

# Enhancement of Technological Functionality of White Wheat Bread Using Wheat Germ Sourdough Along with Dehydrated Spinach Puree

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## ABSTRACT

Bread enrichment with fiber-rich leafy vegetables and fermented by-products of milling has gained more attention in recent years. In the present study, the antifungal lactic acid bacterium isolated from Tarkhineh was used as starter culture in controlled wheat germ sourdough in order to improve quality and safety of the white wheat bread containing wheat germ sourdough along with dehydrated spinach puree. The optimized formulation (10% wheat germ sourdough and 20% dehydrated spinach puree, which were replaced by wheat flour) was determined based on the crumb softness of the enriched breads. Then, the quality characteristics of the supplemented breads were studied in terms of textural properties, antioxidant activity, surface moldiness, phytic acid content and organoleptic acceptability. The sequencing of polymerase chain reaction products led to the identification of *Lactococcus lactis* as the selected antifungal lactic acid bacterium isolate. Texture profile analysis revealed the significant effect ( $P < 0.05$ ) of wheat germ sourdough and dehydrated spinach puree on crumb hardness and loaf specific volume of the produced breads. Bread containing wheat germ sourdough along with dehydrated spinach puree had the highest radical scavenging activity. Green zone of *Aspergillus flavus* growth on wheat germ sourdough supplemented bread and phytic acid content of the aforementioned sample were also remarkably lower than the others. Furthermore, there was no significant difference between bread enriched with wheat germ sourdough and wheat germ sourdough along with dehydrated spinach puree in terms of sensory acceptability compared to the control sample.

**Keywords:** Dehydrated spinach puree, Enriched bread, *Lactococcus lactis*, Tarkhineh LAB.

## INTRODUCTION

Bread is staple food all over the world and, therefore, its enrichment with natural plant-based products is a key strategy to improve its nutritional values and quality characteristics. Among the potential candidates, by-products of cereal milling (such as wheat germ) and green leafy vegetables (like spinach) are the cheapest and locally available bio-additives rich in vitamins and micronutrients. The

positive effects of these supplements on bread sensory and quality attributes, as well as its safety have been also verified. Furthermore, the results of the pharmaceutical investigations revealed that a high-fiber diet may reduce the risk of serious disease (Elleuch *et al.*, 2011; Hobbs *et al.*, 2014).

Wheat germ as a by-product of wheat milling is rich in polyunsaturated fatty acids and, therefore, its removal is aimed at eliminating of flour rancidity and reducing of its anti-nutritional factors such as raffinose and

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phytic acid. Meanwhile, wheat germ is considered as a valuable source of functional compounds including phytochemicals, dietary fiber, minerals,  $\alpha$ -tocopherol and vitamins. Interestingly, it is revealed that the lactic fermentation can improve some nutritional properties of the germ, control the endogenous lipase activity, reduce the concentration of raffinose and increase its phytase activity (Rizzello *et al.*, 2010; Rizzello *et al.*, 2011). Positive effects associated with the use of wheat germ in bread have been detected in Sun *et al.* (2015) survey. Accordingly, addition of appropriate levels (up to 6% of flour) of wheat germ affects quality (sensory and textural) attributes of the supplemented bread without altering its desirable properties.

Rizzello *et al.* (2010) observed that the concentration of free amino acids, protein digestibility, and antioxidant activities were also increased in bread containing freeze dried Wheat Germ Sourdough (WGS: 4% w/w of wheat flour). Furthermore, shelf life, texture and sensory characteristics of traditional wheat bread were enhanced using WGS. Reduction of glutathione, as well as inactivation of lipase and lipoxygenase was observed in wheat germ-stabilized by sourdough fermentation in Marti *et al.* (2014) study. Furthermore, addition of 20% WGS led to a high specific volume and low firmness in produced bread through acidification induced by Lactic Acid Bacteria (LAB).

Production of novel vegetable-enriched bread as a potential vehicle to increase consumption of bioactive ingredients, health-promoting nutrients and antioxidants is also a crucial strategy. It is reported that the use of spinach as a part of bread formulation increases water holding capacity and bread quality, decreases dough stability, improves overall acceptability, and enhances the daily intake of vitamins (B, C, K) and minerals (iron, calcium). Spinach is also rich in folate (natural isoforms of vitamin B<sub>9</sub>), that is synthesized only by the plants and microorganisms, and it is involved in many important metabolic pathways (López-Nicolás *et al.*, 2014).

López-Nicolás *et al.* (2014) indicated that the folate-fortified bread (containing 40% spinach) obtained higher scores in sensory acceptability in comparison with the non-fortified wheat breads. Furthermore, spinach as a fiber-rich leafy vegetable positively influenced bread quality attributes. Khan *et al.* (2015) studied the effect of spinach incorporation on physico-chemical, nutritional and sensory characteristics of chapatti. These researchers revealed that 5% dehydrated spinach powder remarkably improved textural and sensory qualities, as well as mineral, carotenoid and fiber contents of the enriched bread. Sadeghi *et al.* (2019) reported that controlled rice bran sourdough along with pumpkin puree improved techno-functional properties of the supplemented wheat bread. Accordingly, antioxidant capability, *in situ* antifungal activity, and textural properties of the enriched product were significantly better than the control sample.

The aim of the present study was simultaneous application of WGS (fermented with a selected Tarkhineh LAB) and Dehydrated Spinach Puree (DSP) to improve quality characteristics of loaf wheat bread. It should be noted that, based on our best knowledge, there is no report about simultaneous application of these supplements in order to produce supplemented wheat bread. These compounds contain functional ingredients that can modify the sensory properties, antioxidant capacity, textural features and the other techno-functional attributes of the product due to their synergistic interactions.

## MATERIALS AND METHODS

### Raw Materials

Wheat germ flour (by-product of wheat milling with particle size of 500  $\mu$ m) was purchased from a local milling factory. The chemical compositions of the raw materials are as below: White wheat flour (68% extraction rate, 27.2% wet gluten) and

wheat germ flour used in the present study had  $1.37\pm 0.09$ ,  $7.92\pm 0.11\%$  fat;  $12.25\pm 0.20$ ,  $26.43\pm 0.17\%$  protein; and  $0.55\pm 0.03$ ,  $1.74\pm 0.05\%$  ash contents, respectively, in accordance with AACC (2010) standard methods. Microbial media (Merck, Germany) and chemical reagents (Merck, Germany; Sigma-Aldrich, USA) were also purchased with analytical grade.

### Isolation, Molecular Identification, and Screening of the Tarkhineh LAB

Tarkhineh (Kashkineh) as a nutritious Iranian traditional fermented food was formulated (50% goats' fermented milk and grounded wheat) and then it was processed (incubation at  $37^{\circ}\text{C}$  for 24 hours). Tarkhineh LAB were also isolated through spread plating of the serially ten-fold diluted samples on de Man, Rogosa and Sharpe (MRS) and M17 agar. Then, the plates were incubated (at  $37^{\circ}\text{C}$  for 24 hours) and subsequently the colonies were streaked plate in the identical conditions to obtain single pure colony of each LAB isolate. After that, the isolates were checked by Gram-staining and catalase assay, and they were screened based on their inhibitory activity against *Aspergillus flavus* (ATCC 9643) through the overlay bioassay (Magnusson *et al.*, 2003). Next step, the selected LAB isolate (with the highest antifungal effect) was identified by a PCR-amplification (Corbett, Australia) of 1500 base pair (bp) target sequence from its *16S rDNA* gene in accordance with Abnous *et al.* (2009). Finally, the PCR products were sequenced (Bioneer, South Korea), and the sequencing results were compared with the data available in National Center for Biotechnology Information (NCBI) by Basic Local Alignment Search Tool (BLAST) algorithm. The phylogenetic evolutionary tree was also drawn with the data using MEGA6 software through neighbor-joining method with 1,000 bootstrap replicates (Tamura *et al.*, 2013).

### Controlled Fermentation of Wheat Germ by the Selected Tarkhineh LAB

After two times activation (24 hours incubation at  $37^{\circ}\text{C}$  on MRS broth) of the selected LAB isolate, its population was adjusted to  $10^8$  Colony Forming Units (CFU)  $\text{mL}^{-1}$ , and then the Tarkhineh LAB was inoculated ( $10^8$  CFU  $\text{g}^{-1}$ ) to mixture of wheat germ and sterile tap water with dough yield (the ratio of dough to flour $\times 100$ ) of 160. Subsequently, the mixture was incubated at  $37^{\circ}\text{C}$  for 24 h, and then the pH (pH meter; Knick, Germany) and Total Titratable Acidity (TTA) of the produced WGS were monitored in accordance with Rizello *et al.* (2010) through titration of 10 g sourdough and 90 mL water mixture against 0.1N NaOH to pH 8.5.

### Preparation of DSP

To produce DSP, spinach leaves were blanched ( $80^{\circ}\text{C}$  for 10 minutes), and then the leaves were mixed by a household steamer-mixer (Philips, Netherland). Subsequently, the produced puree was dehydrated until the final moisture content of approximately 20% by a laboratory heating oven (Diamante *et al.*, 2014). This puree had  $20.65\pm 0.37\%$  protein and  $10.25\pm 0.16\%$  crude fiber (AOAC, 2003).

### Bread Formulations and Baking

To produce control bread, wheat flour, tap water (60%), baker's yeast (2%: Razavi Yeast Co., Iran), sugar (1%) and NaCl (1%: Golha Co., Iran) were mixed, and the mixture was proofed in Behdad Co. (Iran) laboratory incubator (90 minutes at  $36^{\circ}\text{C}$ ). Subsequently, the dough (divided into pieces of  $100\pm 5$  g) was baked (at  $180\pm 5^{\circ}\text{C}$  for  $20\pm 2$  minutes) in an electrical oven (Feller, Germany). Next, screening based crumb softness (instrumental analysis) along with sensory acceptability (panel test) was used to select the best formulation among the



samples containing 5-15% WGS and 10-30% DSP. After selecting the best formulation, the appropriate amount of WGS, DSP or their mixture, as well as Non-Fermented Wheat Germ (NFWG) alone and mixed with DSP were also added to the dough prepared for the control sample before the proofing step. After that, these samples were processed in identical conditions, and their textural and sensorial characteristics, as well as their antioxidant activities and phytic acid contents were determined in comparison with the control. It should be noted that water absorption of the control dough (wheat dough) was determined using farinography (Brabender, Germany). Then, the moisture content of the other dough samples (supplemented with WGS, NFWG, DSP or their mixtures) was determined in accordance with AACC (2010) standard method. Subsequently, amount of water for each formulation was calculated considering its moisture content and water content of the control sample (based on dry-basis weight). Accordingly, the amount of water added to each formulation was sample-specific.

### Crumb Textural Properties

The crumb textural properties (hardness: force required for a pre-determined deformation, springiness: rate at which a deformed sample returns to its original size and shape, and cohesiveness: strength of internal bonds in the sample) were investigated using a Texture Profile Analysis (TPA) system (Plus Stable Micro Systems, England) with a cylindrical aluminum probe (25 mm diameter) as described by Rizello *et al.* (2010), 2 hours after baking. In TPA assay, the test speed was 1.0 mm/s to compress the center of the crumb a distance of 50% (three uniform slices of 25 mm thickness). Specific volume of the loaf was also evaluated according to the rapeseed displacement method (AACC, 2010). Bread specific volume was obtained by dividing

the volume by the loaf weight (expressed as  $\text{cm}^3 \text{g}^{-1}$ ).

### Surface Moldiness

Surface moldiness on the produced breads was studied through measurement of the *A. flavus* mycelia and its green zone growth according to Gerez *et al.* (2009) method with slight modifications. In brief, a sterile paper disc (6 mm) was placed on the produced breads. Then, the fungal spores ( $10^6$  spores  $\text{mL}^{-1}$ ) were inoculated on the disc. Subsequently, the diameter of the mold growth was determined around the disc. The impact of the formulations on bread pH and water activity ( $a_w$ ) was also investigated through measuring the pH and  $a_w$  ( $a_w$  meter; Novasina LabSwift, Switzerland).

### Antioxidant Activity

The scavenging effect of WGS, DSP, WGS-DSP, NFWG and NFWG-DSP was assayed using 1,1-DiPhenyl-2-PicrylHydrazyl (DPPH) free radical on methanolic (10% w/v in 80% methanol) extract of the supplemented breads compared to the control wheat bread and Butylated HydroxyAnisole (BHA) as a reference through measuring of absorbance using a spectrophotometer (PG Instruments, England) at 517 nm (Yu *et al.*, 2003). The total phenol content of the methanolic extract was also determined using Folin-Ciocalteu's reagent and expressed as gallic acid equivalent. To 50 mL of each sample, 2.5 mL 1/10 dilution of the reagent and 2 mL of  $\text{Na}_2\text{CO}_3$  (7.5% w/v) were added and incubated at 45°C for 15 minutes. The absorbance of the samples was measured at 765 nm (Chlopicka *et al.*, 2012).

### Phytic Acid Content

Phytic acid contents of the produced breads were determined in accordance with

the method described by Haug and Lantzsch (1983). Briefly, each sample was extracted with 0.2N HCl. Then, 1 mL of ammonium iron III solution was added to 0.5 mL of this extract. Subsequently, the mixture was heated (boiling water bath for 30 minutes) and cooled to the room temperature. Next, it was centrifuged (Hanil Combi, South Korea; 3,000×g for 30 minutes), and 1 mL of the supernatant was mixed with 1.5 mL of 2,2'-bipyridine solution. Finally, the absorbance was measured at 519 nm. The amount of phytic acid was determined using a standard curve calibrated with the sodium salt of phytic acid.

### Sensory Acceptability

Bread (a slice of crumb and crust together with approximately 30 mm thickness) sensory acceptability was determined 2 hours after baking (under white light at room temperature) in terms of chew ability, color, flavor, shape and aroma through a 5-point hedonic scale (with 1 and 5 as the lowest and the highest scores, respectively) by semi trained panelists (5 males and 5 females aged between 22 and 42 years) in accordance with Rizello *et al.* (2010). Finally, the average of scores was recorded.

### Statistical Analysis

All the experiments were done in triplicates, and the data were statistically

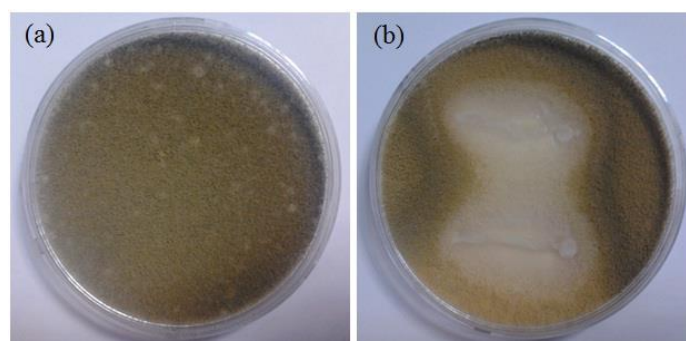
analyzed using one-way Analysis Of Variance (ANOVA). The means were also compared through the Least Significant Difference (LSD) post hoc by SPSS (version 20) software at  $P < 0.05$  statistical significance. The data were also reported as mean±standard deviation.

## RESULTS AND DISCUSSION

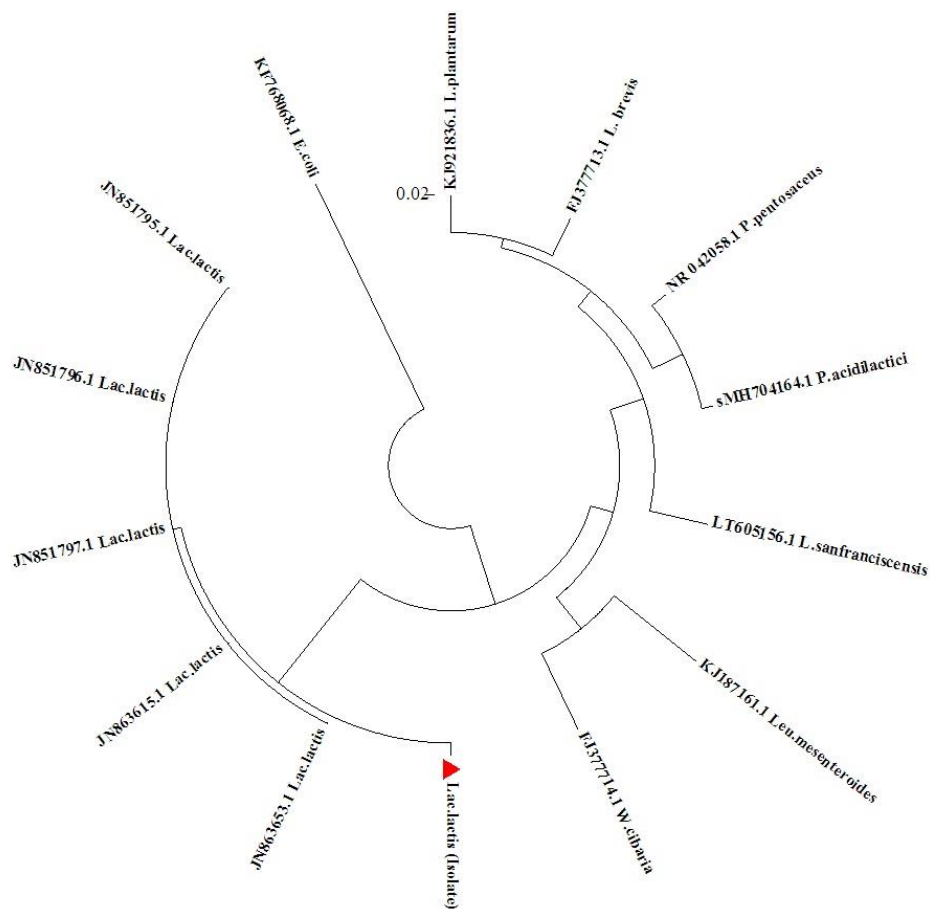
### LAB Isolation, Selection and Identification

Among the different catalase-negative and Gram-positive LAB isolated from Tarkhineh, potent antifungal activity of the selected LAB isolate was verified (Figure 1). This cocci isolate was identified as *Lactococcus lactis* based on the sequencing results of the PCR products. The selected isolate was a member of *Lactococcus* genus, and *L. lactis* JN863653.1 was the nearest LAB to the isolate in phylogenetic evolutionary tree (Figure 2).

Tarkhineh is a mixture of grounded wheat and goats' fermented milk. This fermented ecosystem harbors LAB with proper techno-functional capabilities. Therefore, Tarkhineh is a good resource to isolate functional LAB with unique characteristics (Vasiee *et al.*, 2014). In order to use an isolate as starter or adjunct culture, the functionalities of the isolate are more important than the isolation source (Ebrahimi *et al.*, 2020). There are some reports for isolation of sourdough



**Figure 1.** Inhibitory effect of the selected LAB isolate (b) against *A. flavus* in overlay bioassay, compared to the control (a).



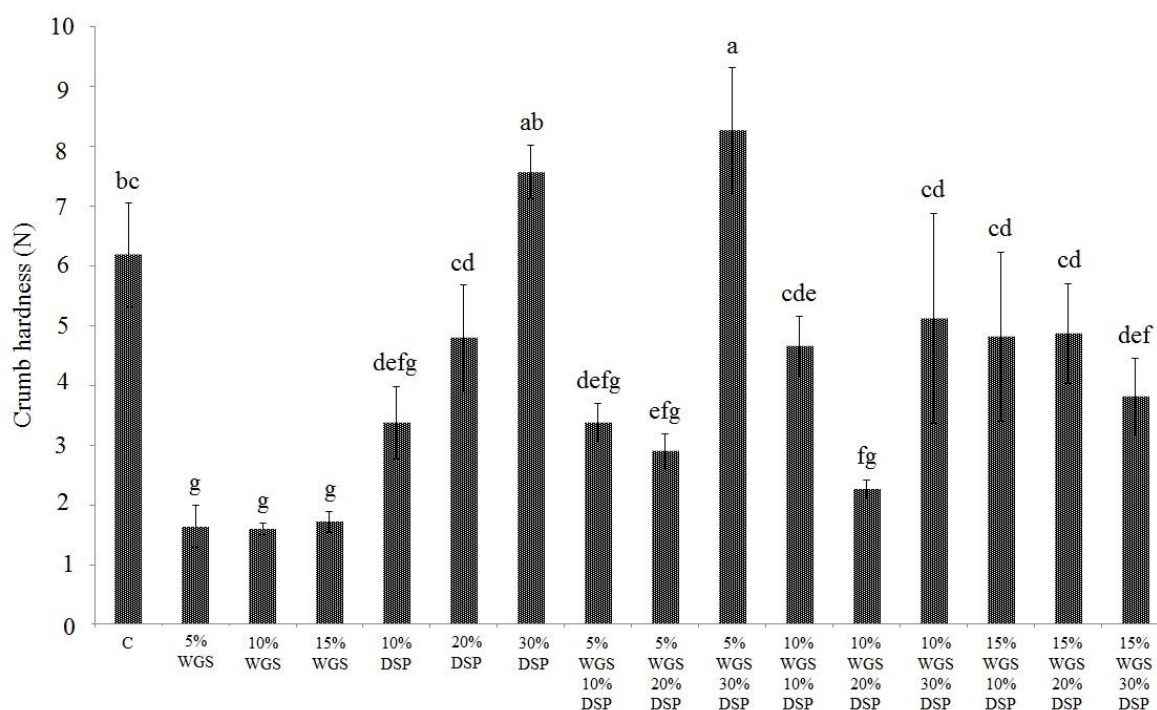
**Figure 2.** Phylogenetic evolutionary position of the LAB isolate obtained from its *16S rRNA* sequence analysis using neighbor-joining method. The bar indicates 2% sequence divergence

potential starter cultures from fermented substrates that were different from the ingredients used in bread making. For example, Plessas *et al.* (2005) used kefir grains as baker's yeast in bread processing. Accordingly, the positive effect of kefir grains on technological functionality of the kefir-leavened bread was approved.

### Crumb TPA and Specific Volume

The pH and TTA amounts of WGS were  $4.68 \pm 0.12$  and  $6.79 \pm 0.05$ , respectively. Considering the direct and indirect effects of crumb texture on bread quality attributes in terms of sensory properties, shelf life and even nutritional value of the product, the

sample with the least crumb hardness (Figure 3) was selected for further studies. It should be noted that the sensory acceptability of the selected sample had no significant difference ( $P > 0.05$ ) with the control. Blending wheat flour with 5-15% WGS generally did not alter the crumb hardness. With the increase of the DSP from 10 to 30%, the hardness of bread crumb decreased slightly, but significantly (especially at 30% level). Meanwhile, the hardness of DSP-enriched breads was lower than the control sample, except for bread with 30% DSP. Furthermore, the sample containing 5% WGS along with 30% DSP had the highest hardness, and hardness of the supplemented bread with 10% WGS along with 20% DSP was the least (selected



**Figure 3.** Crumb hardness of the wheat breads containing different amounts of Wheat Germ Sourdough (WGS: 5-15% w/w) and Dehydrated Spinach Puree (DSP: 10-30% w/w) as alone and mixed compared to the control sample (C). Different letters indicate significant difference at  $P < 0.05$

formulation). Quality characteristics of the supplemented wheat breads with the selected amounts of WGS and DSP are shown in Table 1. The control sample and bread containing WGS had the highest and the least amounts of crumb hardness, respectively. Furthermore, there was no significant difference between supplemented breads with WGS and WGS-DSP in terms of hardness. The highest amount of loaf specific volume was also observed in bread supplemented with WGS, whereas, the effects of NFWG and WGS on springiness were significantly ( $P < 0.05$ ) different. In addition, cohesiveness of the supplemented breads with WGS, DSP and their mixture was significantly higher than the other samples.

Majzooobi *et al.* (2012) reported that the increasing NFWG level (5-15% w/w) had negative effect on bread volume and softness. Khan *et al.* (2015) found that the hardness and chewiness of enriched

chapatties were increased with the increase of spinach powder; whereas, springiness was decreased. The increase in water absorption in enriched bread with spinach may be attributed to the hydroxyl groups present in the spinach fiber. Furthermore, the effect of spinach on bread texture is related to the interactions between the fiber structure and the wheat proteins, as well as the partial disruption of the gluten network and the change in hydration. The crucial effects of sourdough fermentation and fiber-rich leafy vegetables on bread softness have been approved. There are three main different phenomena influencing sourdough bread texture. The first one is acidic environment, which has its primary (direct effect on dough rheology and structure) and secondary (influence on activity of endogenous enzymes) fundamental effects on gluten network. The second one is microbial enzymes, and the third one is the other microbial metabolites like

**Table 1.** Quality characteristics of white wheat breads enriched with the selected amounts of Wheat Germ Sourdough (WGS: 10% w/w), Dehydrated Spinach Puree (DSP: 20% w/w) and Non-Fermented Wheat Germ (NFWG: 10% w/w) as alone and mixed. <sup>a</sup>

Bread samples	Hardness (N)	Springiness	Cohesiveness	Specific volume (cm <sup>3</sup> g <sup>-1</sup> )	DPPH scavenging activity (%)	Total phenol content (mg 100 g <sup>-1</sup> )	Phytic acid content (mg 100 g <sup>-1</sup> )	Overall acceptability
Control	6.19 ± 1.06 <sup>a</sup>	0.96 ± 0.01 <sup>a</sup>	0.58 ± 0.02 <sup>b</sup>	2.35 ± 0.28 <sup>b</sup>	17.04 ± 0.98 <sup>d</sup>	2.04 ± 0.34 <sup>b</sup>	235.68 ± 3.23 <sup>b</sup>	4.13 ± 0.14 <sup>b</sup>
WGS	1.59 ± 0.11 <sup>d</sup>	0.94 ± 0.02 <sup>ab</sup>	0.77 ± 0.01 <sup>a</sup>	3.89 ± 0.28 <sup>a</sup>	73.10 ± 3.90 <sup>b</sup>	2.89 ± 0.49 <sup>b</sup>	201.99 ± 3.12 <sup>d</sup>	3.93 ± 0.29 <sup>bc</sup>
NFWG	3.14 ± 0.53 <sup>c</sup>	0.88 ± 0.00 <sup>c</sup>	0.58 ± 0.02 <sup>b</sup>	2.60 ± 0.64 <sup>b</sup>	61.79 ± 3.82 <sup>c</sup>	5.31 ± 0.96 <sup>a</sup>	253.23 ± 2.89 <sup>a</sup>	3.15 ± 0.11 <sup>c</sup>
DSP	4.80 ± 1.09 <sup>b</sup>	0.96 ± 0.01 <sup>a</sup>	0.81 ± 0.01 <sup>a</sup>	2.95 ± 0.11 <sup>ab</sup>	61.52 ± 0.38 <sup>c</sup>	2.92 ± 0.83 <sup>b</sup>	232.61 ± 2.05 <sup>b</sup>	4.65 ± 0.20 <sup>a</sup>
WGS+DSP	2.26 ± 0.18 <sup>cd</sup>	0.91 ± 0.03 <sup>bc</sup>	0.80 ± 0.01 <sup>a</sup>	2.73 ± 0.38 <sup>ab</sup>	81.92 ± 3.52 <sup>a</sup>	3.07 ± 0.08 <sup>b</sup>	221.76 ± 4.20 <sup>c</sup>	3.60 ± 0.24 <sup>bc</sup>
NFWG+DSP	3.33 ± 0.65 <sup>c</sup>	0.90 ± 0.00 <sup>c</sup>	0.61 ± 0.01 <sup>b</sup>	2.83 ± 0.46 <sup>ab</sup>	72.67 ± 2.06 <sup>b</sup>	3.06 ± 0.49 <sup>b</sup>	251.88 ± 2.75 <sup>a</sup>	3.49 ± 0.15 <sup>bc</sup>

<sup>a</sup> (a-d) Different letters in each column indicate significant difference at P < 0.05.

exopolysaccharides and arabinoxylans. These metabolites reduce the starch retrogradation or increase the water holding capacity of the produced bread leading to its softness (Arendt *et al.*, 2007). Furthermore, addition of high amount of fiber (approximately 15%) would alter dough's rheological properties and change textural features of the produced bread. However,

the numbers of hydroxyl groups of fiber (which depend on the type of fiber) determine its impact on the product quality. This effect is due to the thickening of the walls surrounding the air bubbles in the crumb, and water binding capacity of fiber that avoids water loss during storage (Wang *et al.*, 2002).

### Surface Moldiness

There was no significant difference (P > 0.05) between bread containing WGS and NFWG in comparison with the control sample in terms of surface moldiness (Table 2). However, green zone of the mold growth (as an indicator of fungal sporulation) on wheat bread containing WGS was remarkably prevented compared to the other samples (Figure 4). The pH of wheat bread containing WGS was significantly (P < 0.05) lower than the other samples, but there was no significant difference among the produced breads in terms of a<sub>w</sub>.

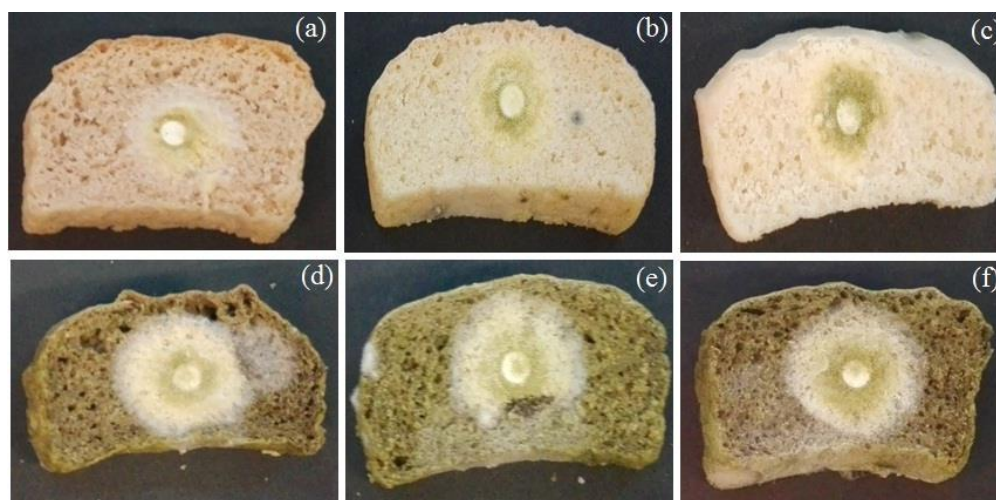
Moldiness is responsible for huge economic losses and a serious safety problem in bakery products. There are several approaches to control fungal spoilage in bakeries; nevertheless, this problem remains unsolved. Currently bio-preservation capability of controlled sourdough fermented with selected antifungal LAB, a promising strategy, has been introduced with pivotal effects on quality and safety of the product (Ebrahimi *et al.*, 2020). Researchers realized that antagonistic activities of LAB take place through their competition with unwanted microorganisms for nutrients and synthesis



**Table 2.** pH,  $a_w$  and percentage of mold growth on the produced wheat breads containing Wheat Germ Sourdough (WGS: 10% w/w), Dehydrated Spinach Puree (DSP: 20% w/w) and Non-Fermented Wheat Germ (NFWG: 10% w/w) as alone and mixed. <sup>a</sup>

Bread samples	pH	$a_w$	Surface moldiness (%)	Green zone of the mold growth (%)
Control	5.46 ± 0.04 <sup>a</sup>	0.96 ± 0.01 <sup>a</sup>	15.85 ± 3.09 <sup>b</sup>	10.33 ± 0.74 <sup>a</sup>
WGS	4.31 ± 0.04 <sup>d</sup>	0.94 ± 0.02 <sup>a</sup>	18.14 ± 0.89 <sup>b</sup>	4.78 ± 0.07 <sup>d</sup>
NFWG	5.20 ± 0.03 <sup>b</sup>	0.93 ± 0.01 <sup>a</sup>	16.61 ± 3.04 <sup>b</sup>	11.03 ± 0.43 <sup>a</sup>
DSP	5.22 ± 0.03 <sup>b</sup>	0.97 ± 0.01 <sup>a</sup>	33.92 ± 2.15 <sup>a</sup>	8.10 ± 0.12 <sup>b</sup>
WGS+DSP	4.84 ± 0.06 <sup>c</sup>	0.95 ± 0.02 <sup>a</sup>	36.34 ± 2.76 <sup>a</sup>	6.64 ± 0.51 <sup>c</sup>
NFWG+DSP	5.18 ± 0.01 <sup>b</sup>	0.95 ± 0.03 <sup>a</sup>	31.10 ± 7.07 <sup>a</sup>	7.07 ± 0.40 <sup>bc</sup>

<sup>a</sup> (a-d) Different letters in each column indicate significant difference at  $P < 0.05$ .



**Figure 4.** Surface and green zone of *A. flavus* growth on white wheat breads supplemented with 10% wheat germ sourdough (a), 10% non-fermented wheat germ (b), 20% dehydrated spinach puree along with 10% wheat germ (c)

of a wide variety of bioactive antimicrobial metabolites like proteinaceous inhibitory compounds, mixture of organic acids, phenyl lactic acid, and antioxidants (Dalié *et al.*, 2010). Furthermore, the decrease in moldiness may be due to the interaction of antifungal metabolites and or inhibitory compounds released from substrates during fermentation, as well as less water available for initial growth and sporulation of the fungus (Debonne *et al.*, 2018). In the present study,  $a_w$  of the wheat and supplemented breads ranged from 0.93 to 0.97, and it is similar to values reported in literature (Debonne *et al.*, 2018). Similarly,  $a_w$  value of the bread was not affected by hop *Humulus lupulus* extract addition in Nionelli *et al.* (2018) study. To interpret

these findings, it should be noted that the pH and  $a_w$  have different effects on mold growth in different conditions. For example, it is hypothesized that reduction of  $a_w$  leads to reduction of fungal growth. Meanwhile, in lower  $a_w$ , the release of inhibitory compounds will be decreased. Accordingly, these factors have a very complex effect on the mold growth in bread.

### Antioxidant Activity

All the supplemented samples containing WGS and DSP scored notably higher than the control in terms of DPPH scavenging activity (Table 1). Bread supplemented with WGS along with DSP had the highest



amount of antioxidant activity among the produced samples, and bread containing WGS had the same scavenge effect of 0.5 mg mL<sup>-1</sup> BHA (70%). Furthermore, the effect of fermentation on antioxidant activity was significantly ( $P < 0.05$ ) higher than the spinach.

The interference of WGS on DPPH scavenging activity observed in this study was also verified by Liu *et al.* (2017). The most important effects of LAB fermentation on antioxidant activity are correlated to their ability to produce and/or release of bioactive metabolites, as well as proteolysis activity of the LAB (Đorđević *et al.*, 2010). The type and amount of the phenolics and other antioxidant compounds present in the raw substrates are also important parameters in antioxidant activity of the bakery products. Furthermore, application of antioxidant-rich vegetables along with fermented substrates is not only important for human health but also a promising strategy to produce functional breads. In the present study, total phenol content of bread enriched with NFWG was significantly ( $P < 0.05$ ) higher than the other samples. It is assumed that phenomena such as dilution of the bioactive ingredients and or change of the optimum pH (which is necessary for protease activity) influence release of antioxidant compounds in supplemented breads with WGS-DSP in comparison with the aforementioned sample. It is hypothesized that heat treatment during baking leads to degradation of phenolic compounds in DSP-enriched bread (Ragaei *et al.*, 2014). Accordingly, the trend for DPPH scavenging activity and TPC in the produced breads was not the same.

### Phytic Acid Content

Addition of NFWG led to increase in phytic acid content in wheat bread. Meanwhile, phytic acid content of the wheat bread enriched with WGS was significantly ( $P < 0.05$ ) lower than the other produced breads.

In the same vein, Bilgiçli and İbanoğlu (2007) reported that the phytic acid content of tarhana (a dried mixture made from yoghurt and wheat flour) increased as wheat germ amount increased. However, more than 90% of the phytic acid present in the sample was inactivated by fermentation. The phytase activity in water/salt-soluble extract of WGS-enriched bread was significantly higher than that found in the control sample and the wheat bread supplemented with NFWG in Rizello *et al.* (2010) survey. Phytic acid as an anti-nutritional factor binds to minerals due to its chelating activity. Fortification of staple foods like bread using sourdough can potentially help in alleviating micronutrient malnutrition. Phytase activity of the LAB and/or effect of acidic pH on endogenous phytase are the main phenomena responsible for this capability. The enzymatic degradation of phytic acid requires an optimum pH, which can be provided by controlled fermentation (Gobbetti *et al.*, 2019).

### Sensory Acceptability

Bread supplemented with DSP had the highest sensory acceptability, while the sample containing NFWG had the least one. Furthermore, there was no significant difference ( $P > 0.05$ ) between bread enriched with WGS and WGS-DSP in terms of sensory acceptance. Furthermore, breads containing mixture of WGS-DSP had moderate color compared to the other samples due to the presence of WGS.

According to the published data, the low pH and high temperature worked synergistically to change sensorial attributes. It was reported that with the increase in spinach powder in chapati, there was a significant decrease and increase in brightness and yellowness, respectively (Khan *et al.*, 2015). Oxidation reaction and carbohydrates participated in caramelization, as well as melanoides produced from dietary fibers during Maillard reaction and are responsible for the change of the color in

samples produced with WGS and or DSP. There are evidences for the formation of a complex compound named maillardized insoluble dietary fibers (which consists of dietary fibers, proteins, maillard reaction products, and polyphenols) during thermal processing of bakery products (Pérez-Jiménez *et al.*, 2014).

## CONCLUSIONS

Production of clean label breads free from synthetic improvers and preservatives is very important from consumer health and safety viewpoints. This project had two different steps. At the first step, optimum amounts of WGS and DSP were determined based on the crumb textural properties and sensory acceptability of the supplemented wheat breads containing different amounts of WGS (5-15%) and DSP (10-30%). Then, the quality characteristics of the supplemented wheat breads with the selected amounts of WGS (10%) and DSP (20%) were investigated. It can be seen from the preliminary results that the greater the amount of added DSP, the lower the crumb softness. However, the effect of different amounts of WGS was not significant on crumb hardness. From the discussion, it is evident that using WGS retards mold growth in the supplemented wheat bread, reduces phytic acid content, improves textural and antioxidant attributes of the product, and induces some changes in bread sensory properties, without altering consumer preferences. Accordingly, WGS-DSP supplemented white wheat bread has the potential to serve the valuable source of functional compounds. There are several well-known applications for vegetables and by-products of milling in bakery products; however, production of enriched bread containing dietary fibers, antioxidants, and nutrient supplements is a promising strategy to process not only enriched but also pro-functional breads to enhance the daily intake of bioactive ingredients.

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### بهبود قابلیت‌های فناوری نان آرد سفید گندم با استفاده از خمیر ترش جوانه گندم و پوره اسفناج آبگیری شده

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#### چکیده

در سال‌های اخیر، غنی‌سازی نان با سبزیجات برگی حاوی فیبر و شکل تخمیر شده محصولات جانبی آردسازی از اهمیت بسزایی برخوردار شده است. در پژوهش حاضر، باکتری اسید لاکتیک ضد قارچ جدا شده از ترخینه به عنوان کشت آغازگر در تخمیر کنترل شده خمیر ترش جوانه گندم با هدف بهبود کیفیت و سلامت نان گندم حاوی پوره اسفناج آبگیری شده مورد استفاده قرار گرفت. فرمولاسیون بهینه (نان گندم حاوی ۱۰٪ خمیر ترش جوانه گندم و ۲۰٪ پوره اسفناج) بر اساس میزان نرمی بافت نان غنی شده تعیین گردید. سپس ویژگی‌های کیفی نان‌های تولیدی شامل خصوصیات بافتی، فعالیت آنتی-اکسیدانی، گسترش سطحی قارچ، محتوای اسید فیتیک و پذیرش حسی مطالعه شد. توالی‌یابی محصولات واکنش زنجیره‌ای پلیمرز منجر به شناسایی لاکتوکوکوس لاکتیس به عنوان جدایه لاکتیکی منتخب ضد قارچ گردید. آنالیز الگوی بافت نیز تاثیر معنی‌دار ( $P < 0.05$ ) خمیر ترش جوانه گندم و پوره اسفناج را بر سفتی بافت و حجم مخصوص نان‌های تولیدی آشکار ساخت. نان حاوی مخلوط خمیر ترش جوانه گندم و پوره اسفناج، دارای بیشترین قابلیت مهار رادیکال آزاد بود. هاله سبز رنگ ناشی از رشد آسپرژیلوس فلاووس بر روی نان حاوی خمیر ترش جوانه گندم و محتوای اسید فیتیک این نان به نحو بارزی از سایر نمونه‌ها کمتر بود. علاوه بر این، تفاوت معنی‌داری بین پذیرش حسی نان حاوی خمیر ترش جوانه گندم و مخلوط خمیر ترش جوانه گندم و پوره اسفناج در مقایسه با نمونه شاهد وجود نداشت.