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Effects of controlled germination on selected physicochemical and functional properties of whole-wheat flour and enhanced γ-aminobutyric acid accumulation by ultrasonication

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ABSTRACT

Using hard red spring (HRS), hard white (HW), and soft white (SW) wheat, this study examined how germination time affected the functionality of whole-wheat flour (WWF) and enhancement of γ-aminobutyric acid (GABA) content through ultrasonication. The falling number values significantly decreased and the glucose content increased by 227–357% after 15 h of controlled germination. The setback value of WWF paste decreased from 654 to 6 cP (HW), 690 to 9 cP (SW), and 698 to 7 cP (HRS), respectively, showing significant decreases of starch retrogradation in an aqueous system. The gluten quality and dough mixing performance of WWF after 5–15 h of controlled germination was enhanced since gluten is less weakened during the dough heating stage of Mixolab testing. After a 72 h germination, the GABA content increased by 339% of the non-sprouting counterpart. Furthermore, the GABA content in the ultrasound-treated SW sample was 30.7% higher than that without ultrasound treatment.

1. Introduction

Wheat is the most important staple food crop for more than one-third of the world's population. In recent years, whole-wheat products have received widespread interest from the food industry and consumer market, due to their health-promoting components. Germinated grain products are a new addition to the food industry owing to their increased nutritional value, improved nutritional absorption, and better flavour and taste (Nelson, Stojanovska, Vasiljevic, & Mathai, 2013). The application of controlled germination as a method of improving the nutritional value and flavour of grain products is of emerging interest. Reports have documented increases in reducing sugar and free amino acids, including γ-aminobutyric acid (GABA) (Ding et al., 2016), bioaccessible minerals (Platel, Eipeson, & Srinivasan, 2010), soluble dietary fibre (Koehler, Hartmann, Wieser, & Rychlik, 2007), phenolic compounds and antioxidant capability (Hung, Hatcher, & Barker, 2011) during grain germinating. Among the micronutrients that are increased during germination, GABA as a health-related compound has drawn interest because it is a bioactive constituent in germinated grains with reported health benefits, such as reducing blood pressure (Inoue et al., 2003), inducing relaxation and enhancing immunity (Abdou et al., 2006), improving brain function, and postponing intelligence degradation (Palmer et al., 2012).

GABA produced in germinated wheat was 18 times of that in ungerminated whole grain dark northern spring wheat (1 mg/100 g) (Nagaoka, 2005). The highest GABA content and antioxidant activity of germinated brown rice were attained after sprouting at 34 °C for 96 h (Caceres, Martinez-Villaluenga, Amigo, & Frias, 2014). During germination, controlled elicitation can be used to improve the nutritional value of sprouted grains, especially with reported increase in GABA content. Chung, Jang, Cho, and Lim (2009) found that the GABA content of germinated barley in anaerobic storage with nitrogen was 4 times higher than that of the control (Chung et al., 2009). Youn, Park, Jang, and Rhee (2011) exposed sprouting spring wheat to nitrogen gas for 2 h, followed by a heat shock at 120–140 °C for 30 s, and reported an increase of GABA content from 1.2 mg/100 g to 47.4 mg/100 g (Youn et al., 2011). Treatment with ultrasound is a new method to stimulate seeds for accumulation of health-promoting compounds. Ultrasound treatment was shown to increase the sprout length and GABA content in soybean sprouts (Yang, Gao, Yang, & Chen, 2015). No study,

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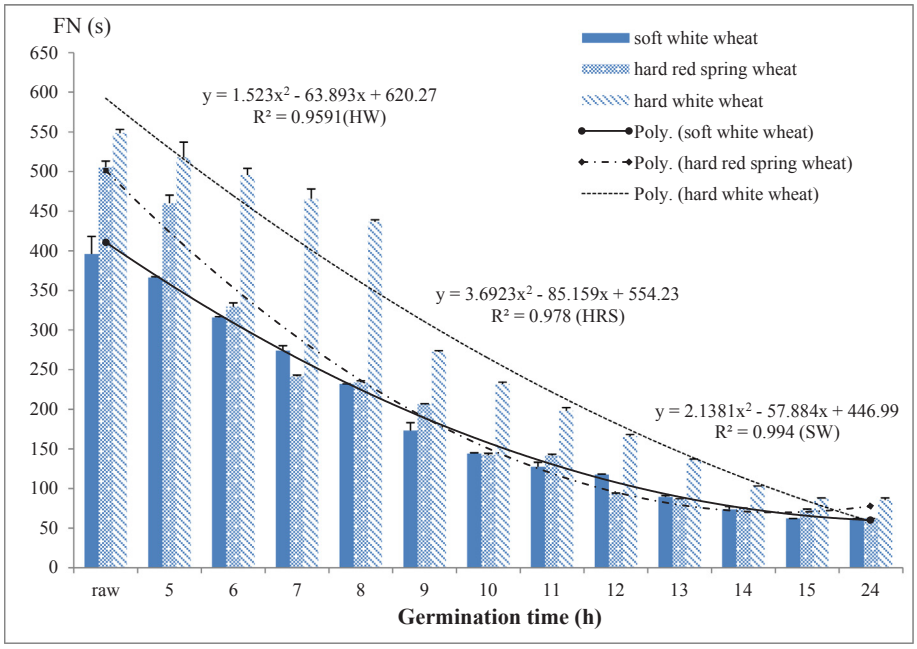

Fig. 1. Changes in the falling number (FN) values (14% moisture basis) of whole-wheat flours from control-germinated HW, HRS, and SW at 28 ± 2 °C for 24 h with a relative humidity of 95 ± 3%. Abbreviations: falling number, FN; hard white, HW; soft white, SW; hard red spring, HRS.

however, has reported the use of ultrasound for enhancing GABA content in sprouted wheat products.

Besides enhanced nutritional value, the functional properties of whole-wheat flour (WWF) from sprouted wheat are also an important consideration in the food industry. Early reports have shown that uncontrolled pre-harvest sprouting, sprouting during storage period, or over sprouting negatively impacted grain products due to increased hydrolytic enzyme activities, resulting in degradation of carbohydrates and proteins into smaller fractions and hence decreasing the functional properties of the dough (Edwards, Ross, Mares, Ellison, & Tomlinson, 1989). Controlled germination, on the other hand, was reported to produce flours with improved functional properties, including higher loaf volume, better texture, improved sensory scores for bread, higher elasticity and plasticity for pasta (Shafqat, 2013), as well as better sensory acceptability for whole-wheat tortillas (Liu, Hou, Cardin, Marquart, & Dubat, 2017). A few reports have documented changes in selected functional parameters in germinated wheat. Shafqat (2013) investigated the effect of steeping and germination times on alpha-amylase activity of soft white winter wheat and hard red winter wheat, and recorded changes using Rapid Visco Analyzer (RVA), Farinograph, GlutoPeak Tester (gluten quality), SDS-PAGE (protein structure), and an antioxidant capacity assay (Shafqat, 2013). The work of Singh, Singh, Kaur, and Saxena (2001) examined the effects of soaking time and germination temperature on falling number (FN), water absorption index, and water solubility index of three local wheat cultivars in India after germination using a regression analysis (Singh et al., 2001). These studies have shown that the sprouting duration and conditions have a significant impact on the rheological properties and functional properties of the WWFs from selected sprouted wheat. However, there is a lack of information on how major U.S. wheat classes react to controlled germination treatments and the changes in functional properties of WWF from the germinated wheat determined by, for instance, falling number and Mixolab analysis under different germination conditions.

This study was undertaken to investigate the effects of degree of controlled germination on the functional and nutritional properties of three wheat classes in the United States, i.e., soft white (SW), hard red spring (HRS) and hard white (HW), and to examine if more severely sprouted (72 h) SW can be used as a natural health promoting food ingredient by treating sprouting SW with ultrasound for GABA content and antioxidant capacity enhancement. Three sets of quality parameters were examined. First, the functional properties of three WWFs from 5-24 h germinated wheat, including FN values, Mixolab dough mixing parameters, and RVA starch pasting properties, were assessed. Second, the nutritional value of three WWFs, including contents of glucose and dietary fibres, were determined. Third, the effects of germination time (24–72 h) and ultrasonication treatment (5 and 30 min) on GABA content of SW-WWF were evaluated. This is the first study to systematically evaluate the changes of both nutritional and flour functional properties in three major U.S. wheat classes during controlled germination process. The ultimate aims of this study are to determine the degree of sprouting on the functional property of WWF in end-product application and to explore the potential use of more severely sprouted wheat (using SW wheat as an example) as a natural nutritional ingredient in food processing.

2. Materials and methods

2.1. Wheat kernels

Three wheat classes in the United States, including HRS, HW, and SW, were supplied by Palouse Brand (Pullman, WA, USA), which were harvested, cleaned and packaged in Palouse (Eastern Washington) in August 2014. Their initial moisture contents of wheat berries are 10.72% for SW, 9.12% for HRS and 9.09% for HW.

2.2. Preparation of sprouted whole-wheat flour

Three classes of wheat kernels (200 g per batch) were soaked in water at 26 ± 2 °C for 6 h, reaching a moisture content of 22.72% for SW, 23.08% for HRS, and 24.24% for HW. The samples were then placed in germination trays that were placed in a controlled growth chamber (Z-3-1-H/AC, Cincinnati Sub-Zero, Cincinnati, OH, USA). The germination was performed at 28 ± 2 °C, and relative humidity of 95 ± 3% with moisture supplied by an ultrasonic humidifier (mist maker) (SPT SU-2020, Sunpentown, City of Industry, CA, USA). The sprouted wheat was removed from the germination (growth) chamber at selected germinating times from 5 to 24 h. The sprouted wheat was dried (OHG 100, Gallenkamp, London, UK) at 80 °C for 2 h to reach a final moisture content of 8–12%. The methods of Wang, Hou, Kweon, and Lee (2016) were used to prepare WWF of fine particle sizes (100–120 μm for hard wheat and 90–100 μm for soft wheat) by a Perten 3100 laboratory mill (Perten Instruments, Sweden) equipped with a

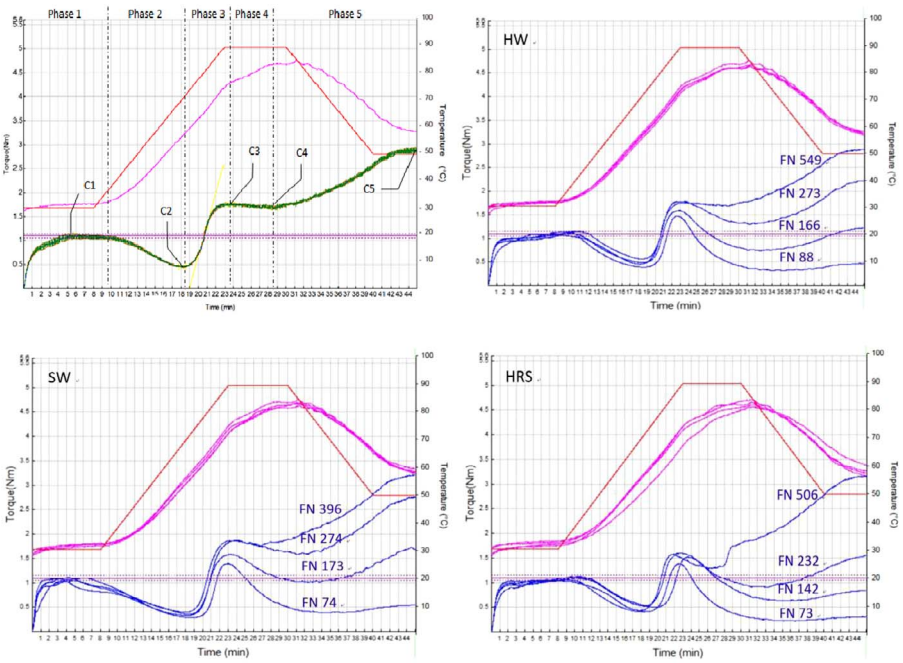

Fig. 2. Five phases (C1–C5) during a typical Mixolab test with the "Chopin Wheat^{+}" protocol. Mixolab test results for WWF from sprouted HW, SW, and HRS wheat with FN values in the ranges of < 100, 100–200, 200–300, and > 300 s. *HW 0/9/12/15 h, FN 549/273/166/88 s; SW 0/7/9/14 h, FN 396/274/173/74 s; HRS 0/8/10/15 h, 506/234/142/73 s. Abbreviations: whole-wheat flour, WWF; hard white, HW; soft white, SW; hard red spring, HRS.

0.8 mm metal mesh, then passed through a 0.6 mm screen (Wang et al., 2016).

2.3. Ultrasound treatment

The method of Yu, Engeseth, and Feng (2016) (Yu et al., 2016) was used with slight modifications to treat wheat samples. The system consisted of an ultrasound generator (25 kHz) and a stainless-steel water tank (height 500 mm × length 660 mm × width 460 mm). Two transducer boxes (1 kW each, 0.625 W/cm^2, height 400 mm × width 400 mm × thickness 90 mm) were installed face to face to the inner walls of the tank in an upright position, with a space of 480 mm between them. This setup allowed a uniform ultrasound treatment (100% output, 16 W/L), as shown in Fig. S. Wheat samples after soaking stage were sonicated for 5 min and 30 min.

2.4. Functionality evaluation of sprouted whole-wheat flour

2.4.1. Hagberg falling number (FN) test

The falling number (FN) value was determined using the AACC International Method 56-81.03 with an FN 1500 System (Perten Instruments, Sweden) with a flour sample size of 7 g (14% moisture basis) in 25 ml of water.

2.4.2. Rapid Visco Analyzer (RVA) starch pasting properties

The starch pasting properties were measured with a Rapid Visco Analyzer (RVA-4 series, Newport Scientific, NSW, Australia), following the AACC International Method 76–21.01. Flour samples (3.5 g each, 14% moisture) and 25 ml water were mixed to form slurries that were homogenized with an aluminum paddle right before a RVA test. The slurry in the analyzer was stirred at 960 rpm for 10 s and then at 160 rpm for the remainder of the test. The heating temperature was initially set at 50 °C, held for 1 min, and raised to 95 °C over 3.75 min. Then the slurry was held at 95 °C for 2.5 min, cooled to 50 °C over 3.75 min, and held at 50 °C for 2 min. The RVA results were expressed in cP. Starch pasting viscosity parameters include the highest viscosity of the paste after gelatinization (peak viscosity), the shear-thinned viscosity of the paste (trough viscosity), and the final viscosity (Zhang, Niu, Eckhoff, & Feng, 2005).

2.4.3. Mixolab mixing properties

The Mixolab (Chopin) test of 50 g flour samples was performed to assess the dough mixing properties of selected WWFs with FN values in the ranges of 50–100, 100–200, 200–300, and > 300 s, as shown in Fig. 2. The AACC International Method 54-60.01 with the "Chopin-Wheat^{+}" protocol was used, which measured the torque of the dough during mixing with an increase in temperature. The moisture of the sample was determined first. A preliminary hydration determination was performed to ensure the maximum torque during Phase 1 (C1) is within the target range of 1.1 ± 0.07 Nm. Five distinct phases (C1–C5) can be observed on the Mixolab curves, and the phase definition and the parameters measured in each phase were summarized by Dubat (2010) (Dubat, 2010). A typical Mixolab output obtained using the "Chopin^{+}" or "ChopinWheat^{+}" protocol is shown in Fig. 2. The green curve in Fig. 2 indicates the torque recorded by the sensor in Nm, the red curve represents the mixer temperature in °C, the pink curve denotes the dough temperature in °C, and the purple horizontal line shows the target consistency (1.1 Nm) that must be achieved during the hydration determination. On a typical Mixolab chart, Phase 1 is for the initial mixing, where the parameters include the percent of water required for the dough to produce a torque within the target range 1.1 ± 0.07 Nm (water absorption,%), maximum torque during Phase 1 (C1, Nm), the time to reach the C1 at 30 °C (T1, dough development time, min), and the mixing time that the torque stayed within the target range (stability, min). Phase 2 represents protein weakening, with a minimum torque at 30–60 °C (C2, Nm). Phase 3 features starch gelatinization, with minimum consistency at 60–90 °C (C3, Nm). Phase 4 highlights the stability of the hot-formed gel, with minimum consistency at 90 °C (C4, Nm). Lastly, phase 5 shows starch retrogradation during the cooling phase from 90 °C to 50 °C (C5, Nm).

2.5. Physicochemical analysis

Moisture content was determined using the AACC International Method 44-15.02. Total nitrogen content was determined by the Dumas combustion method (AACC International Standard Method 46-30, 2000) by a LECO FP528 Nitrogen Combustion Analyzer (Leco, St. Joseph, MI), and the protein content of WWF was calculated based on total nitrogen content using a nitrogen-to-protein conversion factor of 5.7. The glucose content was measured using a Glucose (GO) Assay Kit

(Sigma-Aldrich, MO, USA). The contents of soluble dietary fibre, insoluble dietary fibre, and total dietary fibre were determined following the Chinese standard method GB 5009.88-2014.

2.6. Determination of γ-aminobutyric acid (GABA) content

Freshly sprouted wheat samples were frozen in liquid nitrogen, freeze-dried, ground to powders with an electric blade grinder (BODUM-11160, New York, NY, USA), and stored at −18 °C for later analysis. The GABA concentration was determined using the protocol of Yang, Guo, and Gu (2013), with slight modifications (Yang et al., 2013). A high-performance liquid chromatography (HPLC) with a ZORBAX Eclipse AAA reversed-phase column (3.5 μm, 4.6 × 150 mm) was used to quantify GABA content. The HPLC run time for the separation of GABA from other compounds was less than 30 min with a column temperature of 35 °C. To extract GABA, 1 ml of sample was mixed with 1 ml $NaHCO_3$ (1 M, pH 9.0) and 1 ml dansyl chloride (1 mg/ml, in acetone) in a umber tube, and react at 69 °C for 10 min. The reaction was stopped by putting the tubes into an ice bath. The GABA peak and peak area were detected and quantified at 425 nm using UV–vis diode-array absorbance detection (DAD).

2.7. Statistical analysis

Results are reported as mean ± standard deviations (n = 3). The significance of differences among treatment means was determined using the one-way analysis of variance (ANOVA) calculated by SPSS version 12 (SPSS Institute, Chicago, IL, USA) with a significance level of $P < .05$.

3. Results and discussion

3.1. Effect of germination time on falling number (FN)

The Falling Number Standard Method is a viscometric assay that involves a rapid gelatinization of a flour suspension in a boiling water bath. Previous studies reported that sprouting decreased FN due to an increase in alpha-amylase activity in wheat grains (Olaerts et al., 2016). The alpha-amylase is commonly considered as one of the primary factors for bread production, it hydrolyzes large starch molecules, resulting in a dramatic reduction in viscosity and an inverse curvilinear relationship between its enzymatic activity and the FN value (Mares & Mrva, 2008). WWFs with different FN values after different germination times could be blended with non-germinated WWF (high FN) to obtain flour with a FN value suitable for specific end products.

In this study, the changes of FN value as affected by germination time was determined in a controlled germination process by sampling hourly from 5 to 15 h and at 24 h, as shown in Fig. 1. The FN values (14% moisture) before wheat germination were 506 (HRS), 549 (HW), and 396 (SW) s, but they decreased to 62, 86, and 62 s for germination of 24 h, respectively. The FN values are known to be influenced by wheat genotype and germination time (Olaerts et al., 2016). Among the three classes of wheat used in this study, the FN results exhibited different responses to sprouting (Fig. 1). The FN is generally determined at grain receival to assess the wheat quality and as an indication of alpha-amylase activity. Grain receivers do not want to receive low FN wheat, because breads produced from wheat flours of low FN values have poor fermentation and baking quality (Edwards et al., 1989), whereas an over-high FN wheat will not make good bread, unless bakers add malted barley flour or alpha-amylase tablets to increase enzyme activity and bread volume. The HW wheat grain had the highest FN value among the three wheat classes before germination, with the SW wheat grain having the lowest FN value (Fig. 1). The FN values of the three wheat classes exhibited a monotonic decrease with sprouting time, displaying an inverse curvilinear relationship between germination time and FN value. This is in line with the report of Singh et al. (2001), who found a similar decrease of FN value during sprouting (Singh et al., 2001). The changes of FN values with sprouting time can be described by a second order polynomial equation (Fig. 1) with high coefficients of determination, especially for the SW samples. These polynomial equations could be used to predict and estimate FN values of wheat under this controlled germination condition.

3.2. Effect of germination time on the RVA pasting and gelation properties

The alpha-amylase activity in wheat flour is important for the quality of resulting bakery products; this activity increases significantly during sprouting, causing substantial breakdown of starch molecules and other side effects associated with sprouting (Mares & Mrva, 2008). RVA is widely used as an indirect method to estimate the amount of alpha-amylase in barley by measuring the viscosity of the barley/water slurry. It is often used as a practicable method to detect and measure the degree of germination in barley (Edney, Legge, Izydorczyk, Demeke, & Rossnagel, 2013). Sprouted WWF with high amylolytic activity, low FN value and viscosity, contains higher fermentable sugars, which can be readily utilized by yeast for improved fermentation (Ziegler et al., 2016). Germinated WWF may be used as a natural ingredient to partially replace baking enzymes in bread baking. For instance, adding 2.5 g per 100 g sprouted rye flour shortened the sourdough fermentation process by 8 h (Diowksz, Kordialik-Bogacka, & Ambroziak, 2014). In this study, the RVA test was performed to assess the starch pasting properties of selected WWFs of three

Table 1
Effects of germination time on the RVA starch pasting properties of WWF from germinated HW, SW and HRS wheat with the FN values in the ranges of < 100, 100–200, 200–300, and > 300 s.

WWF	FN, s	Peak Viscosity, cP	Trough Viscosity, cP	Breakdown, cP	Final Viscosity, cP	Setback, cP	Peak Time, min
HW	549	845 ± 9[a]	593 ± 6[a]	259 ± 2[a]	1247 ± 16[a]	654 ± 12[a]	5.4 ± 0[a]
	273	479 ± 17[b]	224 ± 8[b]	246 ± 8[b]	548 ± 14[b]	324 ± 7[b]	5.1 ± 0[b]
	166	206 ± 5[c]	36 ± 8[c]	170 ± 6[c]	96 ± 8[c]	60 ± 1[c]	4.7 ± 0[c]
	88	56 ± 3[d]	−21 ± 4[d]	77 ± 6[d]	−11 ± 4[d]	6 ± 2[d]	3.8 ± 0.1[d]
SW	396	828 ± 64[a]	605 ± 35[a]	284 ± 39[a]	1295 ± 69[a]	690 ± 34[a]	5.4 ± 0.1[a]
	274	625 ± 23[b]	365 ± 10[b]	260 ± 16[b]	858 ± 22[b]	493 ± 17[b]	5.2 ± 0[b]
	173	270 ± 19[c]	79 ± 5[c]	191 ± 14[c]	203 ± 11[c]	124 ± 9[c]	4.8 ± 0.1[c]
	74	71 ± 3[d]	−15 ± 3[d]	86 ± 3[d]	−6 ± 7[d]	9 ± 5[d]	3.6 ± 0.1[d]
HRS	506	825 ± 23[a]	616 ± 18[a]	209 ± 4[a]	1314 ± 25[a]	698 ± 7[a]	5.2 ± 0[a]
	234	287 ± 8[b]	113 ± 4[b]	174 ± 4[b]	274 ± 10[b]	161 ± 11[b]	4.8 ± 0[b]
	142	117 ± 3[c]	16 ± 1[c]	106 ± 4[c]	49 ± 1[c]	31 ± 4[c]	4.5 ± 0[c]
	73	67 ± 5[d]	8 ± 3[d]	54 ± 5[d]	12 ± 1[d]	7 ± 2[d]	3.7 ± 0.5[d]

*Differences in each RVA property between different germination time, for each wheat class, are highlighted by different letters at $p < .05$.
Abbreviations: whole-wheat flour, WWF; hardwhite, HW; soft white, SW; hard red spring, HRS.

wheat classes with FN values in the ranges of 50–100, 100–200, 200–300, and > 300 s. The results of RVA test are shown in Table 1.

As can be seen in Table 1, for all the wheat classes, the peak viscosity, trough viscosity, breakdown, final viscosity, setback, and peak time decreased sharply after germination ($P < .05$) when compared to the raw wheat samples. The average peak viscosity decreased from 845 to 56 cP (HW), 828 to 71 cP (SW), and 825 to 67 cP (HRS) within sprouting for 15 h (Table 1). The sprouted WWF samples of each wheat class experienced a dramatic decrease in trough viscosity and final viscosity, similar with the trend that was observed in the FN tests (Fig. 1). The reduction of peak viscosity of sprouted WWF might be due to the degradation of starch and reduction of swelling index of starch caused by germination. The average breakdown value decreased from 259 to 77 cP (HW), 284 to 86 cP (SW), and 209 to 54 cP (HRS) within sprouting for 15 h (Table 1). Similarly, the average setback value decreased from 654 to 6 cP (HW), 690 to 9 cP (SW), and 698 to 7 cP (HRS) after 15 h of sprouting (Table 1). The decrease in breakdown value suggested a higher stability of the starches (Breakdown value = Peak viscosity − Trough viscosity). Generally, setback and final viscosity are important parameters when WWF is used as an ingredient of grain-based foods because the quality of end-products, such as texture and physical properties deteriorates due to starch retrogradation (Bhat, Wani, Hamdani, Gani, & Masoodi, 2016). Setback value (Setback value = Final viscosity − Peak viscosity) measures the retrogradation ability of the paste and reflects the reordering of starch molecules. The decrease in the setback and final viscosity is mainly due to reordering of amylose and amylopectin, which causes a decrease by the degradation of amylopectin branched chains. Decrease in setback could be an indicator of the improvement in texture quality of WWF end-products like breads and buns (Bhat et al., 2016).

3.3. Effect of sprouting time on the dough mixing properties

Water absorption capacity is one of the main functional properties of flours, which is a key parameter in the Mixolab test (Rosell, Santos, & Collar, 2010). In this study, water absorption (hydration) of these selected sprouted WWF samples in each of the three wheat classes (HW, SW, and HRS) generally decreased and followed the same trend as in the FN tests when the germination time increased. Corresponding to the FN values (Table 1), the water absorption (hydration) values are HW (66.0%, 62.0%, 62.3%, 61.0%), SW (60.5%, 59.0%, 58.5%, 56.0%), and HRS (64.0%, 61.0%, 60.0%, 59.5%) (Table 2). The decrease of water absorption may be attributed to enzymatic hydrolysis of large molecules, such as starch and storage proteins, during germinating (Ohm, Lee, & Cho, 2016). Liu et al. (2017) also reported that the sprouted WWF had lower water absorption, and decreased particle size of WWF resulted in slightly lower water absorption (Liu et al., 2017). The relationship between germination and the change of particle size of WWF remain to be examined.

As can be seen in Table 2, the protein weakening during heating (C2) increased in Mixolab analysis, whereas the C3 (pasting viscosity peak), C4 (pasting viscosity minimum torque), and C5 (retrogradation final torque, end of the blue line) decreased for all three wheat classes when increasing the germination time or decreasing FN. During Phase 2, heating caused a breakage of the protein links; a higher C2 means gluten is less weakened so that a high value of C2 means more stable gluten structure during dough heating stage (Erukainure et al., 2016). The increase of the dough temperature during Phase 3 contributed to the rupture of starch granules, resulting in lower pasting temperature and higher paste consistency; C3 indicated the maximum viscosity during Phase 3. The high dough temperature and water released by the denatured proteins caused the starch to swell and burst, thereby inducing a slightly increased dough consistency (Erukainure et al., 2016). A reduction in viscosity caused by the physical breakdown of starch granules is observed in the Phase 4, leading to a minimum value for the torque. During cooling in Phase 5, gelatinized amylose molecules in the dough begin to recrystallize, leading to retrogradation of the end products; a higher C5 means more starch retrogradation, thus the decreased value of C5 portrays longer shelf stability and better texture of the end products. In this study, the decrease in C3, C4, and C5 values followed the same trend as the viscosities in the RVA tests, showing a good correlation between these two analysis methods, although these two methods use two different dough systems.

Dough development time (T1) measures the time between the first addition of water and the time when the dough reaches the optimum elastic and viscous properties. For instance, the 9 h-germination HW samples had the longest T1 of 10.23 min, and that for 8 h-HRS sample was 10.03 min and that for 14 h-SW sample was 5.69 min. As shown in Table 2, the SW dough has a shorter T1 during mixing than HW and HRS doughs, mainly caused by the difference in their protein content. In this study, the crude protein content (14% moisture basis) of the non-germinated WWF was 11.95 ± 0.16 g/100 g for SW, 13.95 ± 0.06 g/100 g for HRS, and 14.86 ± 0.03 g/100 g for HW. For the sprouted HW- and HRS-WWF, a slight decrease of T1 was observed after 9 h-germination for HW and 8 h- germination for HRS. During germination, the stability time for the SW samples increased significantly from 3.29 min (control) to 7.76 min (germinating for 15 h), while that for HW increased from 9.38 min (control) to 10.77 min (germinating for 9 h), and increased from 9.72 min (control) to 11.71 min (germinating for 8 h) for the HRS samples. The stability time of 8 h-germination and 10 h-germination for HRS has no significant difference ($P > .05$) (Table 2), indicating that the suitable sprouting duration time to achieve the longest stability time should be 8 h for HRS, 9 h for HW and 10 h for HRS. In short, well-controlled germination process could

Table 2
Main parameters in the Mixolab tests for WWF from sprouted HW, SW and HRS wheat with the FN values in the ranges of < 100, 100–200, 200–300, and > 300 s.

WWF	FN, s	Water absorption,%	T1, min	C1, Nm	C2, Nm	C3, Nm	C4, Nm	C5, Nm	Stability, min
HW	549	66.0	5.55 ± 0.04^{d}	1.09 ± 0.01^{b}	0.45 ± 0^{c}	1.76 ± 0.01^{a}	1.71 ± 0.04^{a}	2.82 ± 0.07^{a}	9.38 ± 0.11^{b}
	273	62.0	10.23 ± 0.07^{a}	1.14 ± 0.01^{a}	0.55 ± 0.01^{a}	1.77 ± 0.01^{a}	1.29 ± 0.01^{b}	2.21 ± 0.03^{b}	10.77 ± 0.19^{a}
	166	62.3	9.46 ± 0.20^{b}	1.12 ± 0.01^{ab}	0.50 ± 0.01^{b}	1.61 ± 0.01^{b}	0.76 ± 0.03^{c}	1.27 ± 0.06^{c}	9.70 ± 0.64^{b}
	88	61.0	9.05 ± 0.33^{c}	1.11 ± 0.04^{ab}	0.38 ± 0.01^{d}	1.46 ± 0.02^{c}	0.29 ± 0.04^{d}	0.43 ± 0.06^{d}	9.47 ± 0.33^{b}
SW	396	60.5	3.61 ± 0.16^{d}	1.08 ± 0.05^{a}	0.37 ± 0.01^{a}	1.88 ± 0.01^{a}	1.63 ± 0.16^{a}	3.24 ± 0.04^{a}	3.29 ± 0.20^{d}
	274	59.0	4.13 ± 0.14^{c}	1.11 ± 0.01^{a}	0.39 ± 0.01^{a}	1.85 ± 0.03^{a}	1.55 ± 0.04^{b}	2.71 ± 0.07^{b}	5.27 ± 0.16^{c}
	173	58.5	4.84 ± 0.01^{b}	1.08 ± 0.04^{a}	0.35 ± 0.02^{a}	1.61 ± 0.04^{b}	1.03 ± 0.03^{c}	1.74 ± 0.05^{c}	6.41 ± 0.06^{b}
	74	56.0	5.69 ± 0.08^{a}	1.10 ± 0.01^{a}	0.28 ± 0^{b}	1.39 ± 0.01^{c}	0.39 ± 0.01^{d}	0.55 ± 0.01^{d}	7.76 ± 0.20^{a}
HRS	506	64.0	7.69 ± 0.86^{c}	1.08 ± 0.04^{ab}	0.45 ± 0.01^{c}	1.59 ± 0.01^{a}	1.32 ± 0.04^{a}	3.17 ± 0.01^{a}	9.72 ± 0.64^{b}
	234	61.0	10.03 ± 0.64^{a}	1.10 ± 0.01^{ab}	0.53 ± 0.01^{a}	1.60 ± 0.01^{a}	0.90 ± 0^{b}	1.54 ± 0.01^{b}	11.71 ± 0.13^{a}
	142	60.0	10.00 ± 0.21^{a}	1.12 ± 0.01^{a}	0.51 ± 0.01^{b}	1.52 ± 0.01^{b}	0.61 ± 0.03^{c}	0.87 ± 0.04^{c}	11.63 ± 0.07^{a}
	73	59.5	8.86 ± 0.13^{b}	1.08 ± 0.01^{b}	0.41 ± 0^{d}	1.38 ± 0.01^{c}	0.22 ± 0^{d}	0.31 ± 0^{d}	9.96 ± 0.27^{b}

*Differences in each parameter between different germination time, for each wheat class, are highlighted by different letters at $p < .05$.
Abbreviations: whole-wheat flour, WWF; hard white, HW; soft white, SW; hard red spring, HRS.

Table 3
Fibre and glucose content of WWF from sprouted HW, SW and HRS wheat with the FN values in the ranges of < 100, 100–200, 200–300, and > 300 s.

WWF	FN, s	Soluble fibre, g/100 g	Insoluble fibre, g/100 g	Total fibre, g/100 g	Glucose, mg/100 g
HW	549	1.97 ± 0.05[b]	7.60 ± 0.10[b]	9.55 ± 0.03[b]	74.21 ± 3.04[d]
	273	2.12 ± 0.03[a]	7.18 ± 0.06[c]	9.30 ± 0.05[c]	155.46 ± 7.66[c]
	166	1.88 ± 0.01[d]	7.17 ± 0.02[c]	9.06 ± 0.04[d]	224.74 ± 7.44[b]
	88	1.92 ± 0.01[c]	7.87 ± 0.05[a]	9.77 ± 0.03[a]	423.58 ± 7.87[a]
SW	396	2.17 ± 0.03[a]	8.13 ± 0.05[a]	10.29 ± 0.10[a]	158.56 ± 7.52[d]
	274	1.92 ± 0.05[c]	7.53 ± 0.04[c]	9.45 ± 0.07[d]	254.95 ± 7.45[c]
	173	1.77 ± 0.07[d]	7.80 ± 0.03[b]	9.57 ± 0.09[c]	375.95 ± 10.39[b]
	74	2.01 ± 0.05[b]	7.81 ± 0.05[b]	9.84 ± 0.05[b]	548.19 ± 8.78[a]
HRS	506	1.66 ± 0.05[d]	8.44 ± 0.05[b]	10.11 ± 0.08[c]	125.65 ± 4.56[d]
	234	1.58 ± 0.05[d]	8.85 ± 0.03[a]	10.12 ± 0.03[b]	187.90 ± 7.72[c]
	142	2.34 ± 0.07[b]	8.51 ± 0.04[a]	10.86 ± 0.04[a]	240.01 ± 10.50[b]
	73	2.47 ± 0.03[a]	8.40 ± 0.07[b]	10.86 ± 0.08[a]	392.43 ± 9.41[a]

[*]Differences in concentrations (14% moisture basis), for each compound, are highlighted by different letters at $p < .05$.
Abbreviations: whole-wheat flour, WWF; hard white, HW; soft white, SW; hard red spring, HRS.

improve the gluten strength of whole wheat dough and enhance its performance in end-product applications.

3.4. Effect of sprouting time on the content of glucose and dietary fibres

During germination, the glucose content in wheat samples increased by 4–37% and 227–357%, respectively, after 5 and 15 h of germination (Table 3). Whole wheat flour is generally considered to have higher bitterness intensity than refined wheat flour, which negatively influences WWF product acceptability by consumers (Jiang & Peterson, 2016), thus sugar is commonly added in the formulation to mask the bitterness taste. With the increased glucose content in WWF from sprouted wheat, additional sugar may not be needed for this purpose. Glucose is also beneficial to drive yeast-mediated bread dough fermentation during bread-making besides maltose (Struyf et al., 2017).

Whole grain, such as whole wheat, is abundant sources of soluble and insoluble dietary fibres, which have health-promoting effects (Rebello, Greenway, & Finley, 2014). Koehler et al. (2007) reported that the content of soluble dietary fibre in wheat remained constant or decreased slightly during the first 96 h of germination at 20-25 °C, then increased afterward, in contrast to the soluble dietary fibre, insoluble dietary fibre decreased with prolonged germination times (after 102 h-germination), but the decrease was less than the increase of soluble dietary fibre, thus resulting in an overall increase of the total dietary fibre (Koehler et al., 2007). In our study, at germination temperature of 28 ± 2 °C, a significant increase of soluble dietary fibre was observed in sprouted HW after 12 h, and higher total dietary fibre was observed in sprouted SW (14 h), HRS (15 h), and HW (12 h), as shown in Table 3.

3.5. Effect of ultrasound treatment on the GABA content

To evaluate the changes in GABA content during longer germination period under more prolonged sprouting conditions, one of the three wheat classes, SW was selected for sprouting with a longer duration up to 72 h. SW was chosen because it is easier to germinate than other classes of wheat in laboratory and has a lower pre-harvest sprouting tolerance in field (Schramm, Nelson, Kidwell, & Steber, 2013). A 72-h germination will produce wheat of very low FN (close to a minimum of 60 s), and its functionality in end-product applications is very limited or none. As a result, much of pre-harvest sprouted wheat harvested each year cannot be used for human consumption and hundreds of million dollars are lost each year in the U.S. Pre-harvest sprouted is a worldwide problem in wheat production, with an estimated annual loss of up to US $1 Billion worldwide (Liu et al., 2013). It is of huge economic importance to add value to the pre-harvest sprouted wheat by utilizing it in food applications.

As shown in Fig. 3, a similar but more pronounced increase in GABA was observed during SW germination, with the GABA content doubled in only 24 h (39.76 ± 1.52 mg/100 g), and continued to increase reaching 3.01 times and 3.39 times of the GABA content in the non-sprouting SW (14.68 ± 0.43 mg/100 g) after 48 h (44.16 ± 0.29 mg/100 g) and 72 h (49.72 ± 1.07 mg/100 g) of sprouting, respectively. A similar observation was reported by Hao, Wu, Li, Wang, and Liu (2016) in germination of buckwheat, where the GABA content after a 48 h-germination was doubled compared to the control, and continued to increase monotonically until it reached a peak at 144 h (Hao et al., 2016). Results of this experiment suggested that severely sprouted SW could be used as natural ingredient to increase nutritional profiles in food.

The kernel hardness of wheat is related to the sensitivity of an environmental stress response (Lesage et al., 2012), especially mechanical stress, thus SW wheat was selected for the ultrasound treatment because of its soft kernel texture. The production of GABA is a signal to induce higher stress in plants (Ramesh et al., 2015). The changes in GABA content after 72 h of germination under acoustic stresses were measured and shown in Fig. 3. Specifically, the sprouting grains were treated with ultrasound (25 kHz) for 0, 5, and 30 min after soaking in a specially designed treatment tank, as shown in Fig. 3. The ultrasound treatment significantly enriched the GABA content in SW wheat, compared to germination under normal conditions. Compared to the untreated WWF undergone 72 h germination (Fig. 3), the GABA content of the 72 h germinated SW was 54.82 ± 6.15 mg/100 g and

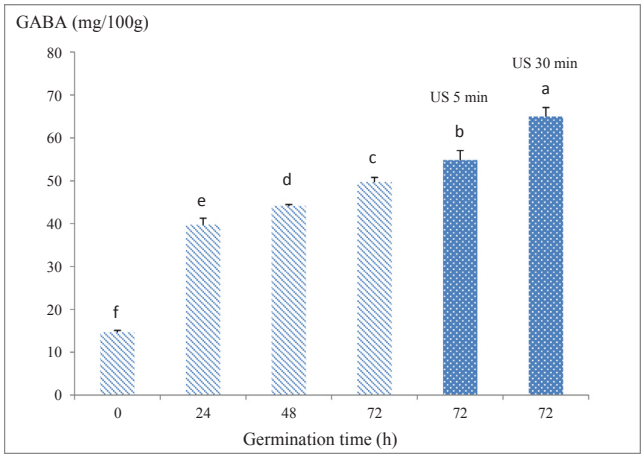

Fig. 3. Changes in the GABA content (DB) of sprouted soft white wheat during germination for up to 72 h at 28 ± 2 °C at relative humidity of 95 ± 3% treated with and without ultrasonication. [*]Differences in concentrations are highlighted by different letters at $p < 0.05$. Abbreviations: gamma-aminobutyric acid, GABA; ultrasonication treatment after soaking, US.

64.98 ± 2.13 mg/100 g after ultrasound treatment for 5 and 30 min, increased by 10.26% and 30.69%, respectively. GABA is produced from glutamate catalyzed by glutamate decarboxylase (GAD; EC 4.1.1.15), which has higher enzymatic activity during germination (Shelp, Bozzo, Trobacher, Chiu, & Bajwa, 2012). Goussous, Samaram, Alqudah, and Othman (2010) reported that ultrasound treatment (40 kHz) increased the germination rates of wheat kernels, which might due to the activated endogenous enzymes (Goussous et al., 2010). Yang et al. (2015) also reported a 43.39% increase of GABA content in soybean sprouts compared to the control when the samples were treated by ultrasound (40 kHz) for 30 min after soaking. Ultrasound treatment (25 kHz) in this study may be one of the effective environmental elicitations to stimulate sprouting grains for producing more health-promoting compounds. Given this, the combination of controlled germination process with power ultrasound treatment may provide an effective and rapid method for enhancing GABA in grain-based products, including sprouted whole-wheat products. Further studies are encouraged to investigate the retention of GABA and antioxidants in sprouted WWF during baking or steaming processes, and to evaluate the performance of germinated WWF in different cereal end-products to improve its functionality or enhance nutritional value.

4. Conclusion

The findings of this study demonstrated that controlled germination for 5–15 h produced WWF with improved flour functionality i.e., increased glucose content, less starch retrogradation during gelatinizing, improved gluten quality with less weakening, and longer mixing stability time during dough mixing. The severely sprouted SW for 72 h enhanced health-promoting bioactive compounds, including GABA and antioxidants, which could be used as natural nutrient enhancer in foods. The controlled germination process could be an alternative natural approach to produce WWF to improve its end-use functionality or enhance health benefits as a food ingredient.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.09.128.

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