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Effects of Partial Substitution of Sprouted Buckwheat (Fagopyrum Esculentum) and Chickpea (Cicer arietinum) Flours on its Functional Properties

Ola A. Da'na[1], Ghaith Habashneh[1], Youngseung Lee[2], Khalid Al-Ismail[1] and Mohammed I. Saleh[1]*

[1] Department of Nutrition and Food Technology, School of Agriculture, The University of Jordan, 11942, Amman, Jordan

[2] Department of Food Science and Nutrition, Dankook University, Cheonan-si 31116, Republic of Korea

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ABSTRACT

This study was conducted to investigate the effects of sprouting buckwheat and chickpeas on their nutritional and physicochemical properties. Lipid content decreased significantly ($P<0.05$) after buckwheat germination but increased significantly ($P<0.05$) after chickpea germination. Protein, vitamin B_6 total phenols, and total flavonoid content increased significantly ($P<0.05$) in sprouted treatments compared to non-sprouted treatments. Water holding capacity was significantly ($P<0.05$) greater for sprouted treatments which could be related to the greater number of proteins after germination. Otherwise, water holding capacity decreased at 55oC for sprouted treatments, which could be due to decreased swelling power at higher temperatures. A shear-thinning model fitted the flow behavior index of sprouted and non-sprouted treatments. Moreover, sprouting also contributed to the decrease in pasting viscosities, except for breakdown viscosity. The use of sprouted buckwheat and chickpea to replace fractions of wheat flour resulted in a significant ($p<0.05$) increase in syneresis during the freeze-thaw cycle of flour, cooked pasta water uptake and solid leaching out due to increasing soluble sugars after germination and a weaker gluten network because of adding gluten-free ingredients.

Keywords: Buckwheat, Chickpeas, Sprouting, Germinated Grains, Functional Characteristics

INTRODUCTION

Germination of grains is believed to improve nutrient bioavailability and physicochemical properties of germinated grains. More specifically, germination positively impacts grains' vitamins and minerals' bioavailability, increases their antioxidant activity, and improves the water-holding, water absorption, and gelation capacities of flours made from germinated grains (Marti et al., 2018; Obinna-Echem, and Barber 2019). Therefore, germinated seeds can be considered among the best examples of functional foods and can be used to

* Corresponding author E-mail: misaleh@ju.edu.jo

reduce the risk factors of many diseases and improve human health (Demir and Bilgiçli, 2020).

Germination generally refers to the process that occurs at the beginning of the development of seeds into plants, during which grains sprout (Rumiyati and Jayasena, 2012). The main elements related to germination include metabolism reactivation, cellular respiration, mitochondrial biogenesis, DNA repair mechanisms, and the arrival of reserve mobilization (Sharma 2020). Furthermore, germination involves changes in the nutritional, biochemical, and sensory characteristics of the food and also reduces the anti-nutritional factors of food by activating some endogenous enzymes, making germinated foods higher in nutritional quality than non-germinated seeds (Nkhata et al., 2018; Zhang et al., 2015).

Farooqui (2018) studied the changes in germinated barley's chemical, nutritional, and mineral composition. The mineral composition of germinated and non-germinated barley flour showed that phosphorus content was 500 and 320 mg/100g, calcium content was 130 and 110 mg/100g, and magnesium, 180 and 160 mg/100g, respectively. The author also reported a significant increase in antioxidant activity and total flavonoids of the germinated flour. In the same manner, Rahman et al. (2018) investigated the effect of germination on the nutritional properties and enzyme activities of three barley (Hordium vulgare L.) varieties, namely BARI Barley-4, BARI Barley-5, and BARI Barley-7. The nutritional compositions and enzymatic activities (α-amylase and protease) in both raw and germinated seeds gradually changed with the germination period. Marti (2018) examined the effects of sprouted wheat under controlled conditions and the effects of enrichment (i.e. 15%, 25%, 33%, 50%, 75%, and 100%) of the related refined flour on dough rheological properties, baking performance, and starch digestibility. Adding sprouted flour significantly decreased dough water absorption and stability during mixing development time, which suggests a weakening of the gluten network.

Although the effect of sprouting grain on its nutritional value has been investigated, there is a limited number of publications investigating how sprouted grains affect flour's functional characteristics. Therefore, this study investigated the impacts of partial substituting wheat flour using fractions with sprouted buckwheat and chickpea flour on its functional characteristics.

Materials and methods

Materials

Wheat flour was obtained from a local mill in Jordan (The Modern Flour Mills and Macaroni Factories Co., Amman, Jordan), buckwheat (Fagopyrum esculentum) and chickpea (Cicer arietinum) grains were purchased from the local market in Amman, Jordan during the harvesting season 2019-2020. The grains were cleaned manually for any foreign matter before use.

Design of the experiment:

A three-factor mixture response surface design, with some modification of the sample numbers, was used to conduct the study (Saleh et al., 2016). A total of two sprouted grain flour combinations were used in each experiment of the study (i.e., buckwheat and chickpeas). Three proportions for each flour type were expressed as a fraction of a mixture, and for each treatment combination, the sum of the component proportions will be equal to one.

A three-factor mixture response surface design, with some modification of the sample numbers, was used to conduct the study (Saleh, et al., 2016). A total of two sprouted or non-sprouted grain flour combinations were used in each experiment of the study (i.e., buckwheat and chickpea) with untreated wheat flour. Three proportions for each flour type were expressed as a fraction of a mixture, and for each treatment combination, the sum of the component proportions will be equal to one (Equation 1), where:

$$Xi = x1 + x2 + x3 \qquad \text{Eq. 1.}$$

The JMP release 10.0 (SAS Institute, Cary, NC) was used to build up the model parameters. Table 1 presents the percentages of the variables for sprouted and non-

sprouted treatments used in the model. Another set of non-sprouted chickpea and buckwheat flour was also included in this study. A total of 10 samples, in addition to the control, were used in this study. The combination flours were used in the fractional replacement of wheat, which was used for functional property evaluation. The control was made entirely of wheat flour. All experiments were performed in duplicate.

Table 1: Mixture response surface model of sprouted and non-sprouted Buckwheat and Chickpea and wheat flour

Treatments	Buckwheat (%)	Chickpea (%)	Untreated wheat (%)
1	66	17	17
2	50	0	50
3	33	33	34
4	17	17	66
5	0	50	50

Germination and treatments:

To test the effect of germination on buckwheat and chickpeas, part of them was germinated and the other part remained raw. After germination and drying of the first part of the grains, non-sprouted and sprouted grains were ground to pass through a 150 µm sieve. For the sprouting, grains of chickpeas and buckwheat were washed with 10% hydrogen peroxide (H2O2) before being soaked in distilled water at room temperature (~25oC) for 12 hours (i.e., 1: 4 ratio grains to water). After that, the water was drained off and the grains were spread on trays covered by filter paper. In a dark place, the grains germinated at 27oC for five days for chickpeas and four days for buckwheat. Grains were sprayed daily with distilled water to maintain an adequate hydration level. The germination process was controlled with good hygienic practices to prevent contamination and microbial growth. The germinated grains were dried at 50oC for 48 hours. Finally, the dried grains were milled to obtain grain sprout flour and stored in the freezer (-20oC) until used (Farooqui et al., 2018; Gao et al., 2019).

Proximate analysis

Moisture, protein, crude fat (ether extract), and ash were determined according to AOAC method numbers 97.28, 983.14, and 920.39 (AOAC, 2011). Total carbohydrates were estimated by subtracting the sum of moisture, protein, fat, and ash from 100. All measurements were performed in duplicate.

B-Vitamins content:

The B vitamins were determined according to the method described by Albawarshi, et al. (2019). In brief, two grams of wheat sprouted and non-sprouted flour types were weighed into a 25 ml centrifuge tube and mixed with 10 ml water. The mixture was vortexed for 1 min, then shacked for 15 min in the water bath shaker (Memmert WB 14, Germany) at 50 °C in the dark, followed by centrifugation (Hermle-Z 206A, Germany) at 6000 rpm for 10 min. The supernatant was collected and the precipitate was re-extracted with 5 ml of distilled de-ionized water, vortexed for 1 min, and then centrifuged at 6000 rpm for 10 min. The supernatant was mixed with the previous extract. The combined extracts were filtered through a 0.45 mm nylon filter and then delivered to the HPLC analysis.

Chromatographic conditions

The concentrations of vitamins in the flour extracts were determined using a Thermo Scientific Dionex UltiMate° 3000 HPLC system consisting of an LPG 3400 SD pump, ACC-3000 autosampler, and a DAD detector. A reverse phase-HPLC with an ACE C18-AR (250 x 4.6 mm; 5µm) column was used. A gradient mobile phase consisting of 0.03% trifluoroacetic acid (TFA) in water (pH 2.6, A) and acetonitrile was studied. The column temperature was 25° C, the injection volume was 20 ml and the flow rate was 0.9 ml/minute. Each vitamin's signal (peak area) was obtained using a photodiode array detector (DAD) at wavelengths 361, 280, 265, and 210 nm. The chromatogram registration and processing were controlled by Chromeleon 6.80 Chromatography Data System (CDS) software.

Phenolic and antioxidant activity of sprouted grains:

Extraction

30 ml of ethanol and 10g of sprouted or non-sprouted flour samples were combined and shaken for 30 min. Samples were then filtered several times, and the extract from the first and second times was combined and used for the analyses.

Total phenolic content:

The phenolic compounds present in methanol, water, and ethyl acetate extracts of sprouted and non-sprouted grains were determined by the Folin-Ciocalteu Reagent (FCR) according to the method of Al-Ismail et al. (2006). Samples of 100 µl of each extract (0.1 mg/ml) were transferred into a 10 ml test tube and the volume will be completed to 3 ml with distilled water. An amount of 0.5 ml of FCR was then added and mixed well. After 3 min, 2 ml of 20 % (w/v) sodium carbonate solution was added. The solution was then made to 10 ml with methanol and then left to stand for 60 min and the absorbance of the sample was then measured at 650 nm using a spectrophotometer (Labomed spectrophotometer, model UVD-2900, Labomed, USA). The concentration of the total phenolic compounds (mg/100g) was calculated by comparison with the absorbance of different concentrations of gallic acid, and the total phenolic compound content of the plant extracts was expressed as gallic acid equivalent.

Total flavonoids content:

The flavonoid content was determined according to Miliauskas *et al*. (2004). In brief, 0.5 ml of each sprouted grain flour extract was mixed with 1 ml of 2 % aluminum trichloride in ethanol solution; the mixture was then diluted with water into a 25-mL volumetric flask and allowed to stand for 40 min at room temperature. The absorbance of the sample was then measured at 415 nm using a spectrophotometer (Labomed spectrophotometer, model UVD-2900, Labomed, USA). The total flavonoid contents (mg/100g) were calculated by comparison with the absorbance of different concentrations of quercetin, and the total flavonoid compound content of the plant extracts was expressed as quercetin equivalent.

Antioxidant capacity

Antioxidant capacity was determined according to Al-Ismail *et al*. (2006). In brief, 0.2 ml of DPPH (25 mg/50 ml) was mixed with different concentrations of the methanolic sprouted grain extract, and then mixtures were completed to a total volume of 4.0 ml with methanol. The mixtures were mixed thoroughly and kept to stand for 45 minutes in a dark place at room temperature. After that, the absorbance was measured at 515 nm using a spectrophotometer (Labomed spectrophotometer, model UVD-2900, Labomed, USA). The DPPH scavenging activity was carried out in duplicate. The radical scavenging activity of the mixtures with different concentrations was expressed as a percent of inhibition according to the following equation:

Inhibition (%) = [(Absorbance of blank – absorbance of sample) / Absorbance of blank] x 100

IC_{50} will be calculated from the equation obtained from their concentration-response curves and it is the concentration of extract in mg/ml needed to scavenge 50% of the DPPH radical.

Water holding capacity (WHC)

The water-holding capacity (%) of flour treatments was determined by Saleh *et al*. (2016). In summary, duplicate flour treatments were dispersed in distilled water and the dispersions were allowed to stand for one hour at 25, 35, 45, and 55oC before centrifuging at 3800 rpm for 30 min. Sediment weights were recorded and were used to calculate WHC (%) according to equation 1.

WHC % =

Weight of sediment/weight of dry solids *100% [Eq. 1]

Rheological measurements

A mixture of 2.5 grams of flour treated and 40 ml of distilled water was prepared in polyethylene plastic tubes for rheological property measurements. Treatments were homogenized in tubes before performing rheological measurements. Two separate samples were used to perform rheological measurements after a set duration at

25.0oC for 1 hr. A rotational viscometer was used to measure the apparent viscosity of treatments during the shear rate of 6– the60s at 25.0oC. The flow behavior index and consistency coefficient of treatments were evaluated. Averages of the two measurements were reported (Saleh, 2018).

The Herschel–Bulkley model, was used to describe the experimental data for flow curves of all samples according to equation 2:

$$\sigma = K\delta^n + \sigma_o \quad \text{[Eq. 2]}$$

Where; σ is shear stress (mPa), σ_o is yield stress (mPa), K is the consistency coefficient (mPa.s^n), δ is the shear rate (S $^{-1}$) and n is the flow behavior index (dimensionless).

Herschel–Bulkley model was used to describe the rheological behavior of treatment functional properties. Flow behavior index (n) is usually used to describe fluid and semifluid behavior with an n value of (1) describing a Newtonian fluid an, n value of less than (1) describing a shear thinning, and an n value of greater than (1) describing a shear thickening fluid behavior.

Freeze-thaw stability

For freeze-thaw stability, aqueous dispersions of flour treatments (5 g treatment/100 g distilled water) were prepared and then gelatinized at 95oC with continuous shaking for 30 min. The gelatinized treatments were then cooled to 25^{o}C and will be subjected to freeze–thaw cycles. The gelatinized treatments were frozen at -22^{o}C for 24 h followed by thawing at 30^{o}C for 2.0 h, then centrifuged at 3800 rpm for 30 min. For each freeze-thaw cycle, separated supernatants were weighed and the degree of syneresis was expressed as the percentage of freeze stability of samples. Three freeze-thaw measurements per cycle were performed, and results were expressed as the average for each cycle (Saleh, 2018).

Pasting measurements

Pasting profile and viscosities (i.e., peak, trough, setback, breakdown, and final) and pasting temperature of treatments were measured and recorded with a Rapid Visco Analyzer (RVA-4 Rapid Visco Analyzer, Foss North America, Eden-Prairie, MN) according to the AACC approved method 76-21 (AACC, 2000) as described by Saleh, et al. (2016). Approximately 3 g of each treatment was mixed with 25 ml of distilled water. Moreover, the slurry was mixed at 50oC for 1 minute at 160 rpm before being heated from 50 to 95oC at a heating rate of 12oC /min. The hot paste was then held at 95oC for 2.5 min. and then cooled down to 50oC at a cooling rate of 12oC/min. Data obtained from the RVA was processed by Thermocline version 1.2 software (Newport Scientific Inc., Warriewood, Australia). All samples were measured in duplicate.

Statistical Analysis

Analysis of variance (ANOVA) was carried out on physicochemical characteristic attribute data using JMP release 10.0 (SAS Institute, Cary, NC). The least significant differences (LSD), at a 5% level of probability, were determined between treatments. A mixture response surface model was fitted using buckwheat, chickpea, and wheat flour as the model factors. The model search was started with the following special cubic equation 3:

$$Y = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 \quad \text{[Eq.3]}$$

Where Y is the predicted response, β's is the parameter estimates models prediction model parameters, x_1, x_2, x_3, x_1x_2, x_1x_3, and x_2x_3 are the linear terms of the flours used, and the cross product terms, respectively. The model chosen was based on its significance ($p < 0.05$), the insignificance of the lack of fit, and the highest R2 according to Cornell 1986.

Results and Discussion

Proximate composition of wheat flour substituted with various fractions of sprouted and non-sprouted buckwheat and chickpea

The proximate composition of sprouted and non-sprouted buckwheat and chickpea treatments are

presented in table 2. Lipid content decreased significantly in treatments with 66% and 50% of sprouted buckwheat flour from 5.67 and 4.43 to 5.03 and 3.78 %, respectively. Similarly, Zhang et al. (2015) reported that after germination for 72 hours, the crude fat content of buckwheat decreased from 30.68 to 25.26 mg/g. Results were attributed to lipid degradation to provide energy to seeds during germination. As for chickpea treatments, lipid content was significantly increased in sprouted compared to non-sprouted treatments. The decrease in lipid content suggests higher metabolic activity during germination. Additionally, the increases in reducing sugars, sucrose, and starch would result in a decrease in the oil content. Furthermore, lipid content in oil seeds has been shown to be due to the conversion of fatty acids into carbohydrates through the glyoxylate cycle (Offem et al., 1993). The difference in lipid content between chickpeas and buckwheat during sprouting was attributed to the variation in their chemical composition and structural integrity. Similar results were reported by Vasishtha and Srivastava (2012), who indicated an improvement in the fatty acid profile of sprouted chickpeas with a decrease in saturated fatty acids.

Protein content increased significantly in the treatments containing different ratios of sprouted buckwheat and chickpea flours (i.e., ranged from 14.57 to 20.70 %) compared to treatments containing the same ratios of non-sprouted flours that ranged from 13.86 to 19.44 %. The changes in protein content during germination were considered a dynamic regulation process that depended on the effect of proteolysis and protein synthesis. Zhang et al. (2015) indicated that protein synthesis outpaced the effect of proteolysis during buckwheat germination.

Sprouted and non-sprouted treatments had an ash content significantly greater than the control (i.e., 0.76%). The non-sprouted chickpea (i.e., N0B50C50W) treatment had the greatest ash content (1.96 %) among treatments. These results were attributed to the leaching out of some water-soluble minerals during the soaking process. Table (2) also presents the carbohydrate content of sprouted and non-sprouted treatments. Results indicated a decrease, although not significant ($P>0.05$), in carbohydrate content in treatments having 66% and 50% sprouted buckwheat (i.e., 75.85 and 80.36 %, respectively) compared to treatments had 66% and 50% non-sprouted buckwheat (i.e., 76.38 and 80.32, respectively). Changes in carbohydrates during germination were also related to the increased amylase activity, which breaks down the carbohydrates usually used to provide energy for seed growth (Ohtsubo et al., 2005).

Vitamins, phenols, and flavonoids content of wheat flour substituted with various fractions of sprouted and non-sprouted buckwheat and chickpea

Table 3 presents the contents of B1, B3, and B6 vitamins, phenols, and flavonoids of wheat flour substituted with various ratios of sprouted and non-sprouted buckwheat and chickpea flour fractions. Vitamin B1 (Thiamin) decreased significantly in sprouted compared to non-sprouted treatments. Žilić, et al. (2014) and El-Adawy, (2002) related the decrease in thiamine during germination to the leaching out of water-soluble vitamins during the soaking. Furthermore, thiamine is one of the most heat-labile B vitamins, so it can be degraded during the drying process (Ariahu and Ogunsua, 2000; Hucker et al., 2012). Furthermore, germination significantly improved B6 vitamin content. For example, the S66B17C17W treatment had the greatest B6 vitamin content (i.e., 0.374 mg100g) ($P<0.05$) among all treatments. Gan, et al. (2017) observed that vitamin B6 was about 11.8 mg/100 g in buckwheat sprouts while it could not be detected in raw seeds, which could be due to the biosynthesis of vitamin B6 during germination.

Table 3 also shows that sprouted treatments had a significant (P 0.05) increase in total phenols and total flavonoid content when compared to each non-sprouted flour treatment. For total flavonoids, N17B17C66W and N33B3334W had flavonoid content of 28.6 and 28.9 mg/100g, respectively, and were equivalent to the control. Tanwar et al. (2019) reported that the total phenolic content of germinated buckwheat flour increased to 26.12 % compared to non-germinated buckwheat. The increase in the total phenolic content was attributed to the fact that

the level of gallic, vanillic, coumaric, and ferulic acids in the free form increased during germination. Wu et al. (2012) showed that germination could significantly improve total phenolic content in the nine legume seeds, including chickpea, which had the greatest total phenolic content among germinated seeds. Furthermore, germination was shown to significantly increase iso-flavonoid content in germinated chickpeas compared to non-germinated chickpeas. in total flavonoids after germination (Yiming, et al., 2015).

Moreover, the concentration needed to inhibit 50 % of DPPH radical (IC50) of non-sprouted buckwheat and chickpea (78.4 and 1704.3 µg/ml, respectively) was significantly greater than sprouted buckwheat and chickpea (62.6 and 788.4 µg/ml, respectively), which indicates that the antioxidant activity of buckwheat and chickpea flour changes positively during germination.

Water holding capacity of wheat flour substituted with various fractions of sprouted and non-sprouted buckwheat and chickpea

Table (4) presents the water-holding capacity (WHC) of wheat flour substituted with various ratios of sprouted and non-sprouted buckwheat and chickpea flour fractions. WHC ranged from 67.9 % for control (100% wheat) held at 25oC to 331.7 % for the control held at 55oC. The increase in holding temperature from 25oC to 35oC resulted in a significant decrease in WHC. For example, WHC decreased from 100.3 to 92.4 % for S66B17C17W and from 96.5 to 94.9 % for S33B33C34W. However, WHC increased from 97.3 to 102.5 % for S0B50C50W. Additionally, the increase in holding temperature from 35oC to 45oC resulted in a significant increase in WHC except for the treatments having 66% sprouted buckwheat flour S66B17C17W and/or 66% wheat flour S17B17C66W and N17B17C66W. Moreover, each treatment significantly increased when the holding temperature increased from 45oC to 55oC.

Our study generally indicates that germination of buckwheat and chickpea increased WHC at 25, 35, and 45oC. Similarly, Fernandez and Berry (1989) studied the effect of germination on the WHC of chickpeas and reported a significant increase in WHC of germinated chickpea starch of 98.35% compared to 83.82% for the non-germinated chickpea starch. The greater WHC value of germinated compared to the non-germinated treatments was related to the dextrin formation during germination; causing an increase in surface area and more association with water. Furthermore, germination was reported to trigger some changes in the internal arrangement of the starch granules through hydrolysis, causing them to absorb water, and swell at a much lower temperature (Fernandez and Berry 1989). Germination was also indicated to enhance the disruption of polysaccharides that led to more damaged starch and thus retained more water. The increase in WHC was also attributed to the greater protein content in sprouted treatments than in non-sprouted treatments. Protein content in legumes tends to increase the tendency to absorb more water (Chauhan, et al., 2015). Traynham, et al. (2007) also reported that increasing the amount of total soy protein in flour blends allowed more interactions with water to occur, resulting in increased WHC of soy-flour blends.

Different trends of WHC at 55oC were reported (table 4) with a significant decrease in the WHC of sprouted compared to non-sprouted of each treatment (from 331.7 to 124.1%). Results suggest that non-sprouted treatments have greater swelling power than sprouted treatments at 55oC. Sprouting may have changed protein and carbohydrate structure, resulting in WHC changes. The reported increase in protein content and possibly damaged starch during germination could retain more water. The increase in water-holding capacity was also attributed to an increase in polar amino acid residues during germination, which was reported to increase the attraction of germinated chickpea flour to water molecules (Sreerama, et al., 2012).

Pasting properties of wheat flour substituted with various fractions of sprouted and non-sprouted

Table (5) presents pasting properties including peak, trough, breakdown, final, and setback viscosities (cP) and pasting temperature (^{o}C) of wheat flour substituted with various ratios of sprouted and non-sprouted buckwheat

and chickpea flour fractions. Results demonstrated a decrease in the pasting properties of sprouted treatments compared to non-sprouted treatments except for breakdown viscosity (Figure 1). For example, the peak viscosity of the S66B17C17W treatment significantly decreased from 1020.5 cP of N66B17C17W to 311.5 cP of the S66B17C17W treatment. The greatest peak viscosity was reported for the control (i.e., 1356.5 cP) and the lowest was for the S66B17C17W (i.e., 311.5 cp). Similarly, the trough viscosity of the S0B50C50W treatment significantly decreased from 484.5 cP to 657.5 cP for the N0B50C50W treatment. Furthermore, sprouted S17B17C66W and S0B50C50W treatments had significantly higher pasting temperatures (84.0 and 86.2oC, respectively) than their non-sprouted counterparts. The increase in pasting temperature during germination could be related to the forming of a more rigid crystalline structure that is not easy to swell (Li et al, 2017).

The breakdown of S33B33C34W treatment increased significantly from 118.8 to 234.5 cP compared to that of non-sprouted N33B33C34W treatment. Similarly, the non-sprouted treatments N50B0C50W had the highest final and setback viscosities (i.e., 2,271.5 and 1,045.0 cP, respectively), while the sprouted S66B17C17W had the lowest (i.e., 333.0 and 118.0 cP, respectively).Final and setback viscosities are related to the aggregation of starch molecules, and therefore there is a low tendency for aggregation of the gelatinized starch molecules during cooling because of the degraded starch structure during germination (Li et al., 2017).

Marengo et al. (2017) indicated that sprouting reduces the pasting properties of chickpeas due to alpha-amylase activity; hence, it increases during sprouting. The decrease in retrogradation in sprouted chickpea flour was attributed to the increased dextrin formation by alpha-amylase and the reduction in starch reorganization, during cooling. More specifically, the endogenous amylases in the flours could efficiently degrade starch molecules to lower their pasting viscosities in the early stage of heating during pasting viscosities before they were inactivated at a temperature above 60°C (Setia, et al., 2019).

Buckwheat, chickpea, and wheat flour affected pasting properties as shown in figure 2 and described in Eq. 4 a-d. For instance, superior pasting viscosities were observed for wheat flour having model coefficients of 1356.5, 519.5, and 1039, respectively. Furthermore, buckwheat and chickpea were positively affected by peak and setback viscosities, having parameter coefficients of (470.3 and 1247.8) and (354. and, 856.2), respectively, while they negatively affected breakdown viscosities, having a coefficient of -61.3 and -321.9, respectively. However, buckwheat × chickpea interaction affected peak viscosity negatively with a model parameter of – 75.8 and affected breakdown positively with a model parameter of 965.5. Moreover, buckwheat × wheat and chickpea × wheat interactions negatively influenced peak and setback viscosities, having model parameters of (– 138.6 and – 2173.6) and (– 317.2 and -1719.4), respectively.

For pasting temperature (i.e., pasting Temp.) (Eq. 4d), the highest was for buckwheat flour with a parameter coefficient of 93.6. Chickpea and wheat flour were also positively affected by pasting temperature, having coefficient parameters of 89.3 and 70.5, respectively. However, buckwheat × chickpea interaction was negatively influenced by pasting temperature with a model parameter of -73.1.

Peak =
470.3x1 + 1247.8x2 + 1356.5 x3 – 75.8x1x2 – 138.6x1x3 – 2173.6x2x3 (Eq. 4a)

Breakdown =
-61.3x1 -321.9x2 + 519.5 x3 + 965.1x1x2 – 169.3x1x3 +354.6x2x3 (Eq. 4b)

Setback =
354.1x1 + 856.2x2 + 1039 x3 + 568.7x1x2 – 317.2x1x3 – 1719.4x2x3 (Eq. 4c)

Pasting Temp.= 93.6x1 + 89.3x2 + 70.5 x3 -73.1x1x2 + 8.4x1x3 + 11.3x2x3 (Eq. 4d)

Freeze-thaw Cycles and viscosity parameters of wheat flour substituted with various fractions of sprouted and non-sprouted buckwheat and chickpea

Table (6) presents three freeze-thaw cycles and viscosity parameters (i.e., flow behavior index (n) and consistency coefficient (K) of wheat flour substituted with various ratios of sprouted and non-sprouted buckwheat and chickpea flour fractions. Results show that sprouted treatments significantly increased water syneresis compared to non-sprouted treatments for all freeze-thaw cycles. For instance, the syneresis of the first cycle significantly increased from 14.2 to 29.2% for N0B50C50W and S0B50C50W, respectively. Furthermore, the syneresis of the second and third cycles for S33B33C34W increased significantly from 21.3 to 41.0 and from 25.2% to 47.0%, respectively.

Results indicated that non-sprouted treatments had significant differences over three cycles compared to the control. N66B17C17W, N33B33C34W, and N0B50C50W, for example, had significantly lower syneresis values than the control (i.e., 8.1, 10.1, and 14.2%, respectively) than the control (i.e., 21.1%). Moreover, all non-sprouted treatments in the second and third cycles ranged from 14.6 to 35.8% and 17.1 to 41.7%, respectively, and had lower freeze-thaw syneresis than the control. Qian *et al*. (1998) showed that buckwheat starch had better freeze-thaw stability than commercial wheat and corn starches for the same storage conditions. The authors indicated that several factors could affect buckwheat gels' stability, including lipid content, molecular weight, and water-binding capacity. In this regard, Abd Elmoneim and Bernhard (2013) reported that flour of germinated sorghum showed higher syneresis than non-germinated sorghum flour over the first three cycles. The higher syneresis of germinated flour was attributed to starch de-polymerization where de-polymerized starch. Retrogradation of starch pastes was correlated with freeze-thaw stability measurement. When starch gels are subjected to a freeze-thaw cycle, the water used to prepare the gels will separate because of the tendency of starch molecules to re-associate, thus forming insoluble aggregates (Abd Elmoneim and Bernhard 2013).

The flow behavior index (n) and consistency coefficient (k) of treatments (i.e., ranged from 0.22 to 0.28 and from 0.5 to 2.2, respectively) were non-significant in sprouted treatments compared to each treatment that has the same ratios of non-sprouted flours.

Conclusion

Substituting wheat flour with sprouted buckwheat and chickpeas increased the water-holding capacity of treatments with a greater capacity than that for non-sprouted flour treatments in a temperature-dependent manner. Sprouting further significantly impacted flour pasting properties and freeze-thaw stability compared to the non-sprouted flour treatments. Additionally, sprouting buckwheat and chickpeas enhanced protein content, phenolic compounds, flavonoids, and vitamins B_3 and B_6, improving flour nutritional values and thus producing functional products. Therefore, substituting wheat flour with different ratios of non-sprouted chickpea and buckwheat flour could improve the flour's functional characteristics.

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Table 2: Proximate composition (i.e., lipid, ash, protein, and carbohydrates) of wheat flour substituted with various ratios of sprouted and non-sprouted buckwheat and chickpea flour fractions

Sample ID	% Lipid	% Ash	% Protein	% CHO
N66B17C17W	5.67 $^{b\ A}$ ± 0.05	1.93 $^{a\ A}$ ± 0.02	16.02 $^{g\ C}$ ± 0.02	76.38 $^{e\ D}$ ± 0.09
N50B0C50W	4.45 $^{f\ D}$ ± 0.02	1.33 $^{cd\ B}$ ±0.02	13.86 $^{j\ D}$ ± 0.02	80.32 $^{b\ B}$ ± 0.07
N33B33C34W	5.40 $^{c\ B}$ ± 0.02	1.93 $^{a\ A}$ ± 0.02	17.70 $^{d\ B}$ ± 0.02	74.97 $^{f\ E}$ ± 0.07
N17B17C66W	4.25 $^{g\ E}$ ± 0.02	1.36 $^{c\ B}$ ± 0.02	15.68 $^{h\ C}$ ± 0.02	78.71 $^{c\ C}$ ± 0.07
N0B50C50W	5.25 $^{d\ E}$ ± 0.02	1.96 $^{a\ A}$ ± 0.02	19.44 $^{b\ A}$ ± 0.02	73.35 $^{h\ F}$ ± 0.08
S66B17C17W	5.03 $^{e\ C}$ ± 0.03	1.81 $^{b\ A}$ ± 0.02	17.31 $^{e\ C}$ ± 0.01	75.85 $^{e\ B}$ ± 0.01
S50B0C50W	3.78 $^{h\ E}$ ± 0.01	1.29 $^{d\ B}$ ± 0.02	14.57 $^{i\ E}$ ± 0.01	80.36 $^{b\ B}$ ± 0.03
S33B33C34W	5.45 $^{c\ B}$ ± 0.02	1.78 $^{b\ A}$ ± 0.02	18.93 $^{c\ B}$ ± 0.01	73.84 $^{g\ E}$ ± 0.02
S17B17C66W	4.23 $^{g\ D}$± 0.06	1.29 $^{d\ B}$ ± 0.02	16.37 $^{f\ D}$ ± 0.02	78.08 $^{d\ C}$ ± 0.02
S0B50C50W	5.91 $^{a\ A}$ ± 0.01	1.78 $^{b\ A}$ ± 0.03	20.70 $^{a\ A}$ ± 0.02	71.61 $^{i\ F}$ ± 0.04
Control	2.0 $^{i\ F}$ ± 0.02	0.76 $^{e\ C}$ ±0.03	13.54 $^{k\ FD}$ ±0.02	83.70 $^{a\ A}$ ±0.07

[1] Treatment: S= sprouted, N= non-sprouted, B= Buckwheat, C = Chickpea, W= Wheat and the number before their letters corresponds to the ratio used in that treatment.

[2] For the different treatments of the same attribute (i.e., column); proximate composition {i.e. lipid, ash, protein, and carbohydrates} (i.e., means ± standard deviations) of treatments having different lower case letter(s) are significantly ($p<0.05$) different according to the least square difference (LSD).

[3] For the different non-sprouted or sprouted treatments of the same attribute (i.e., column); treatments having different upper case letter(s) for sprouted and non-sprouted flours are significantly ($p<0.05$) different according to the least square difference (LSD)

Table 3: Content of B vitamins (B1, B3, and B6), Phenols, and Flavonoids of wheat flour substituted with various ratios of sprouted and non-sprouted buckwheat and chickpea flour fractions

Vitamins (mg/100 g)					
Sample ID	B_1	B_3	B_6	Phenols (mg Gallic /100g)	Flavonoids (mg Quercetin/100 g)
N66B17C17W	0.096 $^{a\ A}$	^{A} 0.219 ^{a}	0.148 $^{g\ A}$	86.7 $^{b\ A}$	36.5 $^{d\ A}$
N50B0C50W	0.062 $^{c\ C}$	^{B} 0.161 ^{b}	0.102 $^{h\ B}$	68.0 $^{c\ B}$	37.0 $^{d\ A}$
N33B33C34W	0.068 $^{b\ B}$	^{B} 0.147 ^{c}	0.158 $^{f\ g\ A}$	52.8 $^{d\ C}$	28.6 $^{e\ B}$
N17B17C66W	0.034 $^{e\ E}$	^{C} 0.089 ^{d}	0.111 $^{h\ B}$	34.2 $^{e\ D}$	28.9 $^{e\ B}$
N0B50C50W	0.040 $^{d\ D}$	^{D} 0.073 ^{e}	0.165 $^{f\ A}$	18.9 $^{f\ E}$	20.5 $^{f\ C}$
S66B17C17W	0.023 $^{f\ B}$	0.229 $^{a\ A}$	0.374 $^{a\ A}$	121.4 $^{a\ A}$	51.0 $^{a\ A}$
S50B0C50W	0.008 $^{g\ C}$	0.171 $^{b\ A}$	0.229 $^{d\ A}$	85.3 $^{b\ B}$	41.2 $^{c\ C}$
S33B33C34W	0.028 $^{f\ B}$	0.146 $^{c\ C}$	0.351 $^{b\ B}$	87.2 $^{b\ B}$	48.7 $^{ab\ AB}$
S17B17C66W	0.014 $^{g\ C}$	0.089 $^{d\ D}$	0.213 $^{e\ B}$	52.0 $^{d\ C}$	39.3 $^{cd\ D}$
S0B50C50W	0.033 $^{e\ A}$	0.064 $^{e\ E}$	0.334 $^{b\ C}$	53.9 $^{d\ C}$	46.8 $^{b\ B}$
Control	0.000 $^{h\ D}$	0.002 $^{f\ E}$	0.016 $^{i\ C}$	14.6 $^{g\ D}$	29.3 $^{e\ B}$

[1] Treatment: S= sprouted, N= non-sprouted, B= Buckwheat, C=Chickpea, W= Wheat and the number before their letters corresponds to the ratio used in that treatment.

[2] For the different treatments of the same attribute (i.e., column); vitamins (i.e., B_1, B_3, and B_6) and Phenols, Flavonoids (i.e. means) of treatments having different lower case letter(s) are significantly ($p<0.05$) different according to the least square difference (LSD).

[3] For the different non-sprouted or sprouted treatments of the same attribute (i.e., column); treatments having different upper case letter(s) are significantly ($p<0.05$) different according to the least square difference (LSD)

[4] All results accomplished in duplicate as dry base

Table 4: Water holding capacity (WHC) of wheat flour substituted with various ratios of sprouted and non-sprouted buckwheat and chickpea flour fractions

Sample ID	WHC (%)			
	25°C	35°C	45°C	55°C
N66B17C17W	85.3 ^{cC} ± 1.4	84.2 ^{dC} ±0.4	87.9 ^{cB} ± 0.6	261.1 ^{dA} ± 0.2
N50B0C50W	70.7 ^{fC} ± 0.7	73.5 ^{gC} ± 0.4	77.2 ^{eB} ± 0.6	259.1 ^{dA} ± 1.7
N33B33C33W	81.9 ^{dB} ± 0.4	84.5 ^{dB} ± 0.4	87.3 ^{cdB} ± 0.8	244.3 ^{eA} ± 4.3
N17B17C66W	74.6 ^{eC} ± 0.6	72.7 ^{gC} ± 0.7	85.4 ^{dB} ± 0.4	313.6 ^{bA} ± 1.2
N0B50C50W	80.9 ^{dC} ± 0.2	81.1 ^{eC} ± 1.0	94.2 ^{abB} ± 1.1	270.2 ^{cA} ± 2.6
S66B17C17W	100.3 ^{aB} ± 0.4	92.4 ^{cC} ± 0.0	93.8 ^{bC} ± 1.1	129.7 ^{gA} ± 0.1
S50B0C50W	75.9 ^{eC} ± 0.1	75.7 ^{fC} ± 0.3	77.1 ^{eB} ± 0.3	126.3 ^{ghA} ± 0.6
S33B33C34W	96.5 ^{bB} ± 0.4	94.9 ^{bC} ± 0.1	95.0 ^{abC} ± 0.3	125.1 ^{hA} ± 0.4
S17B17C66W	75.0 ^{eC} ± 1.3	76.1 ^{fC} ± 0.6	96.1 ^{aB} ± 0.1	124.1 ^{hA} ± 0.2
S0B50C50W	97.3 ^{bC} ± 0.4	102.5 ^{aB} ± 0.1	94.4 ^{abD} ± 0.3	206.8 ^{fA} ± 0.2
Control	67.9 ^{gC} ± 0.4	68.5 ^{hC} ± 0.6	87.7 ^{cB} ± 2,0	331.7 ^{aA} ± 0.1

[1] Treatment: S= sprouted, N= non-sprouted, B= Buckwheat, C = Chickpea, W= Wheat and the number before their letters corresponds to the ratio used in that treatment.

[2] For the different treatments of the same attribute (i.e., column); water holding capacity (i.e., means ± standard deviations) of treatments having a different lowercase letter(s) are significantly ($p<0.05$) different according to the least square difference (LSD).

[3] For the different temperatures of the same treatment (i.e., row); water holding capacity (i.e., means ± standard deviations) of treatments having different upper case letter(s) are significantly ($p<0.05$) different according to the least square difference (LSD).

[4] All measurements accomplished in duplicate as dry base

Table 5: Pasting viscosities [peak, trough, breakdown, final, and setback] and pasting temperature (C) of wheat flour substituted with various ratios of sprouted and non-sprouted buckwheat and chickpea flour fractions

Sample ID	Peak	Trough	Breakdown	Final	Setback	P. T
N66B17C17W	1,020.5 ^{e}	1002.0 ^{b}	18.5 ^{j}	1883.0 ^{b}	881.0 ^{c}	82.3 ^{bc}
N50B0C50W	1,342.0 ^{b}	1226.5 ^{a}	115.5 ^{i}	2271.5 ^{a}	1045.0 ^{a}	83.5 ^{bc}
N33B33C34W	1,075.5 ^{d}	957.5 ^{c}	118.0 ^{i}	1833.0 ^{d}	875.5 ^{c}	78.5 ^{e}
N17B17C66W	1,254.0 ^{c}	947.5 ^{d}	306.5 ^{c}	1883.5 ^{b}	936.0 ^{b}	71.5 ^{f}
N0B50C50W	820.0 ^{f}	657.5 ^{f}	162.5 ^{h}	1212.5 ^{e}	555.0 ^{d}	79.3 ^{de}
S66B17C17W	311.5 ^{k}	145.0 ^{k}	167.0 ^{g}	333.0 ^{j}	188.0 ^{h}	81.7 ^{cd}
S50B0C50W	415.5 ^{j}	157.5 ^{j}	258.0 ^{d}	347.5 ^{i}	190.0 ^{h}	84.8 ^{ab}
S33B33C34W	445.5 ^{i}	211.0 ^{i}	234.5 ^{e}	508.0 ^{h}	297.0 ^{g}	78.7 ^{e}
S17B17C66W	597.5 ^{h}	251.0 ^{h}	346.5 ^{b}	674.5 ^{g}	423.5 ^{f}	84.0 ^{abc}
S0B50C50W	697.5 ^{g}	484.5 ^{g}	213.0 ^{f}	965.5 ^{f}	481.0 ^{e}	86.2 ^{a}
Control	1356.5 ^{a}	837.0 ^{e}	519.5 ^{a}	1876.5 ^{c}	1039.5 ^{a}	70.5 ^{f}

[1] Treatments: S= Sprouted, N= Non-sprouted, B= Buckwheat, C=Chickpea, W= Wheat, and the number before their letters correspond to the ratio used in that treatment. P. T = Pasting temperature.

[2] For the different treatments of the same attribute (i.e., column); pasting viscosities [peak, trough, breakdown, final, and setback] and pasting temperature (i.e., means) of treatments having a different letter(s) are significantly ($p<0.05$) different according to the least square difference (LSD).

[3] All results accomplished in duplicate.

Table 6: Freeze-thaw cycles and viscosity parameters (i.e., flow behavior index (n) and consistency coefficient (K)) of wheat flour substituted with various ratios of sprouted and non-sprouted buckwheat and chickpea flour fractions

	Syneresis (%)			**Viscosity parameters**	
Sample ID	Cycle 1	Cycle 2	Cycle 3	Flow behavior index (n)	Consistency coefficient (K)
N66B17C17W	8.1 ^{i}	14.6 ^{h}	17.1 ^{h}	0.23 ^{e}	1.91 ^{ab}
N50B0C50W	23.2 ^{e}	35.8 ^{c}	41.7 ^{d}	0.25 ^{bcd}	1.02 ^{cd}
N33B33C34W	10.1 ^{h}	21.3 ^{f}	25.2 ^{g}	0.26 ^{abc}	0.91 ^{cd}
N17B17C66W	26.3 ^{d}	31.3 ^{e}	38.1 ^{e}	0.26 ^{abc}	1.04 ^{bcd}
N0B50C50W	14.2 ^{g}	19.2 ^{g}	25.8 ^{g}	0.26 ^{ab}	0.82 ^{cd}
S66B17C17W	37.4 ^{a}	43.2 ^{a}	51.6 ^{a}	0.24 ^{cde}	1.21 ^{bcd}
S50B0C50W	33.1 ^{b}	41.7 ^{b}	47.8 ^{bc}	0.23 ^{de}	1.56 ^{abc}
S33B33C34W	23.3 ^{e}	41.0 ^{b}	47.0 ^{c}	0.28 ^{a}	0.52 ^{d}
S17B17C66W	33.5 ^{b}	44.0 ^{a}	48.6 ^{b}	0.25 ^{bcd}	1.01 ^{bcd}
S0B50C50W	29.2 ^{c}	31.2 ^{e}	33.0 ^{f}	0.22 ^{e}	2.20 ^{a}
Control	21.1 ^{f}	32.4 ^{d}	38.9 ^{e}	0.25 ^{bcd}	1.31 ^{bcd}

[1]Treatments: S= sprouted, N= non-sprouted, B= Buckwheat, C=Chickpea, W= Wheat and the number before their letters corresponds to the ratio used in that treatment.

[2] For the different treatments of the same attribute (i.e., column); freeze-thaw cycles and viscosity parameters (i.e., flow behavior index (n) and consistency coefficient (K) (i.e., means) of treatments having a different letter(s) are significantly ($p<0.05$) different according to the least square difference (LSD).

[3] All results accomplished in duplicate

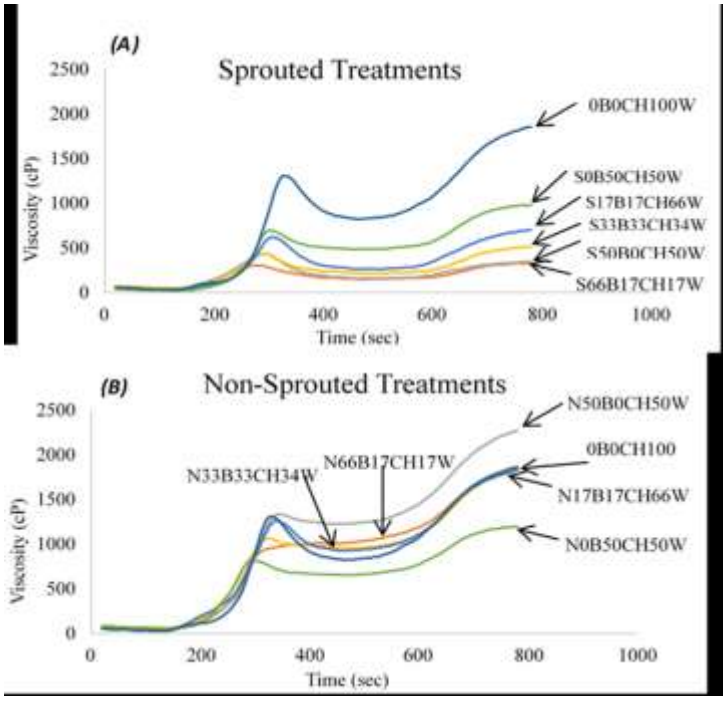

Figure 1: Pasting profile of sprouted (A) (i.e., S66B17C17W, S50B0C50W, S33B33C34W, S17B17C66W, S0B50C50W and 0B0C100W) and non-sprouted (i.e., N66B17C17W, N50B0C50W, N33B33C34W, N17B17C66W, N0B50C50W and 0B0C100W treatments (B)

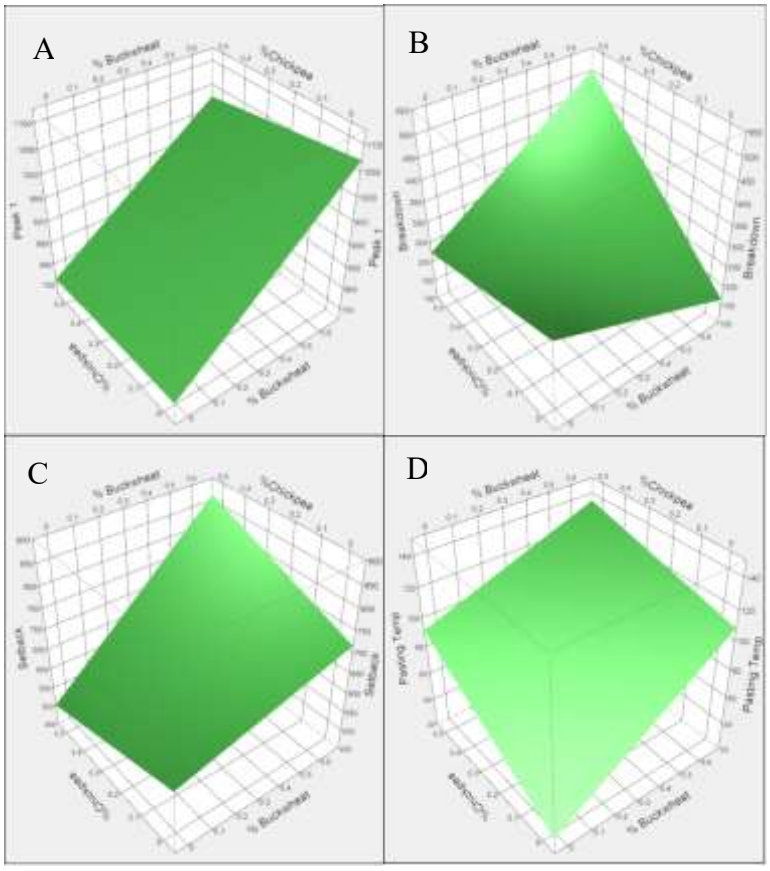

Figure 2: Response surface model for the effect of sprouted buckwheat, chickpeas, and untreated wheat flour on A) peak viscosity, B) breakdown viscosity C) setback viscosity, and D) pasting temperature.

تأثير الاستبدال الجزئي لدقيق الحنطة السوداء (Fagopyrum esculentum) المنبتة ودقيق الحمص (Cicer arietinum)المنبت على خصائصه الوظيفية

علا أ. دعنا[1]، غيث حباشنة[1]، يونغسونغ لي[2]، خالد الإسماعيل[1]، محمد إبراهيم صالح[1]

[1] كلية الزراعة، الجامعة الأردنية، الأردن

[2] جامعة دانكوك، كوريا

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ملخص

أجريت هذه الدراسة لمعرفة تأثير انبات الحنطة السوداء والحمص على الخصائص الغذائية والفيزيائية الكيميائية. انخفض محتوى الدهن معنويا ($P <0.05$) بعد إنبات الحنطة السوداء ولكنه زاد معنويا ($P <0.05$) بعد إنبات الحمص. زاد البروتين، فيتامين ب $_6$، الفينولات الكلية ومحتوى الفلافونويد الكلي زيادة معنوية ($P <0.05$) في المعاملات المنبتة مقارنة بالمعاملات غير المنبتة. كانت سعة الاحتفاظ بالماء أكبر بشكل معنوي ($P <0.05$) في المعاملات المنبتة والتي يمكن أن تكون مرتبطة بكمية أكبر من البروتينات بعد الإنبات. وبخلاف ذلك، انخفضت سعة الاحتفاظ بالمياه عند 55 درجة مئوية للمعالجات المنبتة والتي يمكن أن تؤدي الى انخفاض قوة الإنتاج عند درجات حرارة اعلى. وقد تلائم نموذج ترقق القص مع مؤشر سلوك التدفق للمعالجات المنبتة وغير المنبتة، علاوة على ذلك، ساهم الانبات ايضا في انخفاض لزوجة اللصق، باستثناء لزوجة الانهيار. ادى استخدام الحنطة السوداء المنبتة والحمص المنبت لاستبدال اجزاء من دقيق القمح الى زيادة معنوية ($p<0.05$) في التآزر اثناء دورة تجميد واذابة الدقيق، وامتصاص ماء المعكرونة المطبوخة وترشيح المواد الصلبة والتي تعود الى زيادة السكريات الذائبة بعد الانبات وضعف شبكة الغلوتين بسبب اضافة مكونات خالية من الغلوتين.

الكلمات الدالة: الحنطة السوداء، الحمص، انبات، الحبوب المنبتة، الخصائص الوظيفية.