SUBMITTED TO:

TITLE OF THE PAPER:

Wet Milling of Buckwheat Cultivars and Some Quality Properties of the Fractions

NAME OF THE AUTHORS:

Ayşenur Arslan^{1,2}, Claudia Monika Haros², Erkan Yalçın^{1*}, Ahmet Güneş³

INSTITUTIONAL AFFILIATIONS OF THE AUTHORS:

¹Department of Food Engineering, Bolu Abant İzzet Baysal University, Bolu, Turkey

²IATA-CSIC, Valencia, Spain

³Bahri Dağdaş International Agricultural Research Institute, Konya, Turkey

***Name of the author to whom the correspondence and proofs to be sent:**

Bolu Abant İzzet Baysal University

Faculty of Engineering

Department of Food Engineering

Gölköy Campus, 14280 Bolu, Turkey

GSM Phone : +90 533 610 3698

Fax : +90 374 253 4558

E-mail : **yalcin_e@ibu.edu.tr**

RUNNING TITLE: Wet-milling of Buckwheat Cultivars

Abstract

The Turkish hulled buckwheat cultivars (*Aktaş cv.* and *Güneş cv.*) were wet-milled and then some chemical, yields, colour, functional properties, phenolic compound, antioxidant activity, and pasting, thermal and retrogradation properties of starches were investigated and compared with the wholegrain buckwheat flour (with husk) and buckwheat groat flour (without husk) of the same cultivars. Sodium dodecyl sulphate polyacrylamide gel electrophoresis patterns of buckwheat flours and protein fractions were also examined under reducing and non-reducing conditions. The hull, germ + dietary fibre, protein and starch fractions were finally collected. The total recovery for *Aktaş cv.* and *Güneş cv.* cultivars were 98.1% and 96.1%; total starch yields were 51.6% and 49.7%; pasting temperatures of the starches were found as 83.7 and 85.7°C; and final viscosities of starches were determined as 3.5 and 3.4 Pa·s, respectively. The resistant starch contents of starch fractions of *Aktaş cv.* ve *Güneş cv.* were found as 3.28% and 3.62%, respectively. The highest total phenolic compound contents were detected with dimethyl sulfoxide extraction in the germ + dietary fibre fractions. The highest 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity and trolox equivalent antioxidant capacity were found in the hull fraction (as 81.7%) and germ + dietary fibre fraction (as 11.8 mmol/kg) of *Aktaş cv.* cultivar.

Keywords: Buckwheat, Wet milling, Nutritional Properties, Functional Properties, Pasting Properties, Thermal Properties

1. Introduction

Pseudocereals, which are dicotyledonous plants, can grow in poor soils and growing conditions not suitable for other cereal grains. They are different than cereals, legumes, oilseeds and nuts. The best known pseudocereals are amaranth, quinoa and buckwheat. Although, they have less economic importance in the world, they have significant contributions to human diet. Pseudocereals recently seem to be fine alternatives for their nutritional, functional and interesting technological properties. Pseudocereals may play a role in human nutrition for those who have allergies to traditional cereals, like wheat, rye, barley (Alvarez-Jubete *et al.*, 2010; Fletcher, 2016).

Generally, there are many species of buckwheat in the world, and mainly nine species have agricultural importance. Among these species, only common buckwheat (*Fagopyrum esculentum* Moench) is generally grown, while *Fagopyrum tartaricum* is grown in some mountainous regions (Li & Zhang, 2001; Yu *et al.*, 2018). Buckwheat (*Fagopyrum esculentum* Moench) is a non-glutinous pseudocereal which has been grown for centuries in Europe and it is one of the most important alternative crops (Wronkowska & Haros, 2014). Common buckwheat (*Fagopyrum esculentum* Moench) flour is widely used in food products due to its desirable taste, textural structure and nutrients (Zhou *et al.*, 2009). For many years, the cultivation of buckwheat had declined, but recently there has been a resurgence of interest in its agriculture because the grain is highly nutritious due to high levels of protein, starch, dietary fibre, some minerals (especially iron, manganese, zinc and selenium), vitamins, flavonoids and other bioactive compounds (Krkoskova & Mrazova, 2005; Qin *et al.*, 2010; Yu *et al.*, 2018). Starch content in buckwheat ranges from 59 to 70% on dry weight basis; the fluctuation is caused by the variable climatic and environmental factors (Malik & Saxena, 2016). In addition, buckwheat bran was also a concentrated source of polyphenolics and

phytic acid. Therefore, consumption of large amounts of buckwheat bran was considered to have significant nutritional or medicinal benefits (Hung *et al.*, 2007).

The wet-milling of pseudocereals is based on the existing process for maize with deep modifications due to the specific characteristics of these grains. Wet-milling is an industrial process involving physical, chemical, biochemical and mechanical operations to separate the principal components of different types of grains (Haros *et al.*, 2004; Haros & Wronkowska, 2017). During wet-milling, the chemical components of the grain, such as starch, protein, fibre and oil (germ), are separated. Wet milling starts with steeping (maceration) in water solutions of alkali (sodium hydroxide) or acid (sodium metabisulfite/sulphur dioxide) at a given temperature. During steeping physical and chemical changes occur in the basic constituents, water diffuses into the grain, softens and degrades the intercellular structure, which allows for efficient milling. The objective of this step is to complete dissociation of endosperm cell components with the release of starch granules from the protein network. It is followed by mechanical separation that is applied due to physical properties (density and particle size) of the fractions: starch, protein, germ and fiber. The presence of lactic acid in the steeping water makes the cell walls easier to break, and for better simulates the industrial steeping process at a laboratory level. The changes in starch properties induced by steeping and milling conditions could be important because its physicochemical characteristics and functional properties determine suitability for use in different industrial processes (Pérez *et al.*, 2001; Wronkowska & Haros, 2014; Haros & Wronkowska, 2017). There are limited works (Zheng *et al.*, 1998; Wronkowska & Haros, 2014; Yu *et al.*, 2018) related to wet-milling process of buckwheat in the literature. In this sense, the main aim of this work was to investigate the wet milling procedure feasibility in Turkish buckwheat cultivars (*cv. Güneş* and *cv. Aktaş*) bred at the Bahri Dağdaş International Agricultural Research Institute (Konya, Turkey). Besides, it carried out a comparative study between cultivars in terms of yields of

each fraction and purity, chemical composition (ash, dietary fibre, starch, resistant starch, lipids, proteins, total phenolic compounds, antioxidant activity), and techno-functional characteristics (absorption of oil or water, colour, thermal and rheology properties) according to the fraction. SDS-PAGE patterns of the wholegrain and groat flours and the protein fractions were also examined.

2. Material and Methods

2.1. Material

The buckwheat (*Fagopyrum esculentum* Moench) cultivars (*Güneş cv.* and *Aktaş cv.*), which were harvested in the summer of 2015 growing season, were provided from the Bahri Dağdaş International Agricultural Research Institute (Konya, Turkey). *Güneş cv.* and *Aktaş cv.* buckwheats were prepared as wholegrain buckwheat flours (G-WBF and A-WBF, respectively) and buckwheat groat flours (G-BGF and A-BGF, respectively). Whole grain buckwheat flours were prepared with hull (husk) after dry-milling in coffee grinder (Arçelik, Turkey). Buckwheat groat flours were prepared after cracking the seed and removing the husk, and then buckwheat groats were ground to get fine flours sieved from 212 µ sieves.

2.2. Wet-milling of hulled buckwheats

The laboratory wet-milling of hulled buckwheat cultivars was carried out according to Wronkowska and Haros (2014) and also based on the preliminary studies in the laboratories of Institute of Agrochemistry and Food Technology (IATA-CSIC, Valencia, Spain). The wet-milling of hulled buckwheat kernels was achieved in automatic fermentor (BIOSTAT B PLUS, Sartorius, Switzerland). For analysis, 25 g of hulled buckwheat were steeped in 250 mL of sodium bisulfite solution (to give 0.25% SO₂). The pH of steeping solution was adjusted to 4.0 by using lactic acid, and then the mixture was mixed at 250 rpm at constant pH

for 16 h. The temperature of the fermentor was adjusted to 30°C. At the end of incubation time, the steeping solution was separated and its volume was recorded. Forty mL of the steeping solution was kept at +4°C. The steeped buckwheat samples were ground (Waring, USA) with a small amount of distilled water for 3 minutes. The slurry was manually sieved through a set of stainless screens: 600 (buckwheat with hull), 300, 75 and 53 μ using distilled water in order to separate the fractions. Hull was retained in the first screen, germ and fibre fractions in the second screen, protein fraction in the third and fourth screens. Then, the washing water was centrifuged (Beckman, Avanti Centrifuge J-25, USA) at 7.000 rpm and +4°C for 20 min. After centrifugation, the volume of supernatants was measured and an amount of the supernatant (40 mL) was kept at +4°C. The pure starch and starch with tailings (sediment) obtained from the centrifuge were kept as precipitate. The starch, protein, germ and fibre, and hull fractions were dried in the oven (Binder, Germany) at 40 °C for 24 h. The yield of each fraction was calculated as a ratio of the totally dried isolated fraction to the initial amount of dried buckwheat sample (25 g). The separated steeping and washing waters (1 mL of each) were vacuum-centrifuged (Eppendorf Concentrator plus, Germany) at 250xg and the temperature of 60°C for 3 h in order to concentrate. After vacuum-centrifugation, the supernatants were evaporated and all particules precipitated were recorded for adding them into total amount. This step was repeated four times. The wet-milling was realized three times and the mean values of the yields of fractions and corresponding standart deviations were reported.

2.3. Chemical properties

Moisture contents of the fractions were determined by using a moisture analyser (KERN DBS 60-3, Kern & Sohn GmbH, Balingen-Frommern, Germany). Moisture, ash, crude oil contents of the flours were determined according to the Approved Methods of AACCI Methods No:

44-15.02, 08-01.01 and 30-25.01 (AACCI, 2010), respectively. Protein contents ($N \times 6.25$) of all samples were determined by the micro-Kjeldahl method according to the Approved Method of AACCI Method No: 46-12.01. The total starch contents of all samples were determined by Megazyme Total Starch (AA/AMG) Assay Kit (Megazyme, Wicklow, Ireland), and total dietary fiber, insoluble and soluble dietary fiber contents of all samples were determined by Megazyme Total Dietary Fiber Assay Kit. Enzyme resistant starch contents of all samples were measured by using Megazyme Resistant Starch Assay Kit. All results were expressed in dry weight basis.

2.4. Total Phenolic Compound Content

Total phenolic compound (TPC) contents of the wet-milling fractions and the buckwheat flours were determined as total (free + bound) and free forms on the basis of modified Folin-Ciocalteu reagent method. The TPC contents were determined by using dimethyl sulfoxide (DMSO) extraction solvent according to the methods of Gutfinger (1981) and Singleton *et al.* (1999). The free phenolic compounds (FPC) were determined according to the methods of Ragae *et al.* (2006) and Zielinski and Kozłowska (2000). The FPC contents of the samples were determined using 80% methanol extraction solvent. The absorbance values of extracts were measured in the spectrophotometer (Shimadzu 1700, Japan) at the wavelength of 725 nm. The standard lines with each extraction solvent were prepared by using ferulic acid. The results were expressed on dry weight basis as mg ferulic acid equivalent/g sample.

2.5. Antioxidant Activity

Antioxidant activity of the wet-milling fractions and the buckwheat flours were determined according to the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity (%) method as described by Yu *et al.* (2002). In addition, antioxidant activity was also detected according to

Ragae *et al.* (2006) with slight modifications as trolox equivalent antioxidant capacity (TEAC, mmol Trolox equivalent / kg sample) by using 2,2'-azinobis-3-ethylbenzotiazolin-6-sulfonate (ABTS•+) as free radical compound. The results were reported as an average of triplicate analysis on dry weight basis.

2.6. Colour analysis and whiteness index

Colour analysis was carried out by using Konica Minolta CR-400 Chromameter. 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 a glass sample cup. The colour parameters were defined as: L^* ($L^* = 0$ [darkness] and $L^* = 100$ [lightness]), a^* ($-a^*$ = greenness and $+a^*$ = redness) and b^* ($-b^*$ = blueness and $+b^*$ = yellowness). All measurements were performed in three replicates. Whiteness index (WI) was calculated according to Wronkowska & Haros (2014) as: $WI = 100 - [(100 - L^*)^2 + (a^*)^2 + (b^*)^2]^{0.5}$.

2.7. Functional Properties of Buckwheat Flours

Water solubility (WS, %) and water binding capacity (WBC, %) values of the buckwheat flours were determined based on the method of Singh and Singh (2003) with slight modifications. Water holding capacity (WHC) and oil absorption capacity (OAC) of the buckwheat flours were measured according to the method of Ahmedna *et al.* (1999) with slight modifications. WHC (grams of water per gram of sample) and OAC (grams of oil per gram of sample) were calculated according to Ahmedna *et al.* (1999). The experiments were carried out as triplicate determinations and the mean values and corresponding standard deviations were reported.

2.8. Pasting Properties

Determinations of pasting properties of buckwheat flours and starches were measured according to the Approved Methods of AACCI Method No: 76-21 (AACCI, 2010) and Wronkowska & Haros (2014). A Rapid Visco Analyser (RVA 4500 Model, Perten Instruments, Perkin-Elmer, Sweden) was used in this experiment. 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 10s. The temperature was first maintained at 50°C for 1 min to have a uniform temperature 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 a rate of 12°C/min and finally held at 50°C for 2 min (Wronkowska & Haros, 2014). 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 achieved in triplicate determinations and average value with standard deviations reported.

2.9. Thermal and Retrogradation Properties of Starches

Thermal and retrogradation properties of buckwheat groat flours and starches were studied according to the method of Wronkowska & Haros (2014). Differential scanning calorimetry (DSC) measurements were made with a Perkin-Elmer DSC-7 (Norwalk, CT, USA). Briefly, 18 mg of flour/starch were directly weighed into DSC stainless steel pans and distilled water was added to obtain a water to starch ratio of 3:1 in order to ensure complete gelatinisation. After sealing, pans were heated from 25 to 110°C at a rate of 10°C/min. An empty pan was used as reference. Twelve replicates for each sample were run. The parameters recorded were onset temperature (T_o , °C), peak temperature (T_p , °C) and conclusion temperature (T_c , °C) of gelatinisation. Straight lines were drawn between T_o and T_c and the enthalpies (ΔH , J/g) associated with starch gelatinisation were calculated as the area enclosed by the straight line and the endotherm curve.

For starch retrogradation studies, the heated and then cooled pans were stored at 4°C for 1, 2, 3, 4, 7 and 15 days. Then, they were heated again in the DSC from 25 to 120°C at a rate of 10°C/min. Each starch sample were analysed as two replicates. Small endotherms of 1-6 J/g were observed around 100°C for starch samples. These thermal transitions were attributed to the amylose-lipid complex according to the studies with cereal starches (Haros *et al.*, 2004).

2.10. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE of buckwheat flours and protein fractions was performed based on the original procedure of Laemmli (1970) and also according to the method of Keskin *et al.* (2018). Protein extraction was done according to the method of Keskin *et al.* (2018). The acrylamide concentrations of resolving and stacking gels were 12.5% and 5%, respectively. The gels were stained overnight with Coomassie Brilliant Blue G-250 (Merck, Germany). Apparent molecular weights were estimated using the wide range protein marker (Product No: S8445, Sigma-Aldrich, St. Louis, Missouri, USA).

2.11. Statistics

Data were analyzed by using an analysis of variance (one-way ANOVA) using the SPSS statistical programme (Version 18, USA) at 95% confidential interval. Fisher's Least Significant Difference test was used to determine the differences among means at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Chemical and nutritional properties of the buckwheat flours

The chemical compositions of wholegrain buckwheat flours (A-WBF and G-WBF) and buckwheat groat flours (A-BGF and G-BGF) were shown in **Table 1**. The moisture, protein,

ash and crude oil contents of buckwheat flours changed in the range of 11.4-12.0%, 8.7-13.5%, 1.0-2.0%, and 1.5-3.2%, respectively. The total starch contents of G-WBF, A-WBF, G- BGF and A- BGF were found as 50.3%, 54.1%, 76.0% and 81.1%, respectively. The total dietary fiber (TDF), insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) contents of A-WBF and G-WBF were determined as 33.8 and 32.7%, 29.7 and 28.2%, 4.0 and 4.5%, respectively; whereas for A- BGF and G- BGF, they were found as 8.6 and 8.8%, 4.9 and 4.9%, 3.7 and 4.0%, respectively. It was seen that wholegrain buckwheat flours had higher contents of TDF and IDF in their compositions, mainly due to the presence of cellulose, hemicellulose, lignins (Repo-Carrasco-Valencia, 2017). Yıldız *et al.* (2019) found that protein contents of wholegrain buckwheat flours and buckwheat groat flours were in the range of 14.4-15.4% on dry weight basis. They also determined the average total dietary fiber, total starch and resistant starch contents of wholegrain buckwheat flours and buckwheat groat flours were 29.5 and 7.6%, 52.9 and 78.1%, 4.64 and 4.38%, respectively, on dry weight basis. Qian *et al.* (1998) reported that the range of starch content in buckwheat groats is 37–70% (db), depending on the species. Zheng *et al.* (1998) reported that commercial buckwheats contain about 11-16% (%Nx6.25) protein, they also stated that buckwheat groats contained 75% starch, 13.9% (%Nx6.25) protein, and 4.9% dietary fiber in their study. It was seen that there were not great differences between the proximal compositions of the cultivars, with the exception of the starch contents of the flours which were clearly higher in *Aktaş* cultivar (**Table 1**).

Wet milling fractions: yields, moisture and chemical compositions

The views and yields of the wet-milling fractions obtained from the hulled buckwheat cultivars were presented in **Figure 1** and **Table 2**, respectively. The wet-milling yields of hull, germ and fibre, total protein and total starch of *Güneş cv.* and *Aktaş cv.* cultivars were

determined as 15.9 and 16.3%; 4.8 and 3.9%; 3.2 and 3.6%; 49.7 and 51.6%, respectively. Total solids leached to steeping water were 12.6% and 12.3%, whereas the total solids in washing water were 9.9% and 10.4% for the buckwheat cultivars of *Güneş cv.* and *Aktaş cv.*, respectively. During wet-milling, the buckwheat grains were exposed to sulphur dioxide diffusion that broke the bonds between protein and starch in order to separate them conveniently. The steeping process can also lead to the solubilisation of pectic substances, rapid hydration of starch and protein molecules and increased water penetration resulting in softening of grains. The amount of starch fraction of *Aktaş cv.* (51.6%) was higher than that of *Güneş cv.* (49.7%) as was expected. Zheng et al. (1998) reported that wet-milling yields of starch, protein and dietary fibre in buckwheat groats were 60.2%, 12.4% and 13.4% and stated that starch was the main contaminant in the protein and tailing fractions about 5-10%. The amounts of total solids in steep water were also higher in both cultivars than the amounts of total solids in washing water. Protein and fiber might transfer into the washing water during wet-milling and the soluble dietary fiber contents of both hulled cultivars might increase the solid contents in both steep and washing waters. The total recovery in *Aktaş cv.* cultivar (98%) was also higher than the total recovery in *Güneş cv.* (96.1%, **Table 2**). Similar recoveries (95.6 and 97.3%) were reported by others in wet milling of buckwheat without hulls and with hulls, respectively (Wronkowska & Haros, 2014). The most important product in the wet-milling process is the starch fraction. It was reported that the recovery, purity, thermal and pasting properties of starch achieved in the wet-milling were dependent on many factors, such as steeping time, steeping temperature, organic acid type used for adjusting pH (Wronkowska & Haros, 2014). Wet-milling performance was evaluated on the basis of extraction efficiency and purity of starch fractions in terms of their protein content and whiteness index in this study. High starch extraction efficiency and low protein content in starch were considered as indicators of an effective process (Wronkowska & Haros, 2014). The starch extraction yield

of *Aktaş cv.* (51.6%) was higher than that of the *Güneş cv.* (49.7%). The crude protein contents of the starch fractions with tailings of *Aktaş cv.* and *Güneş cv.* were found as 6.2% and 5.8%, respectively (**Table 2**). The purpose of wet-milling is to keep the protein content of starch as low as possible in order to assure a better separation of protein and starch components of the kernel (Wronkowska & Haros, 2014).

The colour and functional properties

The colour properties of buckwheat kernels, flours and starch fractions were shown in **Table 3**. The colour of starch has an impact on its quality. Any pigmentation in the starch is carried over to the final product and this reduces the quality and acceptability of the starch product. A low value for chroma and a high value for lightness (L^*) are desired for the starch to meet the consumer preference. In this study, the L^* values of the starch fractions of *Aktaş cv.* and *Güneş cv.* were determined as 98.4 and 93.0, respectively. It was obvious that they were higher than that of both types of flours. The whiteness index values of starches were near to white, such as 97.2% for *Aktaş cv.* and 91.5% for *Güneş cv.* The low whiteness index value could be attributed to the dark hull pigment contamination.

Physicochemical and functional properties of plant flours play an important role in estimation of their processing behaviours. The starches and proteins are two main components of plant flours for acting these physicochemical and functional properties. Therefore, water solubility (WS, %), water binding capacity (WBC, %), water holding capacity (WHC, %) and oil absorbtion capacity (OAC, %) properties of all buckwheat flours and the starch fractions of two cultivars were presented in **Table 3**. The WS (%) results of wholegrain buckwheat flours (A-WBF, G-WBF) were found as 5.8 and 5.9% (respectively, $p>0.05$), whereas WS (%) of buckwheat groat flours (A-BGF, G-BGF) were 5.1 and 5.4% (respectively, $p>0.05$). It was seen that WS values of wholegrain buckwheat flours were higher than that of groat flours

($p < 0.05$). The soluble dietary fibre may be included in the steeping water. Similarly, WBC (%) of wholegrain buckwheat flours were higher than that of groat flours, and the difference between both flour types was found as significant ($p < 0.05$). In addition, WBC (%) results of wholegrain buckwheat flours (A-WBF, G-WBF) were found as 149.3 and 147.3% (respectively, $p > 0.05$), whereas WBC (%) of buckwheat groat flours (A-BGF, G-BGF) were 119.9 and 118.2% (respectively, $p > 0.05$). WBC is also defined as swelling power in some literature. Water binding capacity (WBC) indicates the degree of water absorption and association of the molecules with starch granules (Malik & Saxena, 2016) and also dietary fibres. Yu *et al.* (2018) reported that WBC provides information on the strength of starch inter-granular bond; low water binding is attributed to a close association of starch polymers in the native granule. They also indicated that WBC is positively correlated with the damaged starch content.

Water holding capacity (WHC, g H₂O/g flour) values of wholegrain buckwheat flours (A-WBF and G-WBF) were 1.70 and 1.72 g H₂O/g flour, respectively ($p > 0.05$); whereas buckwheat groat flours (A-BGF and G-BGF) had 1.28 and 1.30 g H₂O/g flour, respectively ($p > 0.05$). Wholegrain buckwheat flours had higher WHC values than groat flours, and the difference between both types of flours was found as significant ($p < 0.05$). Since wholegrain buckwheat flours includes higher contents of dietary fibers coming from the hull + bran fractions which are mostly insoluble dietary fibres, than that of the groat flours (Repo-Carrasco-Valencia, 2017). The highest oil absorption capacity (OAC, g oil/g flour) was detected with the flour sample of G-WBF as 1.17 g oil/g flour which was statistically similar with the sample of A-WBF (1.05 g oil/g flour, $p > 0.05$), besides it was higher than the OAC values of groat flours (0.86 g oil/g flour, $p < 0.05$). WS, WBC and WHC values of the starch fractions were almost similar each other ($p > 0.05$), however these values were lower than that of the buckwheat flours ($p < 0.05$). Although the OAC values of the starch fractions were

higher than that of the buckwheat groat flours, there were not statistically differences ($p>0.05$). In addition, the OAC values of the starch fractions were lower than that of the wholegrain buckwheat flours, but the difference between the starch fractions and G-WBF was statistically important ($p<0.05$, Table 3). Since, the wholegrain buckwheat flours included more proteins which had probably hydrophobic character and that increased the OAC values.

SDS-PAGE analysis of buckwheat flours and protein fractions

Wholegrain buckwheat flours, buckwheat groat flours and protein fractions were analysed on SDS-PAGE either with or without reducing agent (5% 2- β -mercaptoethanol) and electrophoretic profiles were shown in **Figure 2**. The electrophoretic results were well in agreement with the results of Radovic *et al.* (1996) and Tang & Wang (2010). The intensity of protein bands in buckwheat groat flours (Lane 3 and 4 in both gels) were higher than that of the wholegrain buckwheat flours. The electrophoretic patterns of buckwheat flours under both conditions exhibited typical buckwheat storage proteins which were indicated on both gels. It was expected that the 13S globulins (consisting of acidic and basic polypeptides linked by disulphide bonds) and 2S albumins were the major proteins in buckwheat flours, whilst the 8S globulins could still be observed (Figure 2 A and B). The albumin (2S) and globulin (13S) proteins could be seen in both type of flours (Lane 1-4, Figure 2 A and B). The protein fractions still included those proteins but their intensity were lower than that of flour proteins (Lane 5-6, Figure 2 A and B). SDS-PAGE analysis under non-reducing (Figure 2 A) and reducing conditions (Figure 2 B) exhibited that disulfide bonds existed in the flours and also in protein fractions, since the separation of the protein groups was clearly achieved.

Buckwheat has gained an excellent nutritional quality and health benefits due to its protein composition. The protein content in buckwheat seeds changes from 8.5-18.9% depending on cultivar. It is reported that albumin and globulin are the major storage proteins in buckwheat

seeds, and prolamin and glutelin contents are very low. *Fagopyrum esculentum* Moench specy has two main protein types which are salt-soluble globulins (13S proteins) with the molecular weight of 280 kDa and water-soluble albumins (2S proteins) with the molecular weight range of 8-16 kDa. The 13S globulin protein, which has a hexameric structure with disulphide-bonded subunits composed of acidic and basic polypeptides, consists of three fractions with molecular weights between 26 and 36, 43 and 68, 57 and 58 kDa. Buckwheat protein has a high biological value due to a well-balanced amino acid composition, like richness in lysine, methionine, tryptophan and arginine. Furthermore, dietary buckwheat protein exhibites remarkable physiological effects, particularly hypocholesterolemic activity, suppressing body fat synthesis and inhibiting mammary carcinogenesis and colon cancerous lesion (Radovic *et al.*, 1996; Radovic *et al.*, 1999; Wijngaard & Arendt, 2006; Guo & Yao, 2006; Tang & Wang, 2010; Tazawa *et al.*, 2014).

Total and free phenolic compound and antioxidant activity of buckwheat products

Total and free phenolic compound (TPC and FPC, respectively) contents, DPPH $\cdot$ radical scavenging activities and TEAC antioxidant capacities of buckwheat flours and wet milling fractions were shown in **Table 4**. The TPC contents determined with DMSO included free and bound forms of total phenolic compound. According to DMSO extractions, germ and fiber fractions of both cultivars had the highest TPC contents among the samples ($p<0.05$). Thus, the highest TPC content with DMSO extraction was found in germ and fiber fraction of *Aktaş cv.* cultivar (12.4 mg FA/g sample) that was followed by germ and fiber fraction of *Güneş cv.* cultivar (10.8 mg FA/g sample, $p<0.05$). The TPC contents of protein fractions of *Aktaş cv.* and *Güneş cv.* cultivars were determined as 8.2 and 7.2 mg FA/g sample ($p<0.05$). The free phenolic compound (FPC) contents of the buckwheat products were determined with 80% MetOH. The highest FPC were found in wholegrain buckwheat flours as 5.1 and 5.2 mg

FA/g sample for *Aktaş cv.* and *Güneş cv.* cultivars, respectively ($p>0.05$). The FPC contents of germ and fiber fractions of *Aktaş cv.* (4.7 mg FA/g sample) and *Güneş cv.* (4.4 mg/g sample) followed those values obtained with wholegrain buckwheat flours ($p<0.05$). The TPC and FPC contents of wholegrain buckwheat flours were higher than that of the buckwheat groat flours in this study ($p<0.05$). The starch fractions obtained from wet milling had the lowest phenolic compound contents in both extraction procedures ($p<0.05$). The highest DPPH radical scavenging activity was determined with the hull fraction of *Aktaş cv.* cultivar as 81.7% ($p<0.05$). The DPPH radical scavenging activity of wholegrain buckwheat flours of *Aktaş cv.* (62.2%) and *Güneş cv.* (59.4%) were higher than that of the buckwheat groat flours of *Aktaş cv.* (53.0%) and *Güneş cv.* (51.9%) ($p<0.05$). The hull fraction of *Güneş cv.* (58.1%) was lower than that of the *Aktaş cv.* in terms of the DPPH radical scavenging activity ($p<0.05$). The highest TEAC value was found in germ and fiber fraction of *Aktaş cv.* as 11.8 mmol/kg sample and it was followed by the TEAC value of the hull fraction of *Aktaş cv.* (11.1 mmol/kg sample, $p<0.05$). The TEAC values of the wholegrain flours were higher than that of the groat flours ($p<0.05$). The starch fractions obtained from wet milling exhibited the lowest phenolic compound contents and antioxidant properties in this study.

Whole buckwheat grains contain more phenolic compounds than that of oats and barley, whereas buckwheat bran and hulls have higher antioxidant activity than that of barley, triticale and oats (Repo-Carrasco-Valencia, 2017). Yıldız *et al.* (2019) found that total phenolic compound contents and antioxidant activity were higher in wholegrain buckwheat flours than that of the buckwheat groat flours. Guo *et al.* (2012) found that free phenolic contents of tartary buckwheat samples were much higher than that of bound phenolic contents. They also found that the fine bran and hull had the greatest free phenolic and bound phenolics, respectively, and flavonoid contents. Sedej *et al.* (2010) indicated that buckwheat flours had better scavenging abilities on free radical compounds compared to wheat flour.

Bioactive compounds are phytochemicals present in plants that can promote health. Phytochemicals in fruit and vegetables can act as antioxidants protecting the cells. Polyphenol compounds, like flavonoids, have been extensively studied health promoting phytochemicals that prevent degenerative diseases (Repo-Carrasco-Valencia, 2017). Pseudocereals are characterized for being rich in many “health-promoting” phytochemicals, such as polyphenols and dietary fiber which exhibit antioxidant and free-radical scavenging activity (Rollán *et al.*, 2019). It is indicated that the primary antioxidants in buckwheat are rutin, quercetin, hyperin and catechins, and buckwheat is the only pseudocereal containing rutin. Buckwheat hulls contained the highest levels of rutin and quercetin (Oomah & Mazza, 1996). Yıldız *et al.* (2019) found that rutin flavonoid contents of the wholegrain buckwheat flours were higher than that of the buckwheat groat flours. An average quercetin flavonoid contents in wholegrain buckwheat flours and buckwheat groat flours were found as 10.8 and 11.23 mg/100 g of flour, respectively (Yıldız *et al.*, 2019). Whole buckwheat and buckwheat hulls and bran have high antioxidant activity compared to oat and barley (Repo-Carrasco-Valencia, 2017).

Pasting properties of buckwheat flours and starch fractions

Pasting properties of the buckwheat flours (A-WBF, G-WBF, A-BGF and G-BGF) and starch fractions obtained from hulled buckwheat kernels in wet milling were presented in **Table 5**. The pasting properties are usually studied by investigating the viscosity of a system containing starch. The pasting curve can be used to indicate the extent of disintegration and reassociation of starch molecules (Malik & Saxena, 2016). The pasting temperatures of buckwheat samples changed in the range of 73.8-88.3 °C. The highest pasting temperature was seen with the sample of G-BGF (88.3 °C) followed by the sample of A-BGF (86.5 °C) and the difference was not statistically significant ($p>0.05$). The pasting temperatures of the

starch fractions of *Aktaş cv.* and *Güneş cv.* were recorded as 83.7°C and 85.7°C, respectively ($p>0.05$). These pasting temperatures were higher than that of the buckwheat starches studied by Yoshimoto *et al.* (2004). The pasting temperatures of the wholegrain buckwheat flours were found as the lowest values and statistically similar ($p>0.05$). Zheng *et al.* (1998) indicated that pasting temperature of native buckwheat starch was 75°C, similar to corn and rice starches. The peak times of A-Starch and G-Starch fractions (6.4 and 6.3 min, respectively) were shorter than the peak times of the flours ($p<0.05$). The peak times of all buckwheat flours were around 7.0 min and statistically similar ($p>0.05$). The peak viscosity values of the starch fractions were found as the highest (2.3 Pa·s) and statistically similar each other ($p>0.05$). They were followed by the peak viscosity values of the A-WBF (1.8 Pa·s) and G-WBF (2.0 Pa·s) wholegrain buckwheat flours ($p<0.05$). The peak viscosity of the buckwheat groat flours were the lowest values (1.0 Pa·s) among the samples and statistically similar ($p>0.05$). Viscosity increase during heating can be attributed to swelling of starch granules. The stable viscosity increase of buckwheat on heating was probably a result of the rigidity of its granules. Hot-paste viscosity (HPV) values of the starch fractions were found as the highest values (2.2 Pa·s) among the samples and they were statistically similar each other ($p>0.05$). They were followed by HPV values of A-WBF (1.7 Pa·s) and G-WBF (1.9 Pa·s) wholegrain buckwheat flours ($p<0.05$). The lowest HPV values ($p>0.05$) were observed with the buckwheat groat flours. The breakdown viscosities of all buckwheat samples were same ($p>0.05$) and found as 0.1 Pa·s. In native buckwheat starch, there was no peak and breakdown viscosity observed during heating process, instead, there was a continue increase in viscosity on holding at 95°C (Zheng *et al.*, 1998). The highest final viscosity was found with the wholegrain flour of *Güneş cv.* cultivar as 3.7 Pa·s, it was followed by the starch fraction of *Aktaş cv.* cultivar (3.5 Pa·s, $p>0.05$). They were followed by the samples of G-Starch (3.4 Pa·s) and A-WBF (3.2 Pa·s) and the difference was statistically significant ($p<0.05$). The final

viscosities of the groat flours of *Aktaş cv.* and *Güneş cv.* cultivars were found as the lowest among the samples and statistically comparable (1.8 Pa·s and 1.7 Pa·s, respectively, $p>0.05$). It was reported that an increase in HPV and final viscosity of starch fractions compared to buckwheat flours may be due to increased intra granular binding force which makes the granule resistant to deformation, an increased final viscosity is due to the reassociation activities of starch molecules (Malik & Saxena, 2016). Qian *et al.* (1998) indicated that buckwheat starch had significantly higher peak viscosity, trough, and final viscosity than did corn and wheat starches. This was due to the higher amylose content and water-binding capacity of buckwheat starch. The highest setback viscosity values were observed in wholegrain buckwheat flours among the samples, for instance G-WBF had the highest setback value (1.8 Pa·s), it was followed by the setback value of A-WBF (1.5 Pa·s) and the difference was statistically found as significant ($p<0.05$). The setback values of starch fractions of *Aktaş cv.* and *Güneş cv.* (1.3 Pa·s and 1.2 Pa·s, respectively) were higher than that of the buckwheat groat flours of *Aktaş cv.* (0.9 Pa·s) and *Güneş cv.* (0.8 Pa·s), and the difference was statistically found as significant ($p<0.05$). The setback viscosity of cooked starch pastes is mainly a result of re-association of leached amylose molecules. Zheng *et al.* (1998) indicated that the setback viscosity of native buckwheat starch was higher than corn and rice starches demonstrating its higher granule rigidity and/or higher amylose leaching. It is known that the lower setback viscosity value causes reducing of retrogradation tendency (Zheng *et al.*, 1998). Shandera & Jackson (1996) also reported that higher levels of SO₂ (from 0.05 to 0.3%) in steeping water reduced the pasting viscosity. However, Singh *et al.* (1999) concluded that SO₂ addition to steeping water could cause only slight modifications in starch pasting properties.

Thermal properties of buckwheat flours and starch fractions

Thermal properties of the buckwheat grout flours (A-BGF and G-BGF) and starch fractions obtained from hulled buckwheat cultivars in wet milling were presented in **Table 5**. The gelatinization temperature of A-BGF was in the range of 63.1-76.4 °C with the peak temperature of 69.5 °C. The gelatinization temperature of G-BGF was in the range of 63.8-77.4 °C with the peak temperature of 70.4 °C. Thermal properties of the both flours were statistically similar ($p>0.05$). Besides, the gelatinization temperature of starch obtained from *Aktaş cv.* was in the range of 60.7-73.8 °C with the peak temperature of 66.5 °C; and the gelatinization temperature of starch obtained from *Güneş cv.* cultivar was in the range of 62.1-76.1 °C with the peak temperature of 68.2 °C. The differences among the DCS parameters (T_o , T_p , T_c) of both starch fractions were statistically found as significant ($p<0.05$). In addition, the onset temperature (T_o) of the starch obtained from *Aktaş cv.* cultivar was the lowest (60.7 °C) compared to the onset temperatures of starch fraction obtained from *Güneş cv.* cultivar and the buckwheat groat flours ($p<0.05$). The G-BGF had the highest peak temperature (70.4 °C), however it was not statistically different than that of the value of A-BGF ($p>0.05$), but the differences with the starch fractions were statistically found as significant ($p<0.05$). The starch fraction of *Aktaş cv.* cultivar had the lowest conclusion temperature (T_c , 73.8 °C) among the samples ($p<0.05$). The gelatinization enthalpies of the starch fractions were higher than that of the buckwheat groat flours. It was observed that the starch fraction of *Aktaş cv.* cultivar had the highest gelatinization enthalpy (11.2 J/g of starch), however it was statistically different than the gelatinization enthalpy of buckwheat groat flours ($p<0.05$), instead it was statistically similar with the starch fraction of *Güneş cv.* cultivar ($p>0.05$). It was noticed that the gelatinization enthalpies of the buckwheat groat flours were similar to each other ($p>0.05$). Qian *et al.* (1998) also found that the ΔH values (10.0 J/g of starch) of buckwheat and wheat starches were same, but was significantly lower than corn starch (11.3 J/g of starch). The gelatinization indexes (PHI) of the starch fractions

were also higher than that of the buckwheat groat flours. The gelatinization index of the starch fraction obtained from *Aktaş cv.* cultivar was the highest among the samples.

Qian *et al.* (1998) reported that gelatinization temperatures, determined by DSC, were 61.1-80.1°C for buckwheat starch compared to 64.7-79.2°C and 57.1-73.5°C for corn and wheat starches, respectively. Zheng *et al.* (1998) reported that DSC analysis of native buckwheat starch revealed a gelatinization temperature range of 63 to 81 °C with a peak at 69 °C, both values being similar to those of corn starch but lower than those for rice starch. The enthalpy value of native buckwheat starch was found as 12.7 J/g. It is reported that the peak gelatinization temperature is changing according to buckwheat cultivar and the protein content in starch also affected the thermal parameters by binding to water rapidly and preventing the hydration of starch. Wijnagaard & Arendt (2006) indicated that the peak gelatinization temperature of buckwheat starch was in the range of 63.7-70.8. T_o , T_p , T_c and ΔH values of native buckwheat starch were reported as 65.2, 70.8, 76.6 °C, and 1.046 J/g, respectively (Malik & Saxena, 2016). In this study, the thermal properties of buckwheat groat flours and starch fractions investigated in DSC had similar tendency as indicated by Zhou *et al.* (2009). They stated that the gelatinization temperatures of buckwheat flour and starch were 67.9°C and 71.9°C, respectively. Furthermore, onset (T_o) and peak (T_p) temperatures found in this study were resemble to the same values of buckwheat starches determined by Yoshimoto *et al.* (2004), however the conclusion (T_c) temperature and gelatinization enthalpy (ΔH) values found in this study were lower than that of the values determined by Yoshimoto *et al.* (2004).

Retrogradation properties of buckwheat starch fractions

The retrogradation properties of starch fractions of buckwheat cultivars were shown in **Table 6**. Retrogradation of buckwheat starches was observed during 15 days storage at +4 °C. The

onset (T_o), peak (T_p) and conclusion (T_c) temperatures and the retrogradation enthalpy (ΔH) values were generally quite similar for each sample during 15 days. ΔH and RI values increased in both starch fractions while increasing the storage time. An increasing of retrogradation enthalpy (ΔH) means that the starch granules become more crystal structure during storage (Yu *et al.*, 2018). Retrogradation index (RI) values in each day were higher for the starch fraction of *Güneş cv.* than that of *Aktaş cv.* cultivar. Some studies showed that cooked native starches can easily retrograde and it has a gelling tendency of pastes, and the syneresis may take place (Malik & Saxena, 2016). Qian et al. (1998) stated that buckwheat starch retrogradation was slower than that of corn and wheat starch at +4°C for 10 days, but it increased as storage time increased, as did that of the other starch pastes.

Conclusions

Turkish hulled buckwheat cultivars (*Aktaş cv.* and *Güneş cv.*) were investigated for their wet milling potential first time in this study. This work demonstrated that starch, protein, hull, germ and fiber fractions could be isolated from hulled buckwheat kernels by wet milling process. The starch extraction efficiency from hulled buckwheat cultivars was could be increased during wet-milling process due to some modifications. Generally, the wet milling method used in this study did not significantly change the properties of isolated starch compared to the buckwheat flours. The proteins fractions obtained from wet milling were examined in SDS-PAGE first time in this research and the protein fractions still included 13S hexameric subunits. Wet-milling fractions contain several nutritional compounds for nuraceutical benefits, such as soluble and insoluble dietary fibers, resistant starch, phenolics and protein. This study concluded that the wet milling fractions of hulled buckwheat could be used as healthy food ingredient after further studies. Further study is especially needed to

know how process conditions affect the structure of protein fractions and their functional properties.

Acknowledgement

The authors would like to thank to the Bahri Dağdaş International Agricultural Research Institute (Konya, Turkey) for providing buckwheat cultivars. This work was financially supported by grants QuiSalhis-Food (AGL2016-75687-C2-1-R) from the Ministry of Science, Innovation and Universities (MICIU), CYTED la ValSe-Food (119RT0S67) and LINCE (PROMETEO/2017/189) by the Generalitat Valenciana (Spain). The fellowship given to Ayşenur Arslan by BAİBU ERASMUS+ Office (EU) is gratefully acknowledged.

Conflict of interest

The authors declare that they have not any conflicts of interest.

References

- AACCI (American Association of Cereal Chemists International) (2010) Approved Methods of Analysis, Metots No: 44-15.02, 08-01.01, 30-25.01, 46-12.01, 76-21.01, 11th edition, The Association: St. Paul, MN, USA.
- Ahmedna M, Prinyawiwatkul W, Rao RM 1999. Solubilized Wheat Protein Isolate: Functional Properties and Potential Food Applications. *Journal of Agricultural and Food Chemistry*, 47: 1340-1345.
- Alvarez-Jubete L, Arendt EK, Gallagher E 2010. Nutritive Value of Pseudocereals and Their Increasing Use as Functional Gluten-Free Ingredients. *Trends in Food Science & Technology*, 21: 106-113.
- Fletcher, R. J. 2016. Pseudocereals: Overview. *Encyclopedia of Food Grains* (Second Edition), Editors-in-Chief: Colin Wrigley, Koushik Seetharaman, Harold Corke, Jon Faubion, Elsevier Ltd., p.274-279.

- Guo, X., Yao, H. 2006. Fractionation and characterization of tartary buckwheat flour proteins. *Food Chemistry*, 98, 90-94.
- Guo, X-D., Wu, C-S., Ma, Y-J., Parry, J., Xu, Y-Y., Liu, H., Wang, M. 2012. Comparison of milling fractions of tartary buckwheat for their phenolics and antioxidant properties. *Food Research International*, 49 (1), 53-59.
- Gutfinger T. 1981. Polyphenols in Olive Oils. *Journal of the American Oil Chemist's Society (JAOCs)*, 58: 966-968.
- Haros M., Perez O.E., Rosell C.M. 2004. Effect of Steeping Corn with Lactic Acid on Starch Properties. *Cereal Chemistry*, 81(1): 10-14.
- Haros, C.M., Wronkowska, M. 2017. Pseudocereals Dry and Wet Milling: Processes, Products and Applications. In "Pseudocereals: Chemistry and Technology", Eds: Claudia Monika Haros, Regine Schoenlechner, Wiley Blackwell, p. 140-162.
- Hung, P.V., Maeda, T., Tsumori, R., Morita, N. 2007. Characteristics of Fractionated Flours from Whole Buckwheat Grain Using a Gradual Milling System and Their Application for Noodle Making. *Journal of the Science of Food and Agriculture*, 87: 2823-2829.
- Keskin, Ş., Yalçın, E., Özkaya, H. 2018. Effects of Storage and Granary Weevil Infestation on Gel Electrophoresis and Protein Solubility Properties of Hard and Soft Wheat Flours. *Journal of Economic Entomology*, 111, Issue 3, 1454-1460.
- Krkoskova B., Mrazova Z. 2005. Prophylactic components of buckwheat. *Food Research International*, 38,5, 561-568.
- Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. *Nature*, 227: 680-685.
- Li, S-Q., Zhang, Q. H. 2001. Advances in the Development of Functional Foods from Buckwheat. *Critical Reviews in Food Science and Nutrition*, 41(6): 451-464.

- Malik, M.A., Saxena, D.C. 2016. Effect on physicochemical and thermal properties of buckwheat (*Fagopyrum esculentum*) starch by acid hydrolysis combined with heat moisture treatment. *Journal of Food Processing and Preservation*, 40, 1352–1363.
- Oomah, B.D., Mazza, G., 1996. Flavonoids and antioxidative activities in buckwheat. *Journal of Agricultural and Food Chemistry* 44: 1746–1750.
- Perez, O.E., Haros, M., Suarez, C. 2001. Corn steeping: influence of time and lactic acid on isolation and thermal properties of starch. *Journal of Food Engineering*, 48, 251-256.
- Qian, J., Rayas-Duarte, P., Grant, L. 1998. Partial Characterization of Buckwheat (*Fagopyrum esculentum*) Starch. *Cereal Chemistry*, 75(3):365-373.
- Qin, P., Wang, Q., Shan, F., Hou, Z., Ren, G. 2010. Nutritional Composition and Flavonoids Content of Flour from Different Buckwheat Cultivars. *International Journal of Food Science and Technology*, 45: 951-958.
- Radovic, S.R., Maksimovic, V.R., Varkonji-Gasic, E.I. 1996. Characterization of Buckwheat Seed Storage Proteins. *Journal of Agricultural and Food Chemistry*, 44, 972-974.
- Radovic, R.S., Maksimovic, R.V., Brkljacic, M.J., Varkonji Gasic, I.E., Savic, P.A. 1999. 2S Albumin from Buckwheat (*Fagopyrum esculentum* Moench) Seeds. *Journal of Agricultural and Food Chemistry*, 47, 1467-1470.
- Ragaei, S., Abdel-Aal, E.S.M., Noaman, M. 2006. Antioxidant Activity and Nutrient Composition of Selected Cereals for Food Use. *Food Chemistry*, 98: 32-38.
- Repo-Carrasco-Valencia, R. 2017. Dietary Fibre and Bioactive Compounds of Kernels. In “Pseudocereals: Chemistry and Technology”, Eds: Claudia Monika Haros, Regine Schoenlechner, Wiley Blackwell, p. 140-162.
- Rollán GC, Gerez CL and LeBlanc JG 2019. Lactic Fermentation as a Strategy to Improve the Nutritional and Functional Values of Pseudocereals. *Frontiers in Nutrition*, Vol. 6, Article 98, 1-16. doi: 10.3389/fnut.2019.00098

- Sedej, I., Mandić, A., Sakač, M., Mišan, A. and Tumbas, V. 2010. Comparison of antioxidant components and activity of buckwheat and wheat flours. *Cereal Chemistry* 87: 387-392.
- Shandera, D.L., Jackson, D.S. 1996. Effect of corn wet-milling conditions (sulphur dioxide, lactic acid, and steeping temperature) on starch functionality. *Cereal Chemistry*, 73 (5), 632-637.
- Singh, V., Haken, A.E., Dowd, M.K., Niu, Y.X., Zou, S.H., Eckhoff, S.R. 1999. Batch steeping of corn: effects of adding lactic acid and sulfur dioxide at different times on starch yields, protein contents, and starch pasting properties. *Cereal Chemistry*, 76, 600-605.
- Singh, J., Singh, N. 2003. Studies on the morphological and rheological properties of granular cold water soluble corn and potato starches. *Food Hydrocolloids*, 17: 63–72.
- Singleton. V.L., Orthofer, R., Lamuela-Raventos, R.M. 1999. Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. *Methods of Enzymology*, 299: 152-178.
- Tang, C-H., Wang, X-Y. 2010. Physicochemical and structural characterisation of globulin and albumin from common buckwheat (*Fagopyrum esculentum* Moench) seeds. *Food Chemistry*, 121, 119-126.
- Tazawa, S., Katayama, S., Hirabayashi, M., Yamaguchi, D., Nakamura, S. 2014. Improvement of Surface Functionalities, Including Allergenicity Attenuation, of Whole Buckwheat Protein Fraction by Maillard-Type Glycation with Dextran Preventive Nutrition and Food Science, 19(4):327-332.
- Wijngaard, H.H., Arendt, E.K. 2006. Buckwheat. *Cereal Chemistry*, 83 (4), 391-401.
- Wronkowska M., Haros M. 2014. Wet-Milling of Buckwheat with Hull and Dehulled-The Properties of the Obtained Starch Fraction, *Journal of Cereal Science*, 60: 477-483.

- Yıldız, N., Yalçın, E., Güneş, A. 2019. Chemical, nutritional and bioactive properties of common buckwheat cultivars bred in Turkey, *Quality Assurance and Safety of Crops & Foods*, 11 (2), 137-143. <https://doi.org/10.3920/QAS2018.1391>
- Yoshimoto, Y., Egashira, T., Hanashiro, I., Ohinata, H., Takase, Y., Takeda, Y. 2004. Molecular Structure and Some Physicochemical Properties of Buckwheat Starches. *Cereal Chemistry*, 81(4):515–520.
- Yu, L., Haley, S., Perret, J., Haris, M., Wilson, J., Qian, M. 2002. Free Radical Scavenging Properties of Wheat Extracts. *Journal of Agricultural and Food Chemistry*, 50: 1619-1624.
- Yu, D., Chen, J., Ma, J., Sun, H., Yuan, Y., Ju, Q., Teng, Y., Yang, M., Li, W., Fujita, K., Tatsumi, E., Luan, G. 2018. Effects of different milling methods on physicochemical properties of common buckwheat flour. *LWT - Food Science and Technology*, 92, 220-226.
- Zheng, G.H., Sosulski, F.W., Tyler, R.T., 1998. Wet-milling, composition and functional properties of starch and protein isolated from buckwheat groats. *Food Research International*, 30, 493-502.
- Zhou, Y.-G., Li, D., Wang, L.-J., Li, Y., Yang, B.-N., Bhandari, B., Chen, X.D., Mao, Z.-H. 2009. Effect of water content on thermal behaviours of common buckwheat flour and starch. *Journal of Food Engineering*, 93, 242-248.
- Zielinski, H., Kozłowska, H. 2000. Antioxidant activity and total phenolics in selected cereal grains and their different morphological fractions. *Journal of Agricultural and Food Chemistry*, 48: 2008-2016.

Figure 1

a.

b.

Figure 1. Wet milling fractions (from left to right are: hulls, dietary fibre, germ, protein and starch) of the hulled buckwheats (**a.** *Aktaş cv.*, **b.** *Güneş cv.*) obtained in this study.

Figure 2

Figure 2. SDS-PAGE profiles of wholegrain buckwheat flours, buckwheat groat flours and protein fractions obtained from wet-milling process in unreduced (A) and reduced (B) conditions. **M:** Wide-range protein markers; **1:** Wholegrain buckwheat flour (*Aktaş cv.*); **2:**

Wholegrain buckwheat flour (*Güneş cv.*); **3**: Buckwheat groat flour (*Aktaş cv.*); **4**: Buckwheat groat flour (*Güneş cv.*) **5**: Protein fraction from *Aktaş cv.* **6**: Protein fraction from *Güneş cv.*

Table 1. Some chemical compositions of buckwheat flours

Buckwheat Flour	Moisture (%)	Protein* (%)	Ash* (%)	Crude Oil* (%)	Total Starch* (%)	TDF* (%)	IDF* (%)	SDF* (%)	Resistant Starch* (%)
A-WBF	12.0 ± 0.03	13.5 ± 0.30	2.0 ± 0.08	3.2 ± 0.14	54.1 ± 3.90	33.8 ± 2.22	29.7 ± 0.41	4.0 ± 1.85	3.34 ± 0.318
G-WBF	11.5 ± 0.04	13.4 ± 0.15	1.9 ± 0.10	2.9 ± 0.09	50.3 ± 0.28	32.7 ± 0.70	28.2 ± 0.75	4.5 ± 0.07	3.14 ± 0.163
A-BGF	11.8 ± 0.20	9.1 ± 0.20	1.2 ± 0.08	1.5 ± 0.08	81.1 ± 0.64	8.6 ± 0.04	4.9 ± 0.15	3.7 ± 0.15	1.13 ± 0.154
G-BGF	11.4 ± 0.04	8.7 ± 0.25	1.0 ± 0.06	1.9 ± 0.08	76.0 ± 0.95	8.8 ± 0.12	4.9 ± 0.13	4.0 ± 0.23	1.35 ± 0.199

*Dry weight basis; Mean ± SD (n = 3)

TDF: total dietary fiber; IDF: insoluble dietary fiber; SDF: soluble dietary fiber

TDF = IDF + SDF; IDF includes Resistant Starch content.

A-WBF: *Aktaş cv.* wholegrain buckwheat flour;

G-WBF: *Güneş cv.* wholegrain buckwheat flour

A-BGF: *Aktaş cv.* buckwheat groat flour;

G-BGF: *Güneş cv.* buckwheat groat flour

Table 2. Yield values (g/100 g of kernels) of the fractions obtained from wet-milling process of hulled buckwheat cultivars and their some chemical compositions (%)

WET-MILLING FRACTIONS	Yields (g/100 g of kernels) & Some Chemical Compositions ^a (%)	
	<i>Güneş cv.</i>	<i>Aktaş cv.</i>
HULL	15.9±0.0	16.3±0.00
Moisture* (%)	17.8±0.55	17.3±0.40
Starch (%)	0.40±0.043	0.34±0.049
Protein (%)	5.0±0.08	5.3±0.63
GERM & FIBRE	4.8±0.5	3.9±0.6
Moisture* (%)	14.5±0.75	15.6±0.58
Starch (%)	9.4±0.37	10.9±0.32
Protein (%)	45,8±0.32	43.0±0.40
Resistant starch (%)	2.61±0.060	2.20±0.151
TOTAL PROTEIN	3.2±0.1	3.6±0.5
Moisture* (%)	13.3±0.29	14.5±1.40
Protein (%)	46.0±0.01	46.1±0.42
TOTAL STARCH	49.7±2.5	51.6±1.1
Moisture* (%)	13.2±0.42	13.8±1.14
Starch (%)	85.0±2.76	87.8±3.73
Protein (%)	5.8±0.21	6.2±0.01
TDF (%)	4.2±0.04	4.5±0.21
Resistant starch (%)	3.62±0.289	3.28±0.118
TOTAL SOLIDS IN STEEP WATER	12.6±0.8	12.3±0.3
TOTAL SOLIDS IN WASHING WATER	9.9±0.7	10.4±0.1
TOTAL RECOVERY OF FRACTIONS	96.1	98.1

^aDry weight basis

*Moisture content after following the wet-milling process

Mean ± sd of wet milling fractions calculated from triplicate analysis (n:3)

Mean ± sd of starch, total dietary fibre and resistant starch calculated from triplicate analysis (n:3)

Mean ± sd of protein calculated from duplicate analysis (n:2)

Table 3. Colour properties, water solubility (WS, %), water binding capacity (WBC, %), water holding capacity (WHC) and oil absorption capacity (OAC) of buckwheat flours and their wet-milling fractions

Buckwheat Sample	Colour properties				Techno-functional properties			
	L^*	a^*	b^*	WI^*	WS (%)	WBC (%)	WHC (g H ₂ O/g flour)	OAC (g oil/g flour)
A-Kernel	46.7 ± 0.03 ^e	2.4 ± 0.08 ^b	10.04 ± 0.03 ^b	45.7 ± 0.03 ^e	-	-	-	-
G-Kernel	42.1 ± 0.02 ^f	4.2 ± 0.02 ^a	11.6 ± 0.01 ^a	40.8 ± 0.02 ^f	-	-	-	-
A-WBF	73.4 ± 0.30 ^d	0.9 ± 0.02 ^d	8.0 ± 0.03 ^c	72.2 ± 0.31 ^d	5.8 ± 0.17 ^a	149.3 ± 7.92 ^a	1.70 ± 0.021 ^a	1.05 ± 0.191 ^{ab}
G-WBF	73.6 ± 0.20 ^d	0.9 ± 0.04 ^{cd}	7.7 ± 0.12 ^c	72.4 ± 0.20 ^d	5.9 ± 0.02 ^a	147.3 ± 12.81 ^a	1.72 ± 0.032 ^a	1.17 ± 0.114 ^a
A-BGF	78.6 ± 0.05 ^c	1.0 ± 0.03 ^{cd}	7.7 ± 0.10 ^c	77.2 ± 0.04 ^c	5.1 ± 0.25 ^b	119.9 ± 2.10 ^b	1.28 ± 0.011 ^b	0.86 ± 0.030 ^c
G-BGF	78.4 ± 0.18 ^c	1.0 ± 0.01 ^c	7.7 ± 0.03 ^c	77.1 ± 0.16 ^c	5.4 ± 0.23 ^b	118.2 ± 2.48 ^b	1.30 ± 0.006 ^b	0.86 ± 0.019 ^c
A-Starch	98.4 ± 0.27 ^a	0.1 ± 0.02 ^f	2.3 ± 0.10 ^e	97.2 ± 0.21 ^a	0.6 ± 0.05 ^c	50.2 ± 3.47 ^c	0.93 ± 0.014 ^c	0.96 ± 0.040 ^{bc}
G-Starch	93.0 ± 4.07 ^b	0.3 ± 0.28 ^e	4.7 ± 2.05 ^d	91.5 ± 4.46 ^b	0.8 ± 0.09 ^c	51.5 ± 3.01 ^c	0.96 ± 0.017 ^c	0.92 ± 0.024 ^{bc}

Mean ± SD (n = 3). ^a L^* , lightness; a^* , red+/green- coordinate; b^* , yellow+/blue- coordinate; WI , whiteness index

Values followed by the different letters in the same *column* are significantly different ($p < 0.05$).

A-Kernel: *Aktaş cv.* kernel (seed);

G-Kernel: *Güneş cv.* kernel (seed)

A-WBF: *Aktaş cv.* wholegrain buckwheat flour;

G-WBF: *Güneş cv.* wholegrain buckwheat flour

A-BGF: *Aktaş cv.* buckwheat groat flour;

G-BGF: *Güneş cv.* buckwheat groat flour

A-Starch: *Aktaş cv.* starch sample;

G-Starch: *Güneş cv.* starch sample

Table 4. Total phenolic compound (TPC) contents, DPPH radical scavenging activities and TEAC antioxidant capacities of buckwheat flours and wet milling fractions.

Buckwheat Flours/ Fraction	TPC*^{ab} (mg FA/g sample)	TPC*^{ac} (mg FA/g sample)	DPPH Scavenging Activity*^a (%)	TEAC*^a (mmol / kg sample)
A-WBF	5.4 ± 0.07 ^e	5.1 ± 0.11 ^a	62.2 ± 4.46 ^b	9.0 ± 0.73 ^d
G-WBF	5.2 ± 0.03 ^e	5.2 ± 0.18 ^a	59.4 ± 2.55 ^{bc}	7.7 ± 0.44 ^e
A-BGF	4.7 ± 0.08 ^f	3.9 ± 0.24 ^d	53.0 ± 3.61 ^d	5.6 ± 0.19 ^{gh}
G-BGF	4.8 ± 0.04 ^f	4.0 ± 0.23 ^d	51.9 ± 2.68 ^d	6.3 ± 0.37 ^f
A-Hull	4.9 ± 0.07 ^f	3.8 ± 0.21 ^d	81.7 ± 1.97 ^a	11.1 ± 0.15 ^b
G-Hull	3.0 ± 0.11 ^g	2.5 ± 0.08 ^e	58.1 ± 1.90 ^c	7.2 ± 0.20 ^e
A-Starch	2.4 ± 0.09 ^h	0.2 ± 0.04 ^h	11.9 ± 1.70 ^h	2.2 ± 0.08 ⁱ
G-Starch	2.4 ± 0.07 ^h	0.2 ± 0.05 ^h	11.8 ± 1.34 ^h	1.9 ± 0.15 ⁱ
A-Protein	8.2 ± 0.11 ^c	1.7 ± 0.08 ^g	23.8 ± 1.02 ^f	6.0 ± 0.29 ^{fg}
G-Protein	7.2 ± 0.12 ^d	2.0 ± 0.08 ^f	18.6 ± 0.77 ^g	5.3 ± 0.69 ^h
A-GDF	12.4 ± 0.25 ^a	4.7 ± 0.06 ^b	36.6 ± 0.76 ^e	11.8 ± 0.31 ^a
G-GDF	10.8 ± 0.18 ^b	4.4 ± 0.07 ^c	33.0 ± 1.12 ^e	9.7 ± 0.21 ^c

*Mean ± SD (n=3). Dry weight basis.

^aValues followed by the different letters in the same column are significantly different ($p < 0.05$). ^bDMSO Extraction, ^c80% MetOH Extraction

A-WBF: *Aktaş cv.* wholegrain buckwheat flour;

G-WBF: *Güneş cv.* wholegrain buckwheat flour

A-BGF: *Aktaş cv.* buckwheat groat flour;

G-BGF: *Güneş cv.* buckwheat groat flour

A-Hull: *Aktaş cv.* hull fraction;

G-Hull: *Güneş cv.* hull fraction

A-Starch: *Aktaş cv.* starch fraction;

G-Starch: *Güneş cv.* starch fraction

A-Protein: *Aktaş cv.* protein fraction;

G-Protein: *Güneş cv.* protein fraction

A-GDF: *Aktaş cv.* germ+dietary fibre fraction;

G-GDF: *Güneş cv.* germ+dietary fibre fraction

Table 5. Pasting (RVA) and thermal (DSC) properties of buckwheat flours and starch fraction obtained from wet-milling process.

Parameter [*]	A-WBF	G-WBF	A-BGF	G-BGF	A-Starch ^a	G-Starch ^a
Pasting temperature (°C)	73.8 ± 0.46 ^c	73.9 ± 0.60 ^c	86.5 ± 2.12 ^{ab}	88.3 ± 0.56 ^a	83.7 ± 2.12 ^b	85.7 ± 3.50 ^{ab}
Peak time (min)	7.0 ± 0.00 ^a	6.9 ± 0.14 ^a	7.0 ± 0.00 ^a	7.0 ± 0.00 ^a	6.4 ± 0.21 ^b	6.3 ± 0.40 ^b
Peak viscosity (Pa·s)	1.8 ± 0.05 ^c	2.0 ± 0.14 ^b	1.0 ± 0.00 ^d	1.0 ± 0.00 ^d	2.3 ± 0.06 ^a	2.3 ± 0.14 ^a
Hot-paste viscosity (Pa·s)	1.7 ± 0.03 ^c	1.9 ± 0.07 ^b	1.0 ± 0.00 ^d	0.9 ± 0.01 ^d	2.2 ± 0.02 ^a	2.2 ± 0.18 ^a
Breakdown viscosity (Pa·s)	0.1 ± 0.03 ^a	0.1 ± 0.07 ^a	0.1 ± 0.00 ^a	0.1 ± 0.02 ^a	0.1 ± 0.05 ^a	0.1 ± 0.06 ^a
Final viscosity (Pa·s)	3.2 ± 0.20 ^b	3.7 ± 0.25 ^a	1.8 ± 0.02 ^c	1.7 ± 0.00 ^c	3.5 ± 0.11 ^{ab}	3.4 ± 0.15 ^b
Setback viscosity (Pa·s)	1.5 ± 0.16 ^b	1.8 ± 0.18 ^a	0.9 ± 0.02 ^d	0.8 ± 0.01 ^d	1.3 ± 0.09 ^c	1.2 ± 0.03 ^c
<i>T_o</i> (°C)	-	-	63.1 ± 0.43 ^a	63.8 ± 0.31 ^a	60.7 ± 0.75 ^b	62.1 ± 1.43 ^a
<i>T_p</i> (°C)	-	-	69.5 ± 0.24 ^a	70.4 ± 0.14 ^a	66.5 ± 0.66 ^c	68.2 ± 0.60 ^b
<i>T_c</i> (°C)	-	-	76.4 ± 0.18 ^a	77.4 ± 0.32 ^a	73.8 ± 0.68 ^b	76.1 ± 0.56 ^a
<i>ΔH</i> (J/g of starch)	-	-	9.5 ± 0.24 ^{bc}	9.0 ± 0.14 ^c	11.2 ± 0.50 ^a	10.5 ± 1.03 ^{ab}
<i>PHI</i>	-	-	1.5	1.4	1.9	1.7

^{*}Mean ± sd. (n=3), ^aMean ± sd (n = 12). Values followed by the different letters in the same row are significantly different ($p < 0.05$).

T_o, onset temperature; *T_p*, peak temperature; *T_c*, conclusion temperature; *ΔH*, gelatinisation enthalphy; *PHI*, gelatinisation index.

A-WBF: *Aktaş cv.* wholegrain buckwheat flour;

G-WBF: *Güneş cv.* wholegrain buckwheat flour

A-BGF: *Aktaş cv.* buckwheat groat flour;

G-BGF: *Güneş cv.* buckwheat groat flour

A-Starch: *Aktaş cv.* starch fraction;

G-Starch: *Güneş cv.* starch fraction

Table 6. Retrogradation parameters of starch fractions stored at +4 °C during 15 days.

Parameters ^a						
Buckwheat Starch	ST (day)	T_o (°C)*	T_p (°C)*	T_c (°C)*	ΔH (J/g of starch)*	RI*
A-Starch	1	39.4 ± 0.6 ^a	48.1 ± 0.4 ^a	58.4 ± 0.2 ^a	1.0 ± 0.1 ^a	9.4
	2	40.8 ± 0.6 ^a	48.0 ± 0.2 ^a	59.8 ± 0.6 ^a	1.2 ± 0.3 ^a	10.3
	3	40.1 ± 0.8 ^a	48.7 ± 0.5 ^a	61.2 ± 0.8 ^a	1.9 ± 0.1 ^a	17.0
	4	37.6 ± 1.3 ^a	46.9 ± 1.2 ^a	60.0 ± 0.5 ^a	3.5 ± 1.0 ^a	29.8
	7	38.2 ± 0.4 ^a	47.9 ± 1.0 ^a	61.0 ± 0.3 ^a	4.0 ± 0.8 ^a	34.4
	15	37.3 ± 1.3 ^a	48.5 ± 0.7 ^a	60.5 ± 0.6 ^a	5.6 ± 0.5 ^a	50.3
G-Starch	1	38.5 ± 0.7 ^a	45.4 ± 0.6 ^b	58.6 ± 0.6 ^a	1.0 ± 0.2 ^a	9.7
	2	40.6 ± 2.0 ^a	48.9 ± 0.5 ^a	60.3 ± 0.2 ^a	1.2 ± 0.1 ^a	11.4
	3	39.4 ± 1.4 ^a	48.6 ± 0.8 ^a	61.8 ± 0.2 ^a	2.1 ± 0.3 ^a	19.5
	4	38.0 ± 1.1 ^a	47.8 ± 0.0 ^a	61.0 ± 1.5 ^a	3.7 ± 0.0 ^a	32.4
	7	38.0 ± 0.2 ^a	47.6 ± 1.1 ^a	61.4 ± 0.2 ^a	3.8 ± 0.9 ^a	37.5
	15	37.5 ± 0.2 ^a	48.9 ± 0.2 ^a	61.0 ± 0.0 ^a	5.2 ± 0.3 ^a	56.6

^a T_o , onset temperature; T_p , peak temperature; T_c , conclusion temperature; ΔH , retrogradation enthalpy;

RI, retrogradation index. ST = Storage time (day). Mean ± SD (n = 2).

*Values followed by the different letters in the *same day in the column* are significantly different ($p < 0.05$).

A-Starch: *Aktaş cv.* starch fraction; G-Starch: *Güneş cv.* starch fraction