Wet-milling of buckwheat with hull and dehulled - the properties of the obtained starch fraction

Małgorzata Wronkowska[1,2], Monika Haros[1]

[1]Cereal Group, Institute of Agrochemistry and Food Technology (IATA-CSIC), Av. Agustín Escardino 7, Parque Científico, 46980 Paterna, Valencia, Spain

[2]Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Division of Food Science, Department of Chemistry and Biodynamics of Food, 10 Tuwima Str., 10-747 Olsztyn, Poland.

Correspondence

Correspondence: Małgorzata Wronkowska, Department of Chemistry and Biodynamic of Food, Division of Food Science, Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, 10-748 Olsztyn, 10 Tuwima Str., Poland; Fax: +48 89 5240124; email: m.wronkowska@pan.olsztyn.pl

Abstract

The buckwheat with or without hull were steeped at 28ºC in SO_2 solution with lactic acid.. Starchy materials obtained by the laboratory wet-milling was characterised by determining starch extraction efficiency, particle size distribution by laser light-scattering methodology and microstructure of granules by scanning electron microscopy (SEM). The isolated starchy material was characterized also by determining pasting properties in Rapid Visco Analyser (RVA), thermal properties in differential scanning calorimetry (DSC). The starch extraction efficiency level was higher for total starch isolated from buckwheat with hull compared

dehulled buckwheat. The mean particle diameter of the pure starch isolated from buckwheat with or without hull was about 18 µm. Microstructure characteristics analysed by SEM showed that buckwheat starch isolated using wet-milling method had a polygonal and irregular shape. The longer time of steeping used for buckwheat with hull caused the decrease in temperature of gelatinization compared to the dehulled buckwheat. Significantly higher enthalpy values for both pure starches compared to the raw material and starches with tailing were noticed. Also the increasing of gelatinisation enthalpy with increasing of the steeping time and the higher proteins amount was observed. Higher RVA results were found for pure starches compared to starches with tailing. The results obtained in this study indicated that the used wet-milling method did not change significantly the properties of the obtained starchy materials compared to raw material.

Key words: wet-milling, buckwheat, starch, hull, DSC, RVA, particle size distribution, SEM

Introduction

Cultivation of buckwheat declined for many years, but recent interest in old, traditional foods and a re-evaluation of typical regional products, has led to resurgence in its cultivation. Buckwheat is a pseudocereal which has been grown for centuries in Europe and now, alongside spelt wheat, is one of the most important alternative crops, suitable for ecological growing, without the use of artificial fertilizers or pesticides. Buckwheat is generally used as human food and as animal or poultry feed. Similarly to cereal crops buckwheat producing seed with high starch content. In addition, buckwheat proteins have a high biological value, and relatively low true digestibility (Skrabanja et al., 2000). Also the content of other nutritionally important fractions, like antioxidative substances, trace elements or dietary fibre is currently under consideration (Wijngaard & Arendt, 2006). Due to the quite

well-known health-promoting values of buckwheat some countries are already have a special health program. In that programs buckwheat is included in the normal diet for children and adults or some of them promoting its healthy properties, like North American Buckwheat Promotion Committee in Canada or project named "Buckwheat Conservation And Utilisation" in Bhutan. Buckwheat dehulling process is carried out by raising the moisture content of raw whole buckwheat kernels followed by simultaneous steaming and heating. Buckwheat hull can be used in food industry. Oomah and Mazza (1996) has reported that buckwheat hull contains 4-times more the phenolic compounds compared to groats. Zielińska et al (2013) found that the buckwheat hull tea showed a lower content of total phenolic compounds and lower antioxidant capacity in comparison to the green tea. Nowadays, the hull is used in the production of therapeutic mattresses and cushions, which are adapts to the position of the body, quickly absorbs the moisture, does not heat up and is always cool. Extremely important feature of these products, the due to the presence of tannins, is inhibiting the development of harmful micro-organisms: mites, mold, bacteria and fungus. The by-products from the processing of buckwheat are characterized by a high content of carbon and hydrogen, that is why it is used as a raw material for the production of granular biofuels.

Wet-milling is an industrial process involving physical, chemical, biochemical and mechanical operations to separate the principal components for different type of grains. This process consist basically of two steps: soaking in water solutions of alkali or acid at a given temperature, followed by mechanical separation that takes advantage of the differences in the physical properties (density and particle size) of the fractions: starch, protein, germ, fibre and hull. During soaking water diffuses into the grain, and softens and degrades the intercellular structure, which allows for efficient milling. Being soaking a diffusive process, its rate will depend on temperature and presence or not of chemicals (acid or alkali). Literature data provide information about the use of wet-milling process for the isolation of fractions from

76 the maize, sorghum, amaranth, wheat or buckwheat (Haros et al., 2004; Buffo et al., 1998;
77 Calzetta Resio et al., 2009; Sayaslan, 2004; Zheng et al. 1998; Loubes et al., 2012). In
78 conventional wet-milling, maize is steeped in an aqueous solution containing sulphur dioxide
79 (0.1-0.2%), a reducing and antimicrobial agent, which solubilised and dispersed the
80 proteinaceous matrix that envelops and bind starch granules. Modification on structural
81 characteristics, physicochemical and functional properties of starch due to steeping and
82 milling conditions were reported (Pérez et al 2001; Beta et al 2001). The presence of lactic
83 acid in the steeping water makes the cell walls easier to break, and for better simulate the
84 industrial steeping process at laboratory level (Pérez et al., 2001). However, scarce
85 information exists concerning the effect of steeping time and the addition of lactic acid on the
86 starch characteristics. Shandera and Jackson (1996) studied the effect of steeping temperature
87 and concentration of lactic acid and sulfur dioxide in the starch functionality. They found that
88 maize kernels steeping conditions affect the physicochemical properties of the starch. In fact,
89 Pérez et al (2001) found that the starch from maize steeped for various time intervals
90 presented an increase in peak temperature and a narrowing of the gelatinization range, due to
91 the annealing produced during the steeping process. The changes in starch properties induced
92 by steeping and milling conditions could be important because its physicochemical
93 characteristics and functional properties determine suitability use in different industrial
94 processes. Moreover, the method used for wet-milling should allow for the best selection of
95 buckwheat fractions, which will be utilize for creation of a new food ingredients.

96 This study was undertaken to determine the recovery of each components of
97 buckwheat with or without hull by wet-milling. The aim of this work was isolation and
98 analysis of buckwheat starch obtained by wet-milling procedure from kernels with and
99 without hull. The starch characterization was analysed by pasting and thermal properties by
100 using the rapid viscoanalyzer (RVA) and the differential scanning calorimetry (DSC),

101 respectively. In addition, damage starch concentration and particle size distribution of starch
102 granules were also assessed.
103
104 **Material and methods**
105 *Materials*
106 Commercial Polish common buckwheat with and without the hull were purchased from a
107 local market (Melvit S.A., Kruki, Poland). The proximal chemical characteristics of
108 buckwheat kernels with hull and without hull in dry basis were: 58.5±0.3% and 69.4±0.3% of
109 starch, 12.3±0.1% and 15.2±0.1% of protein (Nx5.7), and 3.9±0.4% and 1.7±0.1% of ash,
110 respectively.
111
112 *Wet-milling*
113 Applied wet-milling procedure was chosen based on the preliminary studies (data not shown),
114 in which different temperatures, time and pH of steeping water were evaluated (Haros and
115 Suárez, 1999; Perez et al., 2001; Zheng et al., 1998). Buckwheat with hull and dehulled were
116 steeped in 250 ml of sufficient sodium bisulfite solution (Sigma, No 243973) to give a dioxide
117 concentration of 0.25% in distilled water (1:9 w/v) at 28ºC for 16 h and 2 h, respectively. The
118 pH was adjusted to 4.0 by using lactic acid (Sigma, DL-lactic acid solution, No W261114).
119 The steeped buckwheat with or without hull were ground with a blender for 3 min with the
120 small amount of distilled water. The water slurry was manually sieved through a set of
121 stainless screens: 600 (buckwheat with hull), 300, 80 and 53 µm (30, 50, 200 and 270 U.S.,
122 respectively). Hull was retained in the first screen, germ and fibre fractions in the second,
123 protein fraction in the third and four. The sodium hydroxide (4% w/v) was added dropwise to
124 starch slurry passing through 80 and 53 µm sieves, the starchy milk was mixed vigorously for
125 30 min at room temperature. Then slurry was centrifuged 5-times at 20,000 rpm for 20 min at

4ºC, after the centrifugation the pure starch and starch with tailing (sediment) were obtained. The steeping water and the water obtained during the centrifugation were freeze-dried. The pure starch, starch with tailing, protein, germ and fibre, and hull fractions resulting from the wet-milling process were dried for 24 h at 40-45ºC.

The yield of each fraction was calculated as a ratio of the totally dried isolated fraction to the initial amount of dried buckwheat. Extraction efficiencies were calculated by the formula proposed by Zheng et al (1998):

% starch extraction efficiency = [(%fraction yield x % starch content)/% starch content in kernel] x 100

The assays were realized three times.

Fraction chemical characterisation

Moisture was determined by using moisture analyser KERN DBS 60-3 (Kern & Sohn GmbH, Balingen-Frommern, Germany). For a better characterisation of the material, protein (Nx5.7) was measured by the micro-Kjeldahl method (AOAC, 1995). The total starch content was determined by Total Starch (AA/AMG) Assay Kit (K-TSTA) (Megazyme, Irland).

Starch damage analysis and whiteness

The starch damage was carried out with a SDmatic (Chopin, France) which uses the method of analysis based on amperometric method (AACC, 1995). This method consists of measuring the amount of iodine absorbed by the starch granules in a solution at a temperature of 35°C. The instrumental measurement of starchy samples colour was carried out with a ColorFlex (HunterLab, USA), and the results were expressed in accordance with the CIELab system with reference to illuminant D65 and a visual angle of 10°. The measurements were performed through glass sample cup. The parameters determined were: L* (L* = 0 [black]

and L* = 100 [white]), a* (–a* = greenness and +a* = redness) and b* (–b* = blueness and +b* = yellowness). All measurements were performed in four replicates. Whiteness index (WI) was calculated as: $WI = 100 - (100 - L)^2 + a^2 + b^2)^{0.5}$ (Ghanbarzadeh et al., 2010).

Differential scanning calorimetry (DSC)

Differential scanning calorimetry measurements were made with a Perkin–Elmer DSC-7 (Norwalk, CT). Briefly, 10 mg of obtained starchy materials were directly weighted into DSC stainless steel pans (PE 0319-0218) and distilled water was added to obtain a water:starch ratio of 3:1, in order to ensure complete gelatinisation. After sealing, they scanned at a rate of 10ºC/min from 20 to 130ºC. An empty pan was used as reference. Three replicates for each sample were run. The parameters recorded were onset temperature (To), peak temperature (Tp) and conclusion temperature (Tc) of gelatinisation. Straight lines were drawn between To and Tc and the enthalpies (ΔH) associated with starch gelatinisation were calculated as the area enclosed by the straight line and endotherm curve. They were expressed in joules per grams of starch (Haros et al., 2004).

Particle size distribution analysis

The particle size distribution of starch granules was determined by laser diffraction analysis (Malvern Instruments Ltd, Malvern, England) equipped with MS 15 Sample Presentation Unit. Measurements were run in quadruplicate at room temperature. The refraction index of water and starch dispersion was 1.330 and 1.53 with and absorption of 0.1. Size distribution was quantified as relative volume of particles in size bands presented as size distribution curves (Malverin MasterSizer Micro software v. 5.60). Particle size distribution was described by the following parameters: largest particle size (D_{90}), the median diameter (D_{50} - the size at which 50% of particles, by volume, are smaller and 50% are larger), smallest particle size

(D_{10}), Sauter mean diameter (D[3,2]), mean particle diameter (D[4,3]) (Afoakwa et al., 2008; Okechukwu and Rao, 1995).

Rapid visco analyser (RVA)

Determinations of pasting properties samples were measured using a Rapid Visco Analyser (RVA-4; New Scientific, Warriewood, Australia), according to the AACC Method 76–21 (1995). RVA measurements were performed using 3 or 3.5 g of sample on the basis of 14% moisture dispersed in 25 mL of distilled water. Suspensions were stirred thoroughly at 160 rpm for 10 s. The temperature was first maintained at 50ºC for 1 min to have a uniform temperature and then raised to 95ºC at a rate of 12 ºC/min, hold at 95 ºC for 2.5 min, cooled to 50ºC at a rate of 12ºC/min and finally hold at 50ºC for 2 min (Haros et al., 2006). Pasting temperature (P_{temp}), peak viscosity (PV), hot paste viscosity (HPV), final or cool paste viscosity (CPV), breakdown (PV-HPV) and setback (CPV-HPV) were recorded. The experiments were conducted in triplicate.

Scanning electron microscopic examination

The microstructure of starch was analysed under scanning electron microscopy (SEM). Dry samples were fixed to aluminium stubs and coated with gold in a JEE 400 vacuum evaporator (JEOL, Tokyo, Japan). The images were analysed under a JSM 5200 microscope (JEOL, Tokyo, Japan) at 10 keV (Sadowska et al., 1999).

Statistical analysis

The results of the analyses are given as the means and the standard deviation of at least three independent measurements. The data were analysed by one-way ANOVA. Fisher's Least

Significant Difference Test at a significance level of $p<0.05$ was performed for *post-hoc* comparison.

Results and discussion

Wet-milling yields

For the buckwheat with hull the wet-milling yields of total starch, protein, germ and fibre, and hull fractions were: 48.7, 3.2, 1.5 and 20.1%, respectively (Table 1). Total solids leached to steeping water were 9.1% whereas the total solids in water wash were 14.8%. The yields of fraction obtained during the wet-milling of dehulled buckwheat were: 58.8% of total starch, 4.8% of protein, 2.8% of germ and fibre. The total content of solids in steeping water and in washing water were: 10.2 and 19.1%, respectively. Obtained results could be partially explained by the direct (buckwheat dehulled) or not direct (buckwheat with hull) contact of sulphur dioxide with grains during steeping, favouring the sulphur dioxide diffusion through the exposed grains (Dailey, 2002). The main fractions obtained during wet-milling process for both buckwheat (with and without hull) were starch (pure starch and starch with tailing) and protein. However, as it was presented in Table 1 the significant fractions was also the total solids in steeping water and in the washing water of fibre and protein fractions. It could be connected with the content of the soluble dietary fibre in buckwheat (7.7 – 9.2%) which is higher than wheat bran (4.3%) or oat (7.2%) (Krkoškova and Mrázova, 2005).

The most important product in wet-milling process is the starch fraction. Is was described in literature that steeping time can affect recovery, purity and therefore the properties of starch (Haros et al., 2004). Lactic acid as well as steeping temperature could change thermal and pasting properties of maize starch obtained from wet-milling (Brandemarte et al., 2004). Lactic acid decreases some pasting viscosities parameters and starch retrogradation temperatures (Haros et al., 2004). Besides steeping temperatures

promote a stronger annealing in starch granules within the kernels (Perez et al., 2001). In this sense, in the current investigation was studied the parameters which describe buckwheat starch behaviour obtained by wet-milling from kernels with and without hull.

Efficiency and purity of starch fractions

Wet-milling performance was evaluated on the basis of extraction efficiency and purity of starch fractions in terms of protein content and whiteness index (Table 2). High starch extraction efficiency and low protein content in starch were considered as indicators of effective process. The starch extraction efficiency level was higher for total starch isolated from buckwheat with hull (90.9%) than for total starch isolated from dehulled buckwheat (82.8%). The protein content in both pure starch fractions was low and be approximately 0.9% d.m. While protein content in starch with tailings from buckwheat with hull (TBH) and dehulled (TBD) was 14.9 and 10.4%, respectively. However, the protein content in total starch fraction (pure starch + starch with tailings) was approximately 2.2%. The purpose of wet-milling is to obtain starch, so its protein content must be kept as lowest as possible in order to assure a better separation among proteins and starch fractions. For example for amaranth starch, isolated by wet-milling, the reported values of protein content range between 0.13% and 3.66% (Calzetta Resio et al., 2009). The wet-milling procedure used for sorghum grains allowed the isolation of starch fraction with about 0.5% protein content (Wang et al., 2000; Xie and Sieb, 2002). For the maize wet-milling the content of proteins in starch fractions is between 0.9-6.1% (Haros and Suarez, 1997). Such discrepancy results from many differences in materials and wet-milling conditions such as time, temperature and chemicals (alkaline or acid) in steeping water. Park and Baik (2010) found lower starch recoveries and similar purity of starch in wet-milling of whole (unabraded) ground kernel compared to abraded hulless ground kernel of barley.

The colour of starch has impact on its quality. Any pigmentation in the starch is carried over to the final product. This reduces the quality, hence acceptability of starch product. A low value for chroma and a high value for lightness are desired for the starch to meet the consumer preference. In this study the whiteness index values obtained for both pure starches were near to 94%, what indicating a white material. The starch with tailing from buckwheat with hull has the lowest value of whiteness index (67%), what could be connected with the dark hull pigment.

Starch damage and particle size distribution

The lowest level of starch damage was observed for buckwheat flour (2.9%). The highest starch damage values were found for pure starch (SBH) and starch with tailings (TBH) isolated from buckwheat with hull. Starch damage is an important factor to consider when extracting starch, because of its effects on the starch properties (Morrison et al. 1994). Damage to starch mainly come from the milling of the seeds into flour. However, the physical breakdown of starch granules during the milling process result from the various forces used (Karkalas et al., 1992; Morrison and Tester, 1994; Morrison et al., 1994). The flour with a high level of damaged starch generally had high water absorption capacity and was more susceptible to attack by amylase. Chen et al. (1999) reported that the wet-milled rice flour gave the lowest damaged starch level and the finest particle size compared to dry or semi-dry milled flours. Additionally, for the rice the degree of damage is also affected by kernel hardness, mill types, milling methods and the soaking process (Chiang and Yeh, 2002). In this study the higher starch damage observed for fractions isolated from buckwheat with hull could be connected with the longer time of steeping used for that material. According to the results presented by Haros et al. (2004) in the presence of lactic acid the content of damaged maize starch during wet-milling was higher what was also associated with longer steeping

time. Maize starch steeped for a longer time had porous granules with distinct crater-like impressions as it was presented by Haros et al. (2006).

It is known that particle geometry, as well as size distribution, affects the characteristics and behaviour of particulate materials. Several techniques can be used to determine particle size distributions as laser light scattering, microscopy, sieving, sedimentation analysis, permeability of a powder column, or electrical-sensing zone technique. In this work, laser light-scattering methodology was used to measure the particle size distribution (Figure 1 and Table 2). Pure starch fraction (SBH and SBD) presented bimodal distribution, whereas buckwheat flour (BD), and starches with tailings (TBH and TBD) showed a multimodal distribution of particle size (Figure 1). The histograms of pure starches exhibit the particle size distribution fitted by practically two Gaussian curves, being the second one more important. Those fractions obtained from buckwheat with hull or dehulled showed similar particle size distribution (p<0.05), being mean particle diameter (D[4,3]) about 18 µm. Particle size distribution of buckwheat flour was from 13 to 413 µm, with a mean particle diameter of 171 µm. The histogram of buckwheat flour showed at least bimodal distribution. The first peak in Figure 1 presented floury starch fraction, while the second one, which is the melt of two fractions, is starch and endosperm proteins. Starch with tailings obtained after the isolation process is contaminated mainly with proteins (Table 2). Significantly higher values of particle size of starch with tailing obtained from dehulled buckwheat (TBD) was noticed compared to other fractions obtained during wet-milling. The mean particle diameter of this fraction was 162.6 µm, whereas for starch with tailing from buckwheat with hull (TBH) was 113.9 µm. The shape of histograms of starches with tailing looks similar that of buckwheat flour. After buckwheat wet-milling, starch can be separated from endosperm protein, increasing the peak connected with starch (~10 µm) and decreasing the peak connected with endosperm material (~100 µm). For both starches with tailings it was

still observed the second peak as in buckwheat flour, while it disappeared in pure starches. Tailing peak corresponding to the starch fraction is in the same place on the histogram as in the case of the isolated pure starch fractions. For starch with tailing obtained from dehulled buckwheat (TBD) the peak corresponding to the endosperm protein is clearly shifted toward greater particles size (Figure 1). The particle size distribution parameter could be overestimated because particle size was determined in wet form, some variations could be observed in the distribution. Starch increased the particle size presumably due to their partial swelling (Haros and Suarez, 1997) or agglomeration (Figure 2), which is explained above.

Microstructure characteristics

Buckwheat starch granules isolated using wet-milling method (SBH, SBD) had a polygonal and irregular shape and often aggregated, with only a few spherical granules (Figure 2). A normal buckwheat endosperm contains small polygonal starch granules ranging in size from 2 to 19 µm (Neethirajan et al., 2012). The buckwheat starch has smaller granules than those of maize starch (12.2 µm), tapioca starch (18 µm) or potato starch (30.5 µm) (Mishra and Rai 2006). Zheng et al. (1998) found that starch isolated by wet-milling of dehulled buckwheat groats have the mean granule diameter about 6.5 µm. The SEM pictures of pure starch isolated from buckwheat with hull (SBH) showed granules with higher amount of deformed granules compared with the second analysed starch. Obtained results confirm the higher starch damage of starch isolated from buckwheat with hull (Table 2). Both microphotographs of starches with tailing indicate the presence of proteins (Figure 2). Moreover, as indicate the results of particle size analysis (Figure 1), the microstructure of both starches with tailings is similar to the image of buckwheat flour. SEM photo shows a large agglomerated fragments with well visible starch granules and protein fragments for both, buckwheat flour and starches with tailings.

Thermal properties of starch fractions

Thermal properties of investigated buckwheat materials are showed in Table 3. DSC analysis of buckwheat flour revealed a gelatinization starch temperature range from 68 to 81ºC with a peak at 74ºC. The wet-milling of buckwheat with hull, used for pure starch (SBH) and starch with tailing (TBH) isolation, caused the statistically significant decrease of peak temperature compared to buckwheat flour. The highest values of T_p and T_c were found for the fractions isolated from dehulled buckwheat compared to fractions obtained from buckwheat with hull. The enthalpy values were significantly higher for both pure starches compared to the raw material and starches with tailing (Table 3).

Literature data indicate that the different buckwheat cultivar have different peak gelatinization temperatures (Noda et al., 1998). Peak gelatinization temperatures of buckwheat starch ranges from 63.7 to 70.8ºC (Wijnagaard and Arendt, 2006). Zhou et al. (2009) found that isolated buckwheat starch showed similar trends in DSC curves as buckwheat flour. Zheng et al. (1998) showed that gelatinization temperature of starch isolated from the dehulled buckwheat by using wet-milling was range of 63 to 81ºC with a peak at 69ºC. The longer time of steeping used for buckwheat with hull caused a decrease in temperature of gelatinization compared to dehulled buckwheat. For maize wet-milling the elongate of steeping time caused an increase in peak temperature and narrowing in the gelatinization range as found Haros et al. (2004). In this study the increasing of gelatinisation enthalpy with increasing of the steeping time and the higher proteins amount was observed. Similar trends were noticed by Perez et al. (2003) for wet-milling of maize. They explain that probably the higher protein content in starch fraction reduces the water diffusion into the granules during DSC runs, which avoids/prevents the interaction between water and starch.

Pasting properties of pure starch and starch with tailing isolated from buckwheat materials

Peak viscosity (PV) parameter indicated the water-binding capacity of starch and also provides an indication of the viscous load. After reaching PV the swollen starch granules are easily broken and disintegrated by stirring, so the viscosity decreases up to a minimum, the hot paste viscosity (HPV). After the cycle of heating and cooling in the RVA, re-association between starch molecules, especially amylose, occurs. In sufficient concentration this causes gel formation, and the viscosity increases up to a final viscosity (CPV). This phase of pasting curve is commonly referred to as the setback region, and involves retrogradation, whereas the CPV is most commonly used parameter to define the ability to form viscous paste or gel after cooking and cooling (Haros et al., 2006).

Viscosity curves of buckwheat material measured with a RVA are showed on Figure 3. Both pure starches (SBH and SBD) have the similar trend of characteristics of pasting properties. Higher RVA results were found for pure starches (SBH and SBD) compared to starches with tailing (Table 3). Based on the previously discussed results that finding could be related to the higher content of proteins and higher particle size determined for starches with tailing (Table 2). Generally, almost no differences were noticed for both pure starches (SBH and SBD). Only setback values was significantly lower ($p<0.05$) for SBH compared to SBD. In this case the impact of SO_2 on starch granules steeped for a longer time and damage or size distribution of granules might partially explain the differences observed with increasing steeping time (Table 2). Shandera and Jackson (1996) found that higher level of SO_2 (from 0.05 to 0.3%) in steep water reduced pasting viscosity. However, Singh et al. (1999) concluded that the SO_2 addition to steep water cause only slight modifications in starch pasting properties. On the other hand, Serna-Saldivar and Mezo-Villanueva (2003) reported that different steeping periods did not show significant differences in the viscoamylograph properties of starch.

Conclusions

Wet-milling process used in this study caused the higher starch extraction efficiency level for total starch isolated from buckwheat with hull than for total starch isolated from dehulled buckwheat. The mean particle diameter of pure starch isolated from buckwheat with or without hull was about 18 μm. Microstructure characteristics analysed by SEM showed that buckwheat starch isolated using wet-milling method had a polygonal and irregular shape and often aggregated. The steeping time affected the starch properties. Several changes in pasting and thermal properties were observed in starch from buckwheat with hull steeped for longer time than dehulled buckwheat. Generally, wet-milling method used in this study did not change significantly the properties of isolated starch compared to raw material. Hull of buckwheat and steeping time did not provoke important changes in starch properties, which may not affect their final use in processed food. The economic factor of increasing steeping time and the utility of the hull fraction isolated from buckwheat kernels in wet milling process have to be taken into account.

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FIGURE CAPTIONS

Figure 1. Particle size distribution of buckwheat flour (BD) and starch buckwheat fractions: SBH, pure starch from buckwheat with hull; TBH, starch with tailings from buckwheat with hull; SBD, pure starch from dehulled buckwheat; TBD, starch with tailings from dehulled buckwheat.

Figure 2. Scanning electron micrograph of dehulled buckwheat flour (BD) and buckwheat starch fractions: SBH, pure starch from buckwheat with hull; TBH, starch with tailings from buckwheat with hull; SBD, pure starch from dehulled buckwheat; TBD, starch with tailings from dehulled buckwheat.

Figure 3. Viscosity curves of buckwheat flour (BD) and starch buckwheat fractions: SBH, pure starch from buckwheat with hull; TBH, starch with tailings from buckwheat with hull; SBD, pure starch from dehulled buckwheat; TBD, starch with tailings from dehulled buckwheat.

518 **Table 1.** Yield of fractions recovered by wet-milling of buckwheat with hull and dehulled

519

Wet-milling fraction	**Yields, g/100 g of kernels in dry matter**	
	buckwheat with hull	**dehulled buckwheat**
Total starch	48.7±1.8	58.8±3.5
pure starch	35.5±0.2	44.1±6.4
starch with tailings	13.2±1.2	14.7±3.0
Protein	3.2±0.6	4.8±0.9
Germ and fibre	1.5±0.3	2.8±0.4
Hull	20.1±1.1	n.a.
Total solids in steep water	9.1±0.3	10.2±0.9
Total solids in washing water	14.8±1.8	19.1±1.8
Total recovery	97.3	95.6

520 Mean±SD, n=3, n.a. not applicable

521 **Table 2.** Extraction efficiency, damage content and particle size distributions of starch
522 fractions

Parameter	Units	Buckwheat (BD)	Buckwheat with hull		Dehulled buckwheat	
			SBH	TBH	SBD	TBD
Extraction efficiency	%	-	64.63a	26.32b	64.20a	18.58b
Proteins	% d.m.	15.18a	0.89b	14.98a	0.87b	10.45a
Whiteness index	%	83.21b	93.28a	67.37c	94.10a	73.39c
Damage starch	%	2.86c	4.77a	4.45a	3.92b	3.34b
Particle size distribution						
D_{10}	µm	13.00a	4.09d	4.73c	4.01d	5.15b
D_{90}	µm	413.40a	24.82c	351.87b	32.61c	460.81a
D_{50}	µm	115.77a	9.73d	31.88c	10.25d	79.01b
D[3,2]	µm	27.77a	5.34d	8.44c	5.42d	10.25b
D[4,3]	µm	170.91a	18.33c	113.90b	19.89c	162.62a

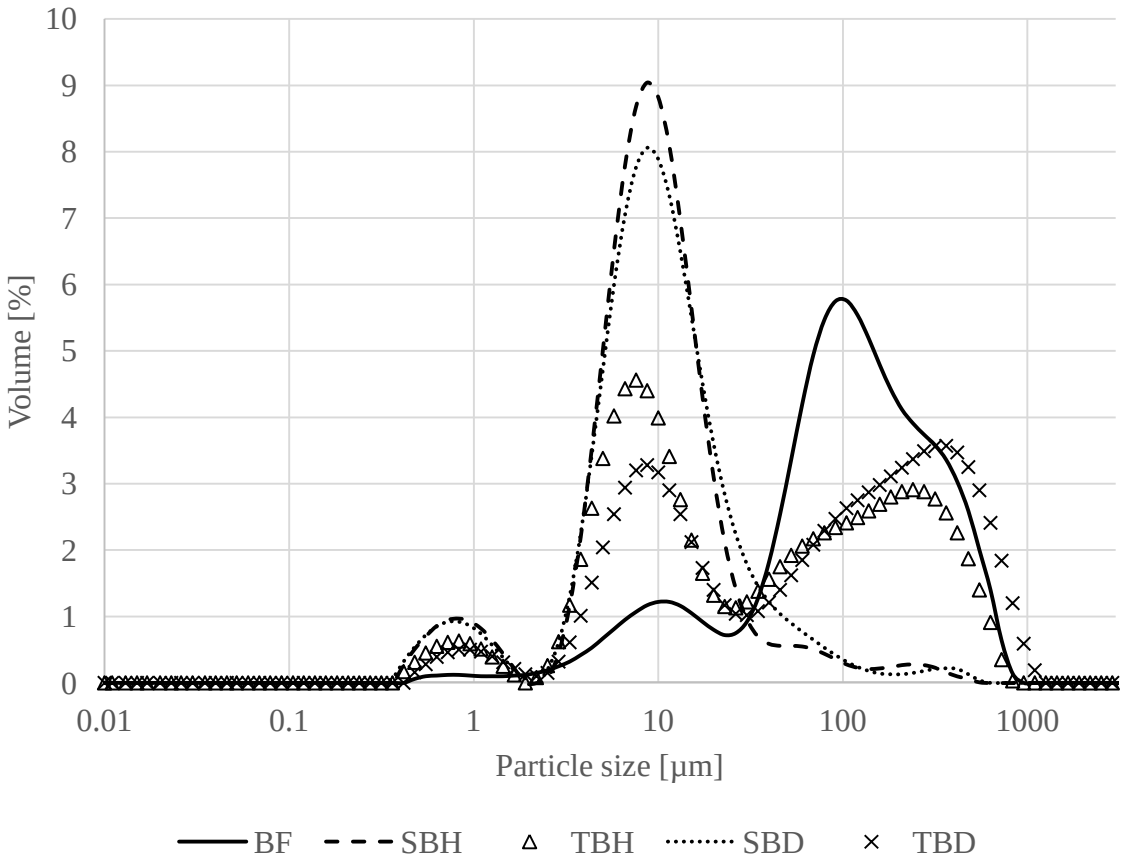
523 Means, n=3. Values within lines followed by the same letter are not significantly different at
524 95% confidence level; d.m. dry matter; D_{90}, largest particle size; D_{50}, the median diameter;
525 D_{10}, smallest particle size; D[3,2], Sauter mean diameter; D[4,3], mean particle diameter.

526 **Table 3.** Thermal and pasting properties of buckwheat starch fractions

527

Parameter	Units	Buckwheat (BD)	Buckwheat with hull		Dehulled buckwheat	
			SBH	TBH	SBD	TBD
Thermal properties (DSC)						
T_o	ºC	67.9a	66.4a	67.2a	68.6a	68.1a
Tp	ºC	74.2a	72.4b	72.6b	75.4a	75.3a
T_c	ºC	80.7b	80.1b	79.7b	84.5a	84.3a
ΔH	J/g of starch d.m.	12.6c	15.1a	14.1b	14.9a	12.5c
Pasting properties (RVA)						
P_{temp}	ºC	61.9a	62.0a	62.3a	62.7a	57.0a
P_{time}	min	7.0a	5.2bc	6.0abc	4.7c	6.5ab
PV	cP	1531a	1686a	866b	1721a	1225a
HPV	cP	1431a	1551a	795b	1461a	1152ab
CPV	cP	2919a	2349a	1326b	2598a	2192ab
Break-down	cP	100a	134a	71b	261a	73b
Setback	cP	1489a	797b	530b	1137a	1040a

528 Mean, n=3. Values followed by the same letter in the same lines are not significantly different
529 at 95% confidence level. DSC, Differential Scanning Calorimetry; T_o, onset temperature; T_p,
530 peak temperature, Tc, conclusion temperature; ΔH, enthalpy of gelatinisation; RVA: Rapid
531 Visco Analyser; P_{temp}, pasting temperature; PV, peak viscosity; HPV, hot paste viscosity;
532 CPV, final or cool paste viscosity; Break-down: PV-HPV; Setback, CPV-HPV; cP,
533 centipoises; d.m., dry matter.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9
8
7
6
5
4
3
2
1
0
Volume [%]
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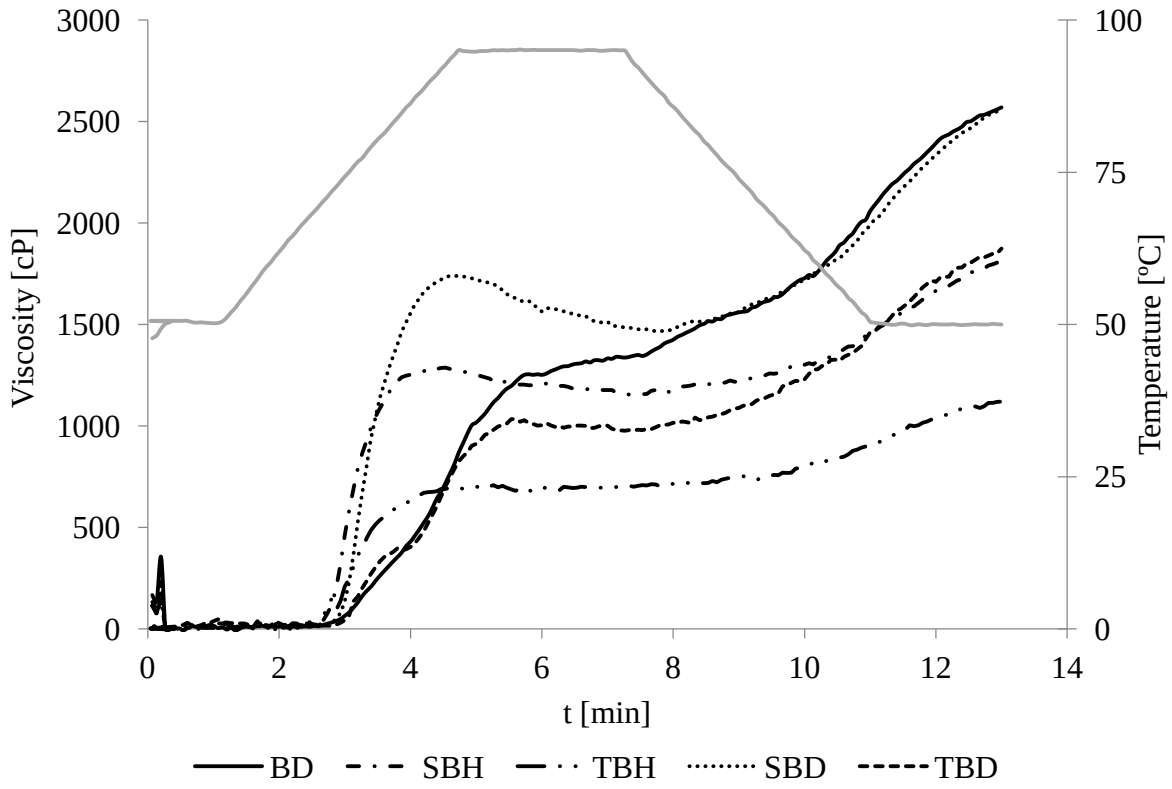
0.1
1
10
100
1000
Particle size [µm]
BF
SBH
TBH
SBD
TBD

534

535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2500
2000
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Viscosity [cP]
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75
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SBH
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536

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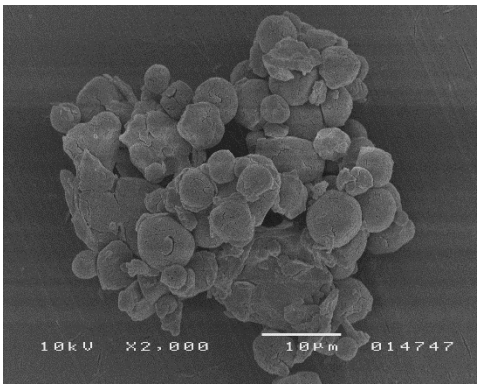
BD



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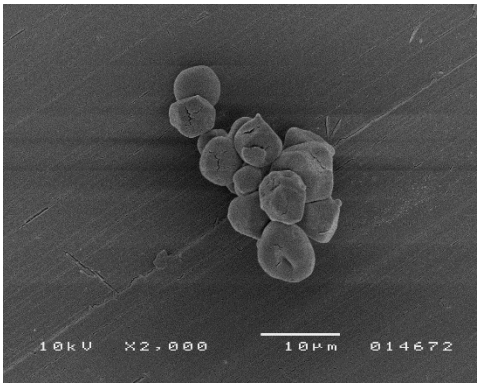

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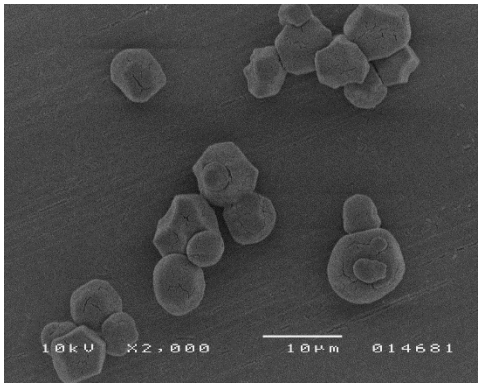

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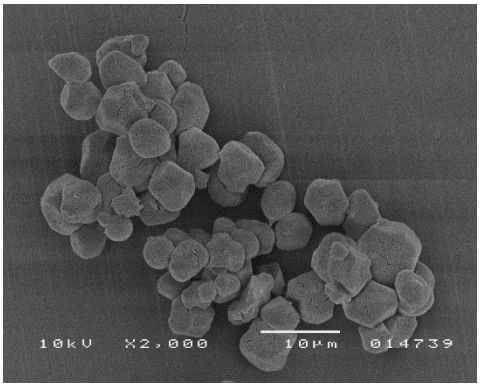

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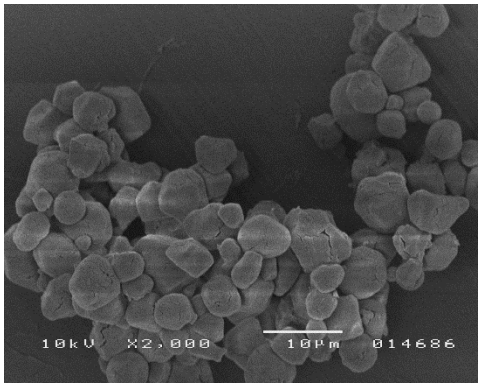

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