet-milling procedure

Previous studies, in which different steeping solution temperatures, times, and pHs were evaluated, were used for reference purposes (Zheng et al., 1998; Calzetta-Resio et al., 2009; Wronkowska and Haros, 2014). Quinoa seeds (50 g) were steeped in 500 mL of sufficient sodium bisulfite to give a sulfur dioxide concentration of 0.25% at pH 5.0, adjusted with lactic acid. The wet-milling tests were performed according to a 3^2 factorial design (Table 1), and each experiment was conducted in duplicate. Two steeping variables – steeping time (1, 5, and 9 hours) and steeping temperature (30, 40, and 50 °C) – were assayed in a laboratory fermenter (Biostat Bplus, Sartorius, Spain) with constant control of temperature, pH at 5.0 adjusted with lactic acid, and stirring at 300 rpm. The steeped quinoa was separated into different fractions in two stages: a) the seeds were milled using a plate mill (Corona, Lambers & Cia, Colombia) to separate the germ fraction by flotation in water. After separation, the germ fraction was washed with ultrapure water (1 L) to remove the residual starch content (Figure 1); b) the degerminated seed slurry obtained after the first milling was scattered/homogenized with a disperser (PT 10/35 GT, Polytron, Lucerne, Switzerland). The homogenization was performed with 200 mL of water at 15,000 rpm for 1 minute three times. Then the homogenate was screened through two sieves (300 and 53 μm), where the hulls and protein fractions were retained, respectively. The fractions were washed with ultrapure water and the resulting suspension was centrifuged at 12,000 rpm for 15 min at 4 °C to obtain the starch fraction. After centrifugation it was possible to separate the pure starch from the tailings, the last were at the top of the pellet and were removed manually with a spatula (Wronkowska and Haros, 2014). The fractions were dried in a forced-air oven at 40 °C overnight, and aliquots of the steepwater (SW) and washing water (WW) were dried at 70 °C

in a forced-air oven to determine the soluble and suspended solids (total solids). All fractions were stored in sealed plastic containers until their analysis in a chamber at 14 °C.

The yield of each fraction, expressed as a percentage, was calculated as the ratio of the totally dried isolated fraction to the initial amount of dried quinoa, as:

$$Yield\ (\%) = \frac{dry\ weight\ of\ separated\ fraction}{initial\ dry\ weight\ of\ grain} x100 \quad (1)$$

The starch recovery was calculated as the ratio of the dry weight of the isolated starch to the dry weight of starch in grain:

$$Starch\ recovery(\%) = \frac{dry\ weight\ of\ isolated\ starch}{dry\ weight\ of\ starch\ in\ grain} x100 \quad (2)$$

2.3. Physicochemical starch characterization

Moisture content was determined following the official assay procedure (Method 925.09, AOAC, 1996). Starch content was measured by the total starch assay procedure (AOAC, 1996). The protein and lipid contents were determined by the Dumas combustion method (Nx5.7) according to ISO/TS 16634-2 (2016) and the Soxhlet technique (Method 30-20, AACC 1995) with petroleum ether under reflux conditions, respectively.

The amylose and amylopectin contents were determined using a commercial assay kit (Megazyme International Ireland Ltd., Bray, Co. Wicklow, Ireland) based on the Concanavalin-A method developed by Yun and Matheson (1990). The instrumental color of the starchy fraction was measured with a digital colorimeter (Chroma Meter CR-400, Konika Minolta Sensing, Japan). The parameters determined were L* (lightness), a* (redness to

157 greenness), and b* (yellowness to blueness), and the whiteness index (WI) was calculated by
158 the following equation: $WI=100–((100–L)^2+a^2+b^2)^{0.5}$. All the analyses were done in triplicate.
159
160 ***2.4. Determination of quinoa starch thermal properties***
161 A differential scanning calorimeter (DSC-7, PerkinElmer, USA) was used to measure the
162 thermal properties of the raw materials and starch fractions, and the amylopectin
163 retrogradation. The DSC was calibrated with indium (enthalpy of fusion 28.4 J/g, melting
164 point 156.4 °C). Samples were weighed into DSC pans (LVC 0319-0218, PerkinElmer), and
165 ultrapure water was added to obtain a water:flour ratio of 3:1 in order to ensure complete
166 gelatinization. After sealing, the pans were left for a few hours to equilibrate the humidity,
167 and then they were scanned at a rate of 10 °C/min from 20 to 130 °C. Subsequently, the pans
168 were stored at 4 °C for 2 days, and then heated again in the calorimeter from 20 to 130 °C at
169 10 °C/min to analyze amylopectin retrogradation. An empty pan (air) was used as a reference,
170 and three replicates of each sample were analyzed. Thermal transitions of starch were defined
171 in terms of onset temperature (T_o), peak (T_p), conclusion temperature (T_c), and enthalpy of
172 gelatinization and amylopectin retrogradation (ΔH_G and ΔH_R, respectively), expressed in J/g
173 of starch (Haros et al., 2004).
174
175 ***2.5. Pasting properties of quinoa starch***
176 The pasting properties of the starch fractions were measured using a Rapid Visco Analyser
177 (RVA-4; Newport Scientific, Warriewood, Australia) according to AACC Method 76-21
178 (1995). Distilled water (25 mL) was added to 3.0 or 3.5 g of sample placed in the aluminum
179 RVA canister. The suspensions were stirred thoroughly at 160 rpm. The temperature was first
180 maintained at 50 °C for 1 min and then raised to 95 °C at a rate of 12 °C/min, held at 95 °C

for 2.5 min, cooled to 50 °C at the same rate, and finally held at 50 °C for 2 min. Pasting parameters evaluated included: pasting temperature (P_{temp}), peak viscosity (PV), peak time (P_{time}), hot paste viscosity (HPV), final or cool paste viscosity (CPV), breakdown (PV-HPV), and setback (CPV-HPV). The experiments were conducted in triplicate.

2.6. Factorial design and statistical analysis

In order to study the effect of steeping conditions on starch recovery and quality, a factorial design was used. The independent factors studied were time (1, 5, and 9 hours) and temperature (30, 40, and 50 °C) at three levels. The run conditions of a 3^2 factorial design are shown in Table 1 in terms of experimental conditions and coded values. The design makes it possible to approximate the experimental data (Y_{obs}) with a response surface model expressed in coded values:

$$Y_{obs} = a_0 + a_1x_1 + a_2x_2 + a_{12}x_1x_2 + a_{11}x_1^2 + a_{22}x_2^2 + \varepsilon \quad (3)$$

In Equation 3, x_1 is the design factor steeping time, and x_2 is the steeping temperature. The coefficients a_1 and a_2 are the main effects of x_1 and x_2, respectively. The square coefficients (a_{ii}) indicate if any of the variables has a maximum or minimum in the experimental domain, whereas the mixed coefficients (a_{12}) represent the interactions between factors. The difference between the experimental data (Y_{obs}) and the model (Y_{calc}) gives the residual (ε). For each response the RS-Q was calculated, which is the fraction of variation of the response explained by the model.

2.7. Statistical analysis

The multivariate analyses (stepwise regressions, multiple way analysis of variance, and correlation matrix) of the yields of quinoa fractions after wet-milling, and the physicochemical, thermal, and pasting properties of the starch fractions were performed using the Statgraphics® software package (Statistical Graphics Corporation, Virginia, Washington, DC, USA).

3. Results and discussion

3.1. Effect of steeping condition on yields

The composition of raw material used in this investigation was : moisture, 10.68±0.02; starch, 67±3; protein, 12.8±1.0; lipid, 7.3±0.6, and ash contents, 2.32±0.04 g/100 g in dry matter (d.m.).

The fraction yields obtained by quinoa wet-milling were: 7.1–13.3% of germ, 9.1–14.6% of fiber, 0.9–2.6% of protein, 54.9–58.5% of starch, 5.2–17.9% of total solids in steepwater, and 4.1–10.8% in washing water, expressed in dry matter. The starch yields in the current investigation were slightly higher than those reported by Wright et al. (2002), and much higher than those obtained by Jan et al. (2017a) using different steeping conditions and wet-milling procedure. Wright et al. (2002) performed the steeping step in 0.30% NaOH at room temperature for 12 h. In the case of Jan et al. (2017b), quinoa seeds were steeped in alkali solution (0.20, 0.25, and 0.30% NaOH) at ~4 °C for 24 h.

The analytical data obtained from the factorial design for the yields of quinoa fractions obtained by wet-milling were fitted to multiple regression equations using three levels of two independent factors (Table 2) in order to estimate the dependence of yields (Eq. 3). The results obtained showed that the steeping time factor significantly affected the yields of the quinoa fractions (p<0.01). In general, when the steeping time increased, the germ yield and

SW solids increased significantly (by 19–46% and 76–93%, respectively), whereas the other yields decreased significantly (fiber, protein, starch, and WW fractions). The total solids (SW) leached in the steepwater increased significantly with steeping time at the expense of degradation of grain components during this step (Perez et al., 2003).

The steeping temperature individually promoted the largest increase in the germ yield (from 7.1-11.4 % (at 30ºC) to 10.5-12.8 % (at 50ºC)) and in the SW solid fraction (from 5.2-13.0 % (at 30ºC) to 8.1-17.9 % (at 50ºC)).

As a global tendency, the starch yields and recoveries decreased with the increase in steeping temperature, as indicated by the a_2 coefficient (Tables 2 and 3, respectively). In contrast, the steeping time, as a single independent variable, did not show any significant effect on the starch yields/recoveries. However, it was reported that corn starch yields increased as the steeping time increased (Perez et al., 2001). These discrepancies could be due to the ability of sulfur dioxide/lactic acid in the steepwater to disperse the protein matrix that envelopes the starch granules. This fact is less significant in quinoa than in corn, which the grain is harder and the starch is strongly linked protein matrix (Dailey, 2002; Perez et al., 2003; Wronkowska and Haros, 2014). In addition, there was a significant effect derived from the interaction between steeping time and temperature (a_{12}, Tables 2 and 3). On the other hand, as a result of the factorial design, the starch yield/recovery presented a maximum value in the domain studied, as represented by the negative quadratic coefficient of steeping time a_{11} (Tables 2 and 3, respectively).

The fiber yields also presented a significant interaction coefficient between the two factors studied (Table 2). The quadratic coefficient of the steeping time factor (a_{11}) was also significant for the fiber and protein yields, in both cases indicating a minimum value of these

252 fractions within the domain studied (Table 2). On the other hand, the quadratic coefficient of
253 the steeping temperature factor (a_{22}) was non-significant for any of the by-products.
254 It is observed that even though the increase of steeping time causes more solids to leach into
255 the steep water, such an increase was particularly pronounced at 50ºC of steeping. It was
256 reported that when lactic acid was present in the steepwater an increment of the proteolytic
257 activity resulting from the action of that chemical (Perez et al., 2001). One of the main
258 contribution to the increase of leached solids could be due to increased solubility of protein by
259 the action of lactic acid, so the protein fraction decreased significantly with the steeping time
260 (a1: -0.497). On the other hand, when the steeping time increased the germ fraction
261 augmented and the fiber fraction decreased probably due to the better separation of embryo
262 and higher soluble fiber lost, respectively.
263
264 ***3.2. Starch recovery and physicochemical characterization***
265 Results of starch recovery and physicochemical characterization in terms of factorial design
266 coefficients are shown in Table 3. In general, as the steeping temperature increased the starch
267 recoveries decreased, whereas the steeping time showing a maximum in this parameter (Table
268 3). However, it is important to take into account that there was also a significant interaction
269 between the two factors, as mentioned earlier. The absolute values of the starch recoveries
270 were within the range 81.9±0.1–87.2±0.7% in dry matter. There are only a few investigations
271 on quinoa wet-milling and the recoveries were not reported. Nevertheless, the results of the
272 current investigation could be compared with previous data for cereals and/or other
273 pseudocereals. The starch recovery/efficiency in corn was around 85.1% d.m. (Perez et al.,
274 2003), in amaranth 67.7% d.m. (Calzetta-Resio et al., 2009), in buckwheat 64.6% d.m.

(Wronkowska and Haros, 2014), and in rice 69.6% (Loubes et al., 2016), at the same order of magnitude as the current investigation.

With regard to the protein and damaged starch contents in the starch fraction, neither steeping time nor steeping temperature affected them significantly (Table 3). The results varied in the range 1.56±0.02–1.9±0.8% protein d.m. and 5.5±0.1–7.5±0.2% damaged starch d.m.

It was also observed that the amylose content of the starch decreased significantly only with the linear factor steeping time. This could be due to the higher hydrolysis of amylose during this step, as evidenced by the significantly higher amount of total solids in steepwater after 9 hours of steeping (from 5.2–8.1 to 13.0–17.9% d.m., for 1 and 9 h, respectively). On the other hand, the starch whiteness index quality parameter was significantly affected by the linear steeping temperature factor (Table 3), which decreased slightly with the increase in temperature (from 91 to 89% for 30 °C and 50 °C, respectively).

The starch physicochemical properties of quinoa obtained under the various steeping conditions by wet-milling were similar to the results reported by Steffolani et al. (2013), and slightly higher than those reported by Jan et al. (2017a, 2017b). In the current study the non-detection of lipids owing to efficient separation of the germ during the wet-milling procedure was a valuable result from the point of view of starch quality and in comparison with other investigations (Wright et al., 2002; Steffolani et al., 2013; Jan et al., 2017a).

3.3. Effect of steeping conditions on thermal properties

The DSC analysis of quinoa starch revealed how the steeping time and steeping temperature affected the thermal properties. Factorial design showed that the initial and peak temperatures of both gelatinization and retrogradation were significantly affected by both steeping factors. In general, they were higher when the steeping time and temperature increased (T_{oG}: from

299 51.2±0.1 °C to 55.4±0.5 °C; T_{pG}: from 58.6±0.1 °C to 61.1±0.1 °C; T_{oR}: from 35.6±0.2 °C to
300 40.0±0.5 °C; T_{pR}: 45.5±0.1 °C to 47.8±0.1 °C), whereas the conclusion temperatures were not
301 affected significantly (TcG: 68.2±0.3–70.1±0.2 and 55.6±0.5–56.8±0.5 °C). In addition, the
302 factors showed a significant interaction in T_o and T_p of gelatinization, as well as a significant
303 effect on the quadratic terms, which indicated the presence of a maximum in T_o (steeping time
304 factor, a_{11}) and a minimum in T_o and T_p (steeping temperature factor, a_{22}) in the response
305 surface, respectively.
306 The increase in T_o and T_p, with a narrower gelatinization temperature range, suggests a partial
307 annealing effect. This behavior was also observed in corn starch (Perez et al., 2001, 2003), in
308 wheat starch (Lorenz and Kulp, 1978), and in rice starch (Lee et al., 2004) with an increase in
309 steeping time. Annealing is defined as the heating of starch in excess water at
310 subgelatinization temperatures, which are the conditions during steeping (Perez et al., 2001;
311 Falade and Ayetigbo, 2015). It may provoke partial melting of some crystals and realignment
312 of starch chains in the amorphous regions, giving rise to more ordered crystals with higher
313 melting points (Perez et al., 2001).
314 The steeping conditions did not significantly affect the enthalpy of gelatinization, as was also
315 observed previously in corn wet-milling and rice wet-milling by other researchers (Perez et
316 al., 2001; Lee et al., 2004). It was reported that annealing has no effect on ΔH_G (Knutson,
317 1990), but it was also stated that the more crystalline structure are before annealing, the less
318 they can be enhanced by the annealing process (Alvani et al., 2012).
319 In the current investigation, the enthalpies of gelatinization and retrogradation were in the
320 range of 12.4±0.3–13.5±0.1 J/g of starch for ΔH_G and 1.2±0.2–1.7±0.2 J/g of starch for ΔH_R,
321 respectively. The results of enthalpy of gelatinization are in agreement with those reported by

Wright et al. (2002) for starches isolated from several varieties of quinoa. On the other hand, Steffolani et al. (2013) reported slightly higher values in quinoa and kañiwa starches.

Results for enthalpy of retrogradation were higher, by ~26%, than those reported by Steffolani et al. (2013) after 14 days of storage at 4 °C. Srichuwong et al. (2017) reported retrogradation temperatures in accordance with our results (36.2–61.7 °C) after 6 days of storage at 4 °C. However, the enthalpy of retrogradation was higher (4.2 J/g starch) than in this study, probably owing to the longer storage time in their case.

3.4. Influence of steeping conditions on quinoa starch pasting properties

The values of the pasting property parameters were: from 3898 to 3064 cP for PV, from 3430 to 2231 cP for HPV, from 4340 to 3557 cP for CPV, from 7.00 to 6.72 min for P_{time}, and from 68.44 to 64.50 °C for P_{temp}. These results are in agreement with those reported by Wu et al. (2017) for starch from several varieties of quinoa. Steffolani et al. (2013) observed similar values, with the exception of P_{time} (5.17–4.97 min) and P_{temp} (62.7 °C), which were slightly lower than our results. In general, variation of steeping temperature did not modify the pasting properties, and no significant differences were found in the RVA parameters of quinoa starch with the exception of the pasting temperature (P_{temp}). This parameter increased slightly with the increase in temperature and presented a maximum (Table 3). It was observed that the linear effect of steeping time significantly affected the coefficients of the PV, HPV, and CPV parameters, causing a decrease in them when the time increased. In contrast, setback increased significantly as the steeping time increased. However, all these tendencies seemed to be significant for the first 5 hours of steeping, whereas longer steeping times did not seem to modify the viscosity profile (Figure 3). The PV decreased by about 11–14% between 1 hour of steeping and 9 hours of steeping. This parameter indicates the starch water-binding

capacity and gives an indication of the viscous load. A drop in its value is usually brought about by partial hydrolysis (Haros et al., 2004; 2006). The steeping chemicals, such as SO_2 and lactic acid, could hydrolyze the starch and reduce the pasting viscosities because they diffuse into the starch granules during their hydration and swelling (Shandera and Jackson, 1996; Haros et al., 2006). After reaching PV the swollen starch granules are easily broken and disintegrated by stirring, so the viscosity decreases to a minimum, the hot paste viscosity (HPV). During the hold period at 95 °C and mechanical shear stress, the starch granules disrupt and amylase molecules leach out into the solution (Haros et al., 2006). The HPV dropped significantly during the first hour of steeping at all the temperatures studied. After the cycle of heating and cooling a reassociation between amylose molecules occurs. If the concentration is sufficient they form a gel and the viscosity increases up to a final viscosity (CPV) which involves the retrogradation phenomenon. In the current investigation CPV also dropped significantly in the starch fraction after steeping, probably owing to the partial loss of amylose. As described above, the amylose content of starch decreased significantly with the steeping time factor (Table 3), from 23.1±0.8–26.5±0.4% to 19.2±0.2–24.0±0.2% for 1 hour and 9 hours of steeping, respectively, which could explain the changes observed in pasting properties (Figure 3).

The values of the pasting property parameters were: from 3898 to 3064 cP for PV, from 3430 to 2231 cP for HPV, from 4340 to 3557 cP for CPV, from 7.00 to 6.72 min for P_{time}, and from 68.44 to 64.50 °C for P_{temp}. These results are in agreement with those reported by Wu et al. (2017) for starch from several varieties of quinoa. Steffolani et al. (2013) observed similar values, with the exception of P_{time} (5.17–4.97 min) and P_{temp} (62.7 °C), which were slightly lower than our results.

3.5. Optimization of steeping conditions

The calculation of the optimum experimental conditions to be used was performed for the recovery parameter, taking into account the starch physicochemical properties of quinoa in terms of protein content, damaged starch, and whiteness index. The effects of time (x_1) and temperature (x_2) on starch recovery were satisfactorily simulated by Equation (3). The maximum expected starch recovery (85.7%) occurred at 6.5 h of steeping time at 30 °C. Expected responses for steeping time and temperature factors in comparison with values reported by other authors are shown in Table 4. In general, the expected values were higher than those reported in other studies. This expected response was corroborated experimentally. The expected responses were tested, and the results were: 86.7±1.6% (d.m.) for starch recovery, 62.3±0.8% for starch yield, 1.74±0.05% of protein, and 91.8±0.08% of whiteness. The differences between the experimental and expected responses presented a deviation of only 2%.

4. Conclusions

The quinoa wet-milling process proved to be a potential procedure for obtaining various valuable components of quinoa grains. The factorial design showed that the variables steeping time and temperature affect the parameters significantly, increasing or decreasing their values, depending on the parameter analyzed. The wet-milling process developed in this study achieved a high level of starch recovery from quinoa. Maximum response values were obtained when the steeping time was set at 6.5 hours and the steeping temperature at 30 °C.

It is still necessary to study quinoa starch more deeply, because its propitious properties may have application potential in areas such as novel food additives, fat replacement, pharmaceuticals, cosmetics, papermaking, and textiles. Finally, the economic cost of steeping

operating conditions and the starch quality/recovery obtained by using a wet-milling procedure should be evaluated in order to find the most suitable conditions at industrial level.

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Figure captions

Figure 1. Laboratory Quinoa Wet-Milling

Figure 2. Influence of interaction between steeping time (x_1) and steeping temperature (x_2) on yields of **A.** Germ, **B.** Fiber, **C.** Protein, **D.** Starch.

Figure 3. Effect of steeping conditions on pasting profile of starch obtained by wet-milling. **A.** 30 °C; **B.** 40 °C; **C.** 50 °C; —— 1 h; — — 5 h; …… 9 h; —— quinoa whole flour.

521 **Table 1.** Factorial design for sampling

522

Run	Name	Steeping Conditions		Coded Value	
		time, h	Temperature, °C	time, x_1	Temperature, x_2
1	WM1-30	1	30	-1	-1
2	WM1-40	1	40	-1	0
3	WM1-50	1	50	-1	1
4	WM5-30	5	30	0	-1
5	WM5-40	5	40	0	0
6	WM5-50	5	50	0	1
7	WM9-30	9	30	1	-1
8	WM-9-40	9	40	1	0
9	WM9-50	9	50	1	1

523

Table 2. Factorial design coefficients of yield of by-products of quinoa wet-milling

Coefficient	Constant	Linear		Quadratic		Interaction	R-SQ
	a_o	a_1	a_2	a_{11}	a_{22}	a_{12}	
Germ	11.866	1.763**	1**				0.83
Fiber	9.444	-1.682**		1.479**		0.82**	0.88
Protein	1.118	-0.497**		0.472*			0.79
Starch	57.636		-0.789*	-1.689**		-1.055*	0.72
SW	13.787	4.565**	2.302**				0.94
WW	6.220	-2.152**					0.70

0.05 (*) and 0.01 (**) indicate statistically significant at the 95 and 99% confidence levels, respectively.

SW: leached solids in steepwater, % in dry matter.

WW: solids in washing water, % in dry matter.

a_1 and a_2 are the coefficients of the main single effects of x_1 and x_2, respectively (x_1 is the design factor steeping time, x_2 is the steeping temperature). The square coefficients (a_{ii}) indicate if any of the variables has a maximum or minimum in the experimental domain, whereas the mixed coefficients (a_{ij}) represent the interactions between factors. R-SQ: adjusted square coefficient of the fitting model.

Table 3. Factorial design coefficients of physicochemical, thermal, and pasting properties of quinoa starch isolated by wet-milling

Coefficient	Constant	Linear		Quadratic		Interaction	R-SQ
	a_o	a_1	a_2	a_{11}	a_{22}	a_{12}	
Physicochemical characteristics							
Recovery, % d.m.	85.898		-1.176*	-2.514**		-1.574**	0.72
Protein, % d.m.	1.674						0.65
Damage, % d.m.	4.481						0.36
Whiteness Index	91.788		-0.703*				0.74
Amylose, % d.m.	22.937	-2.232**					0.66
Thermal Properties							
Gelatinization							
T_o, °C	51.428	0.558**	1.573**	-0.74*	1.67**	0.957**	0.94
T_p, °C	58.656		0.632**		1.594**	0.796**	0.85
T_c, °C	68.719	-0.513**					0.71
ΔH_G, J/g of starch	13.132						0.45
Retrogradation							
T_o, °C	38.161	1.411**	0.694*				0.79
T_p, °C	47.078	0.822**	0.432*				0.81
T_c, °C	55.749						0.21
ΔH_R, J/g of starch	1.179						0.32
Pasting							
P_{temp}, °C	66.396		0.949*	-1.428*			0.64
P_{time}, min	6.915						0.29
PV, cP	3333.39	-230.25**					0.67
HPV, cP	2669.5	-296.0**					0.68
CPV, cP	3845.83	-181.67*					0.60
Breakdown, cP	664.11						0.55
Setback, cP	1170.61	105.0*		-139.17*			0.64

0.05 (*) and 0.01 (**) indicate statistically significant at the 95 and 99% confidence levels, respectively.

DSC, Differential Scanning Calorimetry; T_o, onset temperature; T_p, peak temperature, T_c, conclusion temperature; ΔH_G, enthalpy of gelatinization, J/g in d.m.; ΔH_R enthalpy of retrogradation, J/g in d.m.

RVA: Rapid Visco Analyser; P_{temp}, Pasting temperature; P_{time}, Peak time; PV, Peak viscosity; HPV, hot paste viscosity; CPV, final or cool paste viscosity; Breakdown: PV-HPV; Setback, CPV – HPV; cP, centipoise; d.m., dry matter. a_1 and a_2 are the coefficients of the main single effects of x_1 and x_2, respectively (x_1 is the design factor steeping time, x_2 is the steeping temperature). The square coefficients (a_{ii}) indicate if any of the variables has a maximum or minimum in the experimental domain, whereas the mixed coefficients (a_{ij}) represent the interactions between factors. R-SQ: adjusted square coefficient of the fitting model.

548 **Table 4.** Expected **y**ield, recovery, and physicochemical composition of quinoa starch fractions, and
549 comparison with other investigations
550

Parameter of Starch Fraction	Units	Current Investigation[a]	Jan et al., 2017a,b	Steffolani et al., 2013	Wright et al., 2002
Yield	%.	61.89	48.52	NR	53.3
Recovery	%	85.68	NR	NR	NR
Protein	%	1.78	0.95	1.09	0.46
Lipids	%	ND	0.40	1.94	NR
Whiteness Index	%	91.45	NR	NR	NR
Amylose	%	23.9	12.1	17.4	20.6
Damaged Starch	%	4.65	NR	NR	NR

551
552 [a]expressed in dry matter.
553 ND: Not detected; NR: Not reported.
554 Steeping conditions: Jan et al. (2017a, 2017b): 0.25% NaOH, 24 h; Steffolani et al. (2013): 0.25% NaOH, 12 h;
555 Wright et al. (2002): 0.30% NaOH, 12 h.
556

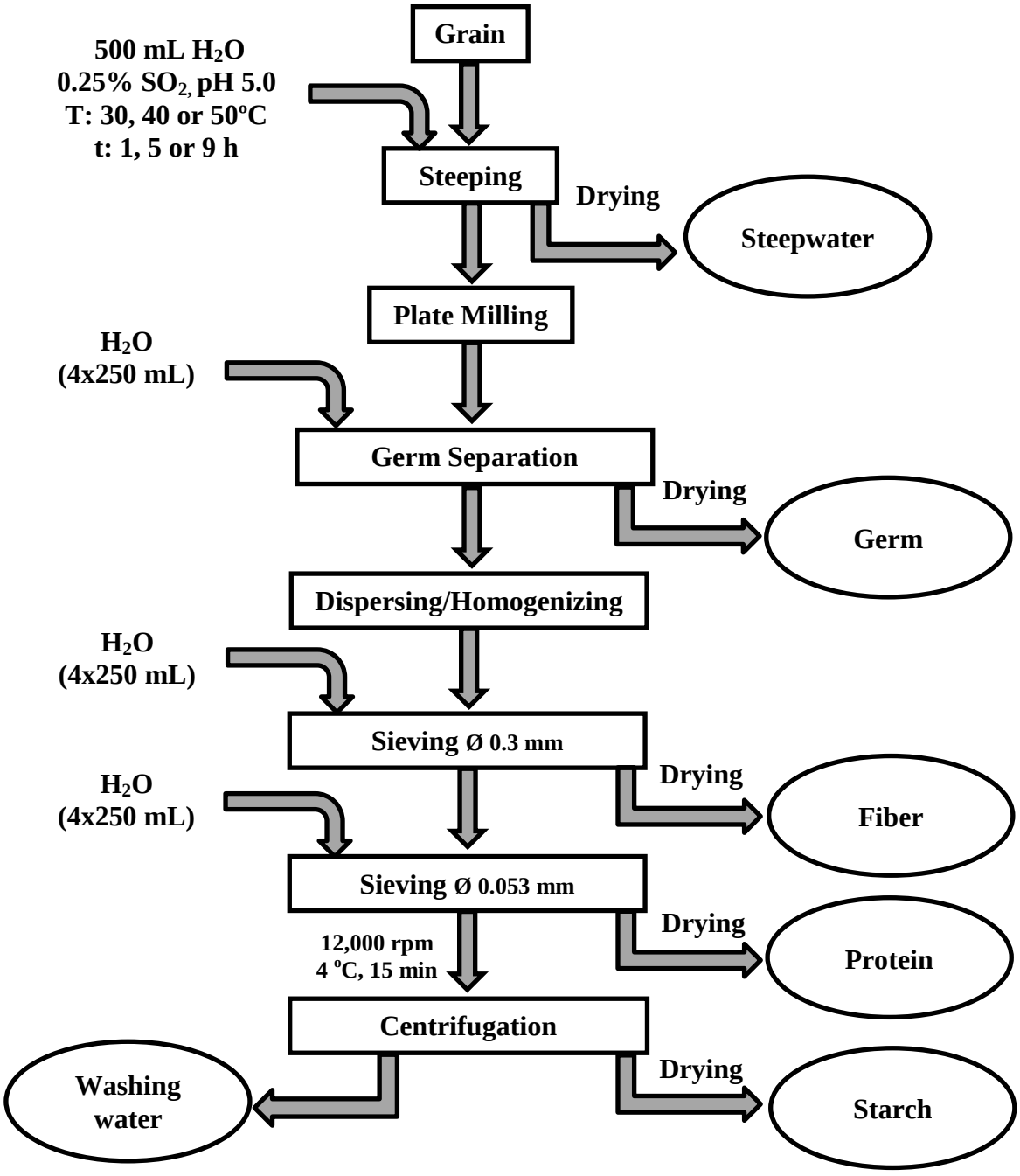

Grain
500 mL H_2O
0.25% SO_2, pH 5.0
T: 30, 40 or 50ºC
t: 1, 5 or 9 h
Steeping
Drying
Steepwater
Plate Milling
H_2O
(4x250 mL)
Germ Separation
Drying
Germ
Dispersing/Homogenizing
H_2O
(4x250 mL)
Sieving Ø 0.3 mm
Drying
Fiber
H_2O
(4x250 mL)
Sieving Ø 0.053 mm
Drying
Protein
12,000 rpm
4 °C, 15 min
Centrifugation
Drying
Starch
Washing
water

Figure 1

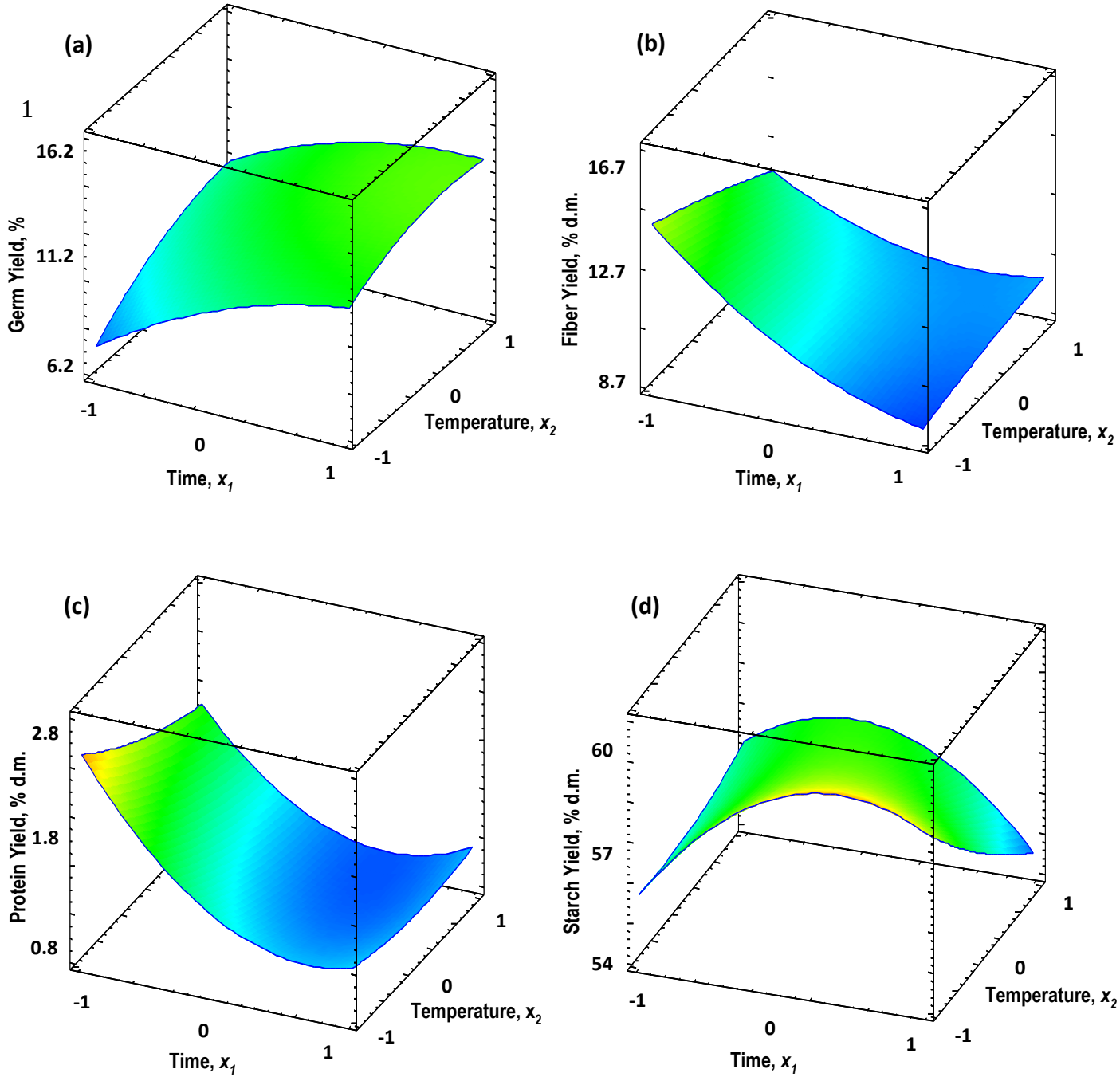
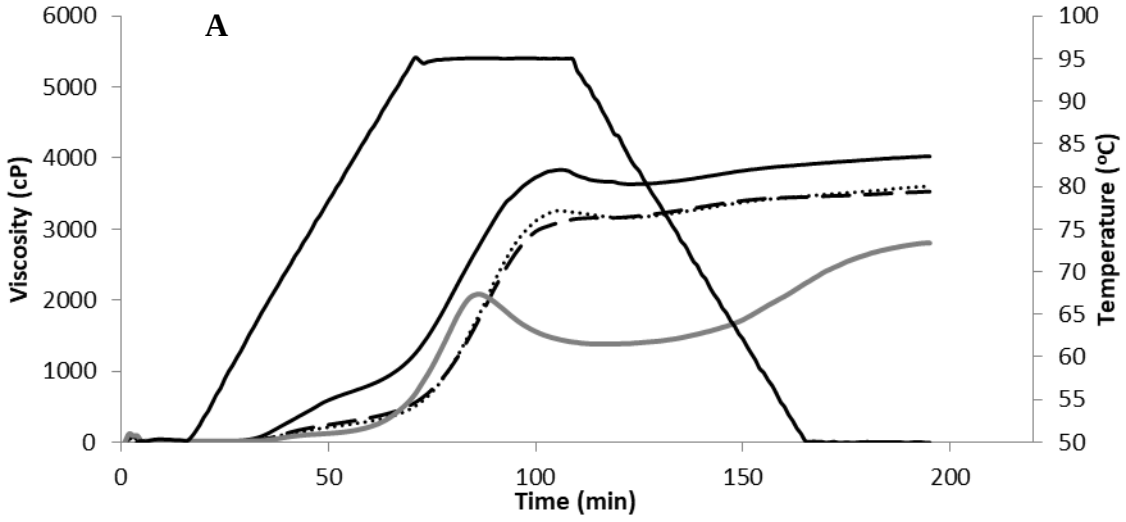
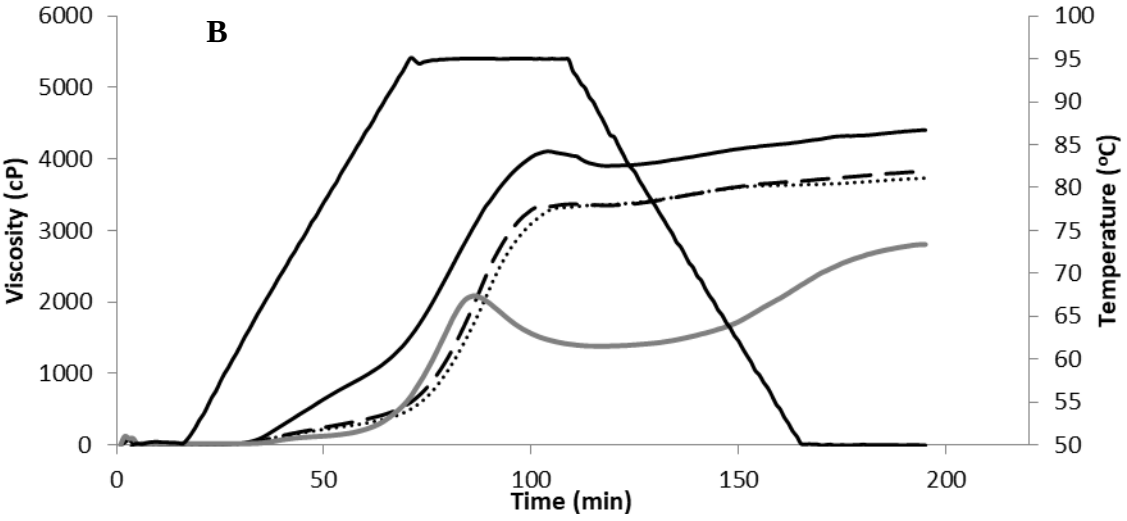
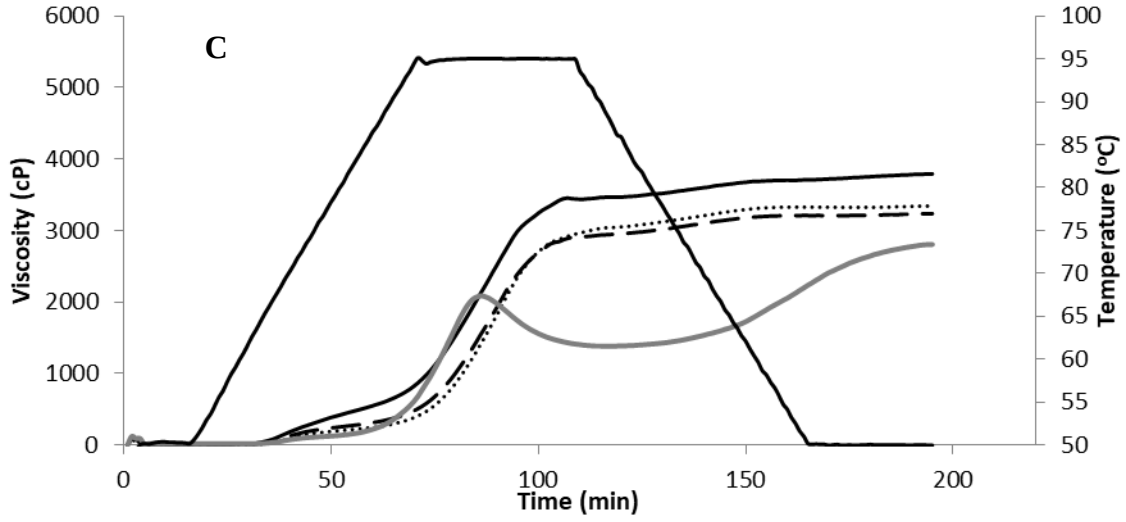

(a)
Germ Yield, %
16.2
11.2
6.2
-1
0
1
Time, x_1
Temperature, x_2
(b)
Fiber Yield, % d.m.
16.7
12.7
8.7
(c)
Protein Yield, % d.m.
2.8
1.8
0.8
(d)
Starch Yield, % d.m.
60
57
54

Figure 2


A
Viscosity (cP)
Temperature (ºC)
Time (min)
0
1000
2000
3000
4000
5000
6000
50
55
60
65
70
75
80
85
90
95
100
0
50
100
150
200


B
Viscosity (cP)
Temperature (ºC)
Time (min)
0
1000
2000
3000
4000
5000
6000
50
55
60
65
70
75
80
85
90
95
100
0
50
100
150
200


C
Viscosity (cP)
Temperature (ºC)
Time (min)
0
1000
2000
3000
4000
5000
6000
50
55
60
65
70
75
80
85
90
95
100
0
50
100
150
200