**Title:**

# Biochemical Constituents and in Vitro Antioxidant Potential of fermented wheat grains using *Bacillus subtilis*

Muhammad Zahaib Ilyas[1], Muhammad Roman[2], Muhammad Waqas Ali[3,*], Muhammad Nadeem[4]

[1] Department of Agronomy, Faculty of Agriculture, University of Agriculture Faisalabad, 38000, Pakistan

[2] School of Soil and Water Conservation, Beijing Forestry University, Beijing, China

[3] School of Applied Biosciences, Kyungpook National University, Daegu 41566, Korea

[4] Soil and Water Testing Laboratory Hafizabad, Punjab, Pakistan

* Correspondence author

Muhammad Waqas Ali

School of Applied Biosciences, Kyungpook National University, Daegu 41566, Korea

E-Mail: waqasali3515@yahoo.com

ORCID: 0000-0003-2826-4545

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17 **Biochemical Constituents and in Vitro Antioxidant Potential of fermented wheat grains**

18 **using *Bacillus subtilis***

19 High antioxidants level in food is gradually becoming popular because of the enhanced risk of
20 oxidative stress in humans. Bread wheat is rich in vital antioxidants but its major bioactive
21 compounds are not available for the human. This study was conducted with the aim to enhance the
22 phytochemical constituents and antioxidative activity of wheat grains by fermenting it with the use
23 of *Bacillus subtilis* KCTC 13241. The antioxidative potential was determined by DPPH (2,2-
24 diphenyl-1-picryl- hydrazyl) and ABTS (3-ethyl-benzothiazo- line-6-sulfonic acid) radical
25 scavenging assay as well by the concentration of amino acids, flavonoids, minerals, carbohydrates
26 and phenolic compounds. Different varieties showed different free radical scavenging potential on
27 fermentation, which was significantly high with respect to their corresponding unfermented wheat
28 varieties. The highest potential was found in a fermented wheat variety named as *Namhae* and this
29 combination can be used in pharmaceutical and food industries.

30 **Keywords:** Antioxidant activity; phenolic contents; fermentation; wheat; minerals

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## Introduction

Globally, cereals are the most important part of the human diet and source of protein, carbohydrate, fibers, minerals and vitamins. Processing of cereal's products is important both for nutritive and sensorial properties. Microbial fermentation, conversion of complex organic molecules into simpler ones like amino acids, peptides etc., has been commonly used to improve the nutritional quality of foodstuff (Liu et al. 2017). These peptides and amino acids are potent natural antioxidants (Dordević et al. 2010). Consumption of food products manufactured by using a whole grain of cereals, unfermented, has been coupled with some chronic diseases like diabetes and cancer etc. (Wojdyło et al. 2007).

Wheat is a staple food of the people of temperate regions and ranks as second in overall production after maize. It contains numerous phenolics namely vanillic, caffeic, ferulic, salicylic, *p*-coumaric, gentisic, sinapic acids and syringic (Naczk and Shahidi 2006). Polyphenolics have diverse biological properties including anti-oxidant, anti-inflammatory and anti-microbial properties (Trouillas et al. 2003). In cereals, mostly, phenolics acids are not present in free form but as conjugates with proteins, sugars and fatty acids. So, the hydrolysis process must be adopted to get more phenolics in the cereal grains (Wojdyło et al. 2007). Some studies reported that total phenolics content significantly enhanced by microbial fermentation (Cai et al. 2012). However, other hydrolysis processes as an enzymatic reaction are not economically feasible because the synthetic enzymes involved in this process are not economically viable.

Fermentation with *Bacillus subtilis* has been employed for making proteinases in past few decades worldwide (Juan and Chou 2010). Anti-oxidants derived from a plant with metal chelating and free-radical scavenging properties can minimize the risk of oxidative stress and keep a balance

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between anti-oxidants and oxidants in the human body (Bhanja et al. 2009). Over the past few decades, phenolics have potentially been used to prevent different chronic disease including cancer, diabetes mellitus, cardiovascular disease, neurodegenerative diseases and osteoporosis (Martins et al. 2011). In microbial fermentation, microorganisms release a number of carbohydrases like xylanase, cellulases, esterase and pectinases convert unavailable phenolics to available form (Bhanja et al. 2009).

Naturally, wheat grains have a sufficient quantity of anti-oxidants but human body unable to use these anti-oxidants because of the presence of these compounds in bound form. So instead of getting health benefits by eating these food items, people suffer from health complications. The main objective of this research is to find a potent bacterial strain by employing wheat grain in order to enhance the antioxidant properties and nutrient status of wheat grain to avoid these health complications. *Bacillus subtilis* has been employed for fermentation but a few results about its activity are available.

## Materials and methods

### Reagents

Acetonitrile, HPLC grade water and methanol were bought from Fisher Scientific (Fairlawn, OH, USA). Ferric chloride, 2,4,6-tripyridyl-s-triazine, sodium acetate, rutin, glacial acetic acid, 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid), Folin–Ciocalteu phenol reagent and potassium per sulfate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All chemicals were of analytical grade.

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## Microorganism and inoculum preparation

Formerly isolated and purified strain, *B. subtilis* KCTC 13241 was employed in the fermentation of wheat grains. Under laboratory conditions, *B. subtilis* was retained on the slants of nutrient agar. For the preparation of inoculums, the activated culture of microbes was streaked on the slants of nutrient agar (as a medium) and incubated, precisely, at 27 $^{o}$C for 24 hours. The newly grown active cells were collected in sterilized distilled water. After adjusting the level 7.43 log CFU mL$^{-1}$ and this suspension were utilized for the microbial fermentation of cooked wheat grains (Ali et al. 2018).

## Preparation of fermented wheat

There were four varieties of wheat used in this experiment named as *Baekjoong*, *Jeokjoong*, *Milseoung* and *Namhae*. Twenty grams of wheat grain were taken in Erlenmeyer flasks of 250 mL, mixed with twenty mL of distilled water, steamed at 130 $^{o}$C for twenty minutes and eventually cooled to room temperature. Steamed substrates were separately incubated with bacterial suspension, mixed thoroughly and incubated at 25 $^{o}$C for 3 days named as fermented wheat (FW), secondly, the same inoculated wheat varieties were frozen instead of incubation and named as cooked wheat (CW) as a control. The experiment was repeated three times.

## Viable cell number

One gram sample plus nine millilitres of 0.85% sodium chloride solution was mixed and diluted bacterial suspension was spread over agar medium, incubated for 36 hours at 27 $^{o}$C for counting of colonies (Ali et al. 2017).

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## Sample extraction conditions

Samples extraction was performed by using the protocol of Xu and Chang (Xu and Chang 2007) with few adjustments. Samples were freeze-dried and ground by using the electric grinder. The prepared samples were extracted by taking ten grams of each sample plus one hundred millilitres of 80% of methanol (v/v) and incubated for twenty-four hours at 25 $^{o}$C. After incubation, the extracts were goes under centrifugation for twenty minutes at 3000 rpm. The collected supernatants were further filtered by using a PVDF filter of 0.45-µm Millipore (Schleicher & Schuell, GmbH, Dassel, Germany). The filtrates were kept at 4 $^{o}$C in dark for proposed analysis.

## Determination of total phenolic content

Total phenolics were estimated by the following protocol of Folin-Ciocalteu (Katsube et al. 2004). The methanolic extracts were filtered by a 0.2 µm syringe filter (Water, Milford, MA, USA). 50 mL methanolic extract plus one millilitre of two percent $Na_2CO_3$ (sodium carbonate) was mixed and left for three minutes. Then mixed with fifty µL of one normal Folin–Ciocalteu phenol reagent and left for thirty minutes at 25 $^{o}$C in the dark. The samples were run on a spectrophotometer (Thermal Fischer Scientific, Vantaa, Finland) to read absorbance on 750 nm wavelength against a blank sample. The concentration of total phenolics was computed by employing standard calibration curve, which was plotted by utilizing gallic acid, and stated as µgGAE/g grain (microgram gallic acid equivalent per gram of wheat grains).

## Evaluation of total flavonoid content

It was determined by the method reported by Adhikari (Adhikari et al. 2018). Fermented wheat grains extract was added into an equal proportion of deionized water and then thirty-five

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117 microliters of 5% sodium nitrate ($NaNO_3$) solution was added and allowed to react for five minutes
118 at 25 $^oC$ followed by the addition of seventy microliters of 10% aluminium chloride ($AlCl_3$).
119 Addition of 175 mL of one molar sodium hydroxide (NaOH) was done after five minutes and
120 absorbance was recorded instantly 505 nm by using spectrophotometer (Thermo Fischer Scientific,
121 Vantaa, Finland). A calibration curve was drawn by using catechin as a standard and total
122 flavonoids stated as catechin equivalent (µgCE/g).

123 **DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging assay**

124 The free radical-scavenging activity of fermented wheat extracts was measured by using protocol
125 illustrated by Bilal (Bilal et al. 2016) with slight modifications. 0.1 mM DPPH solution was freshly
126 prepared in 99.9% pure methanol which was then used for the analysis. An equal amount of
127 methanolic extracts plus 0.1 mM freshly prepared DPPH solution was mixed properly and kept for
128 half an hour in the incubator to measure absorbance at 516 nm and Trolox was treated as a control.
129 The following equation was used to calculate the DPPH radical-scavenging activity and expressed
130 in percentage.

131 DPPH radical-scavenging activity (%) = $[\,1 - (AbS - AbC)] \times 100$

132 Where *AbS* was the absorbance of the test compound, *AbC* was the absorbance of control.

133 **ABTS cation radical-scavenging assay**

134 It was measured by following the protocol illustrated by Bilal (Bilal et al. 2016). Potassium
135 persulphate was used for the oxidation of ABTS to generate $ABTS^{\bullet+}$ in the solution. It was diluted
136 before use to attain an absorbance of 0.7 ± 0.02 at 735 nm by using 50% ethanol. The ABTS
137 reagent was added in the sample, mixed vigorously and absorbance was recorded at 735 nm after

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three minutes of mixing to measure the scavenging activity. Fifty percent ethanol was used as a blank and Trolox as a positive control. The following equation was used to calculate the ABTS radical-scavenging activity.

ABTS cation radical-scavenging activity (%) = $[\,1-(AbS-AbC)] \times 100$

**Free amino acids composition**

It was determined by following the protocol by Ali (Ali et al. 2017). One gram of ground, the sieved sample was hydrolyzed with six molar HCl for 24 hours at 105 $^{o}$C to determine amino acid profile through Hitachi Amino Acid Analyzer (L-8900, Hitachi, Japan). Standard amino acid mixture solution (Type H, Wako Pure Chemical Industries Ltd., Japan) was used for the determination of endogenous amino acid profile. Samples from all treatment were analyzed in triplicate and stated as mg per gram of dry weight.

**Analysis of minerals and carbohydrates**

Minerals were identified and quantified by (Andualem and Gessesse 2014) method with slight modifications. Fifteen milliliters of HNO3 was added into 0.5 g of the freeze-dried sample of FW and CW. An equal volume of distilled water was put for dilution. Plasma-atomic emission spectrometer (ICP AES: Varian Vista, Varian Australia, Victoria, Australia) was used to determine the concentrations of different minerals. Standards for each mineral were used for calibration of the instrument.

The carbohydrates of the samples was identified and quantified according to the method followed by Kang (Kang et al. 2014). Ground dried samples were homogenized with liquid nitrogen and sugar extracted with aqueous ethanol. The ethanol was evaporated by rotary evaporator. The

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residues were dissolved in water and filter was injected to HPLC Waters system (Millipore Crop, Waters Chromatography, Milford, MA, USA) and the sugar signals were detected by Waters refractive index detector. The de-ionized water was used as mobile phase and the flow rate was 0.5 ml/min at 90 °C. Glucose and fructose were measured on the basis of peak areas and comparison with a calibration curve obtained with the corresponding standards.

**Statistical Analysis**

Recorded data were subjected to ANOVA (analysis of variance) by employing SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). The treatment means separation was done by DMRT (Duncan's multiple range tests) at the 95% confidence level. The results were stated as the means ± SD (standard deviation) of three replicates.

## Results and discussion

### Variation in viable microbial population in fermented and cooked wheat

The ability of four different fermented wheat (FW) to support viable microbial cells is shown in **fig. 1**. Among four varieties of wheat, the descending order of bacterial population (log CFU/g) was recorded in FW as *Namhae* (13.05 ± 1.13), *Milseoung* (12.76 ± 1.65), *Baekjoong* (12.34 ± 1.34) and *Jeokjoong* (11.27 ± 0.92) but in cooked wheat (CW), the highest population was recorded in the *Namhae* (8.53 ± 1.04) and lowest was in the *Jeokjoong* (6.32 ± 0.43). The microbial population recorded in *Baekjoong* and *Milseoung* was 7.45 ± 0.76 and 7.98 ± 0.61 respectively. The variation in microbial population within the same factor perhaps due to the difference in nutritional status and size of wheat grains under study and between the factors due to change in the time span of incubation. Results of the previous experiment showed that viable bacterial

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population increased significantly after fermentation (Cho et al. 2011) which is also proved by the present study.

**Total phenolic content (TPC) of fermented and cooked wheat**

The total phenolic contents of the fermented wheat varieties and their corresponding cooked wheat varieties are demonstrated in **fig. 2**. Phenolic compounds are crucial in the food-related fields because of their ability to reduce the process of lipid peroxidation (Wojdyło et al. 2007). The TPC (µg $GAEg^{-1}$) that was measured in four fermented as well as cooked wheat varieties showed that the highest TPC was available in fermented wheat (FW) varieties and their concentrations compared with CW from highest to lowest was as *Namhae* FW (1671.32 ± 32.54), CW (1139.57 ± 23.01), *Milseoung* FW (1597.43 ± 10.87), CW (1106.96 ± 9.43), *Baekjoong* FW (1533.78 ± 20.39), CW (1054.21 ± 12.17) and *Jeokjoong* FW (1321.46 ± 19.17) CW (954.43 ± 14.54). The results of TPC showed that amount of free phenolic compounds were considerably increased according to the potential of above wheat varieties when these were allowed to ferment for 72 hours then cooked wheat. Previous experimental results also narrated that the phenolic content of different cereals was significantly increased when fermented and highest was noted in buckwheat (Dordević et al. 2010). In cereals, fermentation led to break down of cell walls causing synthesis as well as the liberation of various bioactive compounds (Katina et al. 2007) and the above statement is also supported by the experiment. The above results also showed the fermentation potential of *B. subtilis* KCTC 13241 for these four wheat varieties. Previous results revealed that the concentration of available TPC increases in fermented cereals and the extent of increase depends on the species of microorganism (Kariluoto et al. 2006).

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## Total flavonoid contents (TFC) of fermented and cooked wheat

Flavonoids could be used to suppress different diseases including neurodegenerative disorders, cancer as well as cardiovascular diseases (Cai et al. 2012). Consumption of vegetables and fruits rich in flavonoids has been associated with defence against heart diseases and cancer (Juan and Chou 2010). The difference in TFC of FW and corresponding CW is shown in **fig. 3**. All the wheat varieties that were fermented had shown a greater concentration of total flavonoids then its corresponding cooked variety. *Namhae* (FW) had maximum flavonoids content with 142.65 ± 2.25 and its corresponding *Namhae* (CW) showed 76.33 ± 6.31 followed by *Milseoung* (FW) with 137.26 ± 6.73 TFC and its corresponding *Milseoung* (CW) had 65.96 ± 4.76 TFC then *Baekjoong* (FW), which had 124.57 ± 7.94 TFC compared to *Baekjoong* (CW) with TFC of 51.29 ± 4.41. The lowest concentration of TFC was found in the *Jeokjoong* (FW) with 109.21 ± 5.11 and its corresponding had TFC of 43.87 ± 3.27 which is significantly less than FW and it is also supported by the outcomes of past study which narrated that fermented soybeans extract was highly concentrated in TFC than its corresponding cooked soybeans extract (Juan and Chou 2010). The difference in TFC of FW and CW might be owing to the difference in genetic potential of wheat varieties and it is referred by Bilal et al. (2016). Another study showed that the concentration of TFC in soybean significantly enhanced when fermented using microorganisms (Lee et al. 2008). Low TFC in cooked wheat is due to lack of fermentation with bacteria, which causes degradation of complex, bound and unavailable phytochemicals that scavenge free radicals and protect humans from oxidative stress. Xu and Change (2007) also reported the similar results that fermented or flavonoids rich foods reduce the chance of human diseases related to oxidative stress (Xu and Chang 2007).

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225 **DPPH free radical scavenging activity**

226 The DPPH free radical scavenging assay has been commonly used to measure the radical
227 scavenging potential owing to ease and high accuracy of this method (Dordević et al. 2010). The
228 free radicals of DPPH have been employed in the estimation of free radical scavenging potential
229 of different plants extract (Katalinic et al. 2006). The DPPH free radical scavenging potential of
230 the four FW and CW varieties is shown in **fig. 4**. The scavenging potential of FW and CW extract
231 was expressed in percentage and highest percentage was observed in *Namhae* (FW) with 44.36 ±
232 2.15 (%) as compared to cooked *Namhae* (control) with very low scavenging activity of 27.43 ±
233 0.84 (%) followed by the *Milseoung* (FW) with 39.03 ± 1.56 (%) of scavenging potential which
234 was a valuable difference from its corresponding cooked *Milseoung* (control) whose scavenging
235 potential was 25.88 ± 1.23 (%). The DPPH free radical scavenging potential of *Baekjoong* (FW)
236 extract was 35.71 ± 1.39 (%) but its corresponding control (cooked *Baekjoong*) showed a very
237 weak potential to scavenge radical of DPPH that was 23.04 ± 1.45 (%). Among these treatments,
238 the lowest potential was noticed in case of *Jeokjoong* (FW) extract with 32.01 ± 0.99 (%) but in
239 comparison with its corresponding control (20.61 ± 1.76), it showed a considerable potential to
240 scavenge DPPH radicals. Other investigations also demonstrated that some wheat varieties have
241 weak scavenging potential (31%) for DPPH radicals (Yu et al. 2002). There is a strong
242 antioxidative potential of fermented *Namhae* according to the results of our experiment and also
243 reported by the Dordecic et al., (2010) who worked on cereals (Dordević et al. 2010). It is cleared
244 that fermented *Namhae* has enough potential to protect humans against the oxidative stress but in

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comparison with the antioxidative potential of legumes, wheat is placed in lower rank and this is also mentioned by Acosta-Estrada et al., (2014) (Acosta-Estrada et al. 2014).

**ABTS radical scavenging potential of FW and CW extract**

The $ABTS^{\bullet+}$ scavenging potential of FW and CW is shown in the **fig. 5**. The ABTS free radical scavenging assay has been widely used for the determination of the antioxidative potential of food products (Bilal et al. 2016). Free radicals scavenging compounds have more potential to scavenge $ABTS^{\bullet+}$ than DPPH free radical (Sachindra and Bhaskar 2008). The similar case was also found in the following results of our research. The highest $ABTS^{\bullet+}$ scavenging potential (expressed in %) showed by the *Namhae* (FW) was 53.94 ± 2.39 followed by *Milseoung* (FW) with 47.19 ± 2.87 then *Baekjoong* (FW) with 42.45 ± 2.06 and *Jeokjoong* (FW) with least antioxidative potential of 38.83 ± 1.45; the antioxidative potential of their corresponding controls (CW) was 36.49 ± 2.23, 33.28 ± 1.65, 31.17 ± 1.27 and 29.86 ± 1.04 respectively. There was a significant increase in the antioxidant potential of fermented seed extract as compared to unfermented seed extract which was also pronounced by the Starzynska-Janiszewska et al., (2008) (Starzyńska-Janiszewska et al. 2008). The above experimental results were also proof of the results explained by the Dey and Kuhad (2014) in the case of wheat (Dey and Kuhad 2014).

**Amino acid profile of FW and CW**

The concentration of amino acids available in the FW and CW varieties is presented in **table 1**. An enzyme released by the *Bacillus Subtilis* hydrolyzes the protein sources present in the wheat into a free amino acid and the short peptide that then instantly available to the human body (Liu et al. 2017). A quantitative study of fourteen amino acids was carried out both in FW and CW in this

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experiment including seven essential amino acid namely phenylalanine, valine, threonine, methionine, leucine, isoleucine, histidine and seven non-essential amino acids namely arginine, serine, glutamic acid, glycine, alanine, aspartic acid, tyrosine. The concentration of amino acids was expressed in mg/g. The total amount of essential plus nonessential amino acids in FW decreases as *Namhae* < *Milseoung* < *Baekjoong* < *Jeokjoong* and their corresponding concentrations are 116.71 ± 4.64, 115.91 ± 5.17, 102.78 ± 5.32 and 101.93 ± 4.13 respectively; there is a significant total amino acid difference from their corresponding CW as 95.94 ± 5.98, 95.33 ± 6.76, 84.02 ± 4.87 and 81.48 ± 5.32 respectively. Glutamic acid is the most abundant amino acid in FW as well as CW but the highest amount in FW (*Namhae*). Few amino acids like tyrosine, histidine, methionine have been described as antioxidants and especially, histidine shows strong free radical scavenging potential (Udenigwe and Aluko 2011). Amino acids have surplus electrons which can be used to scavenge free radicals. Some amino acids regulate the expression of genes, enhance skeletal muscles' growth, burn non-essential body fat (Wu 2009). Leucine, valine, isoleucine and phenylalanine are act as neurotransmitters which affect the performance of the brain and nervous system (Fernstrom 2013). A high proportion of available amino acid act as a supportive character in quality assurance of food products (Bilal et al. 2016).

**Minerals and carbohydrate contents in FW and CW**

Mineral contents are separated into two main categories micro and macro minerals. Calcium, potassium, sodium, phosphorous, magnesium, and sulfur are macro minerals whereas iron, selenium, copper, iodine, cobalt, zinc, chromium, molybdenum and boron are micro minerals (Lukaski, 2004). In this research, the most important ten minerals were analysed and showed in **table 2**. Wheat cultivars showed the interesting results related to minerals. *Jeokjoong* showed the

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highest contents of iron, calcium, magnesium and potassium in CW as well as after fermentation in FW. *Baekjoong* has the other mineral contents in higher concentration such as phosphorus, manganese and sodium. Overall the total minerals contents after fermentation has been increased. In current study, first time minerals composition was determined in fermented wheat using *Bacillus Subtilis*. This difference in the mineral contents might be due to the interaction of bacterial fermentation with other metabolites breakdown (Maria John et al. 2015). These essential minerals play an important role in the health. For nerve functioning, blood clotting and immune system, calcium plays its role for their functions and also supports to relax and contract the muscles. Sodium helps for regulation of electrolyte balance, heart function and metabolic activities. Magnesium aids to make the proteins, immune system and control constipation while potassium needs for nerve transmission, control blood pressure and muscles shriveling. Iron present in red blood cells and carries oxygen in the body and helpful in metabolism (Gharibzahedi and Jafari 2017). The value of glucose and fructose represented in **table. 2**. *Beakjoong* has the highest value of glucose (0.46 g/100g) in CW and this value increased after fermentation as compared to other varieties. The value of fructose is higher in *Jeokjoong* (CW) and this value increased in *Milseoung* (90.54 g/100g) after the fermentation of wheat grains. These variations in the carbohydrate contents are because of potential of cultivars and due to presence of *Bacillus Subtilis*.

## Conclusion

The results of the present study reveal that microbial fermentation of bread wheat using *Bacillus subtilis* KCTC 13241 is a highly effective technique for quality enhancement both in antioxidant potential and nutritional aspects. Most of the free radicals scavenging compounds are present in bound form in wheat and functionally inert because they are not available to the human body. It

is cleared from the obtained results that there is a significant value addition to fermented wheat varieties because of increase in their potential to reduce the risk of oxidative stress as well as to combat with hidden hunger due to malnutrition. In the present era, the main focus is on the increase of wheat yield which leads towards the dilution in quality characters of wheat. All the time, less attention is paid to utilize the quality parameters up to the potential by employing suitable techniques. Considerable quality gap is found between available and potential status in unfermented wheat varieties during the study. To fulfil this gap, bacterial (*B. subtilis* KCTC 13241) fermentation is the best option that is convenient, reliable and very cheap. Therefore, it is concluded that *Namhae* variety is the most potent regarding the discussed parameters and can be used on a commercial scale for fermentation.

## Conflict of interest

No conflict of interest in this research work.

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417 **Table 1.** The composition of free amino acids during cooked and fermented wheat.

| Amino Acids mg/g | Sample | *Baekjoong* | *Jeokjoong* | *Milseoung* | *Namhae* |
|---|---|---|---|---|---|
| arginine | CW | 4.63 ± 0.98 | 4.93 ± 1.07 | 5.35 ± 1.34 | 5.43 ± 1.49 |
| | FW | 5.14 ± 1.43 | 5.69 ± 1.64 | 6.43 ± 1.13 | 4.76 ± 0.45 |
| threonine | CW | 2.42 ± 0.42 | 2.31 ± 0.36 | 2.65 ± 0.33 | 1.82 ± 0.37 |
| | FW | 3.23 ± 0.67 | 3.73 ± 0.51 | 3.52 ± 1.02 | 2.39 ± 0.62 |
| serine | CW | 3.35 ± 0.30 | 4.13 ± 0.86 | 3.93 ± 0.24 | 4.05 ± 0.56 |
| | FW | 4.24 ± 0.85 | 5.03 ± 1.12 | 4.37 ± 0.53 | 5.35 ± 1.68 |
| glutamic acid | CW | 37.98 ± 3.86 | 35.29 ± 2.43 | 38.74 ± 4.63 | 38.99 ± 5.43 |
| | FW | 45.65 ± 5.39 | 42.32 ± 3.78 | 48.80 ± 5.24 | 47.94 ± 4.94 |
| phenylalanine | CW | 5.15 ± 1.87 | 5.37 ± 1.58 | 5.99 ± 0.71 | 4.65 ± 0.92 |
| | FW | 6.76 ± 1.74 | 6.97 ± 1.49 | 6.54 ± 1.19 | 5.16 ± 1.57 |
| glycine | CW | 3.04 ± 0.68 | 3.45 ± 0.43 | 4.56 ± 0.92 | 4.81 ± 1.03 |
| | FW | 4.72 ± 0.65 | 4.78 ± 1.04 | 5.41 ± 1.23 | 5.49 ± 1.31 |
| alanine | CW | 2.81 ± 0.71 | 2.23 ± 0.50 | 3.89 ± 0.69 | 4.82 ± 1.04 |
| | FW | 3.46 ± 1.13 | 3.93 ± 1.23 | 4.25 ± 1.02 | 5.63 ± 1.78 |
| histidine | CW | 2.96 ± 0.57 | 3.52 ± 1.07 | 4.53 ± 0.67 | 4.21 ± 1.18 |
| | FW | 3.13 ± 0.59 | 4.13 ± 1.16 | 5.37 ± 0..48 | 5.04 ± 1.24 |
| valine | CW | 3.59 ± 0.12 | 4.58 ± 1.21 | 5.01 ± 144 | 4.17 ± 0.72 |
| | FW | 3.99 ± 0.64 | 5.16 ± 1.67 | 6.05 ± 0.89 | 5.99 ± 1.13 |
| methionine | CW | 1.48 ± 0.54 | 1.87 ± 0.67 | 1.95 ± 0.59 | 2.54 ± 0.81 |
| | FW | 2.46 ± 0.52 | 2.47 ± 0.83 | 2.89 ± 0.83 | 3.69 ± 1.48 |
| aspartic acid | CW | 4.67± 0.21 | 3.62 ± 0.16 | 4.98 ± 0.24 | 5.16 ± 0.29 |
| | FW | 5.25 ± 1.54 | 3.98 ± 1.23 | 5.23 ± 1.38 | 5.82 ± 1.67 |
| isoleucine | CW | 2.98 ± 1.23 | 3.26 ± 0.76 | 3.72 ± 0.89 | 4.14 ± 0.51 |
| | FW | 3.87 ± 1.71 | 4.78 ± 0.62 | 4.57 ± 0.82 | 5.68 ± 1.37 |

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| | | | | | |
|---|---|---|---|---|---|
| leucine | CW | 6.45 ± 1.13 | 4.33 ± 0.31 | 6.79 ± 1.37 | 7.15 ± 0.92 |
| | FW | 7.39 ± 1.05 | 5.31 ± 1.87 | 7.75 ± 0.95 | 8.31 ± 1.82 |
| tyrosine | CW | 2.51 ± 0.76 | 2.86 ± 0.63 | 3.24 ± 0.43 | 3.98 ± 0.74 |
| | FW | 3.49 ± 1.15 | 3.65 ± 0.84 | 4.75 ± 1.08 | 5.46 ± 1.39 |
| Total Amino Acids | CW | 84.02 ± 4.87 | 81.48 ± 5.32 | 95.33 ± 6.76 | 95.94 ± 5.98 |
| | FW | 102.78 ± 5.32 | 101.93 ± 4.13 | 115.91 ± 5.17 | 116.71 ± 4.64 |

All values are the average of determinations in three independent experiments. The data were analyzed with a one-way ANOVA followed by Duncan multiple range tests ($P < 0.05$). CW: cooked wheat, FW: fermented wheat

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423 **Table 2.** Minerals and carbohydrate contents during cooked and fermented wheat.

| Minerals mg/100 g | Sample | *Baekjoong* | *Jeokjoong* | *Milseoung* | *Namhae* |
|---|---|---|---|---|---|
| Iron | CW | 3.17 ± 0.23 | 3.26 ± 0.13 | 3.25 ± 0.14 | 3.11 ± 0.22 |
| | FW | 3.98 ± 0.89 | 4.71 ± 0.32 | 4.73 ± 0.28 | 3.26 ± 0.85 |
| Calcium | CW | 25.58 ± 3.83 | 28.43 ± 3.64 | 23.65 ± 4.49 | 23.89 ± 4.63 |
| | FW | 29.87 ± 2.23 | 30.87 ± 2.77 | 27.35 ± 3.43 | 26.39 ± 3.98 |
| Phosphorus | CW | 287.77 ± 7.32 | 285.43 ± 4.92 | 278.49 ± 4.82 | 275.15 ± 5.94 |
| | FW | 315.32 ± 5.75 | 302.54 ± 5.98 | 295.45 ± 5.98 | 300.24 ± 6.47 |
| Magnesium | CW | 120.87 ± 6.41 | 124.29 ± 7.73 | 123.99 ± 5.13 | 123.04 ± 7.15 |
| | FW | 139.11 ± 5.34 | 145.87 ± 9.65 | 140.62 ± 7.60 | 131.82 ± 8.73 |
| Selenium | CW | 71.04 ± 6.21 | 70.65 ± 3.76 | 71.41 ± 3.29 | 70.25 ± 6.37 |
| | FW | 70.65 ± 4.19 | 69.78 ± 4.65 | 72.76 ± 7.13 | 68.92 ± 5.92 |
| Potassium | CW | 356.22 ± 6.94 | 360.32 ± 9.54 | 351.73 ± 7.82 | 349.14 ± 7.81 |
| | FW | 368.71 ± 7.15 | 385.12 ± 10.07 | 388.60 ± 10.54 | 365.84 ± 8.95 |
| Manganese | CW | 3.99 ± 0.43 | 3.28 ± 1.09 | 2.91 ± 0.84 | 2.27 ± 0.32 |
| | FW | 4.13 ± 0.74 | 4.96 ± 0.61 | 3.65 ± 0.73 | 3.99 ± 0.36 |
| Zinc | CW | 1.48 ± 0.54 | 2.19 ± 0.67 | 1.95 ± 0.59 | 2.54 ± 0.81 |
| | FW | 2.46 ± 0.52 | 2.47 ± 0.83 | 2.89 ± 0.83 | 3.69 ± 1.48 |
| Sodium | CW | 2.32 ± 0.19 | 2.26 ± 0.87 | 2.25 ± 0.15 | 2.30 ± 0.95 |
| | FW | 4.78 ± 0.84 | 4.00 ± 0.90 | 4.49 ± 0.36 | 4.02 ± 0.72 |
| Copper | CW | 0.45 ± 0.17 | 0.33 ± 0.09 | 0.32 ± 0.01 | 0.39 ± 0.43 |
| | FW | 0.73 ± 0.07 | 0.63 ± 0.05 | 0.41 ± 0.11 | 0.72 ± 0.17 |
| Glucose g/100g | CW | 0.41 ± 0.06 | 0.46 ± 0.13 | 0.23 ± 0.09 | 0.19 ± 0.10 |
| | FW | 0.42 ± 0.12 | 0.65 ± 0.17 | 0.25 ± 0.08 | 0.21 ± 0.04 |
| Fructose g/100g | CW | 71.02 ± 3.27 | 65.38 ± 5.32 | 67.27 ± 4.63 | 64.12 ± 4.76 |
| | FW | 72.24 ± 3.81 | 75.42 ± 6.87 | 90.54 ± 6.17 | 76.13 ± 5.53 |

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All values are the average of three replicates. The data were analyzed with a one-way ANOVA followed by Duncan multiple range tests ($P < 0.05$). CW: cooked wheat, FW: fermented wheat

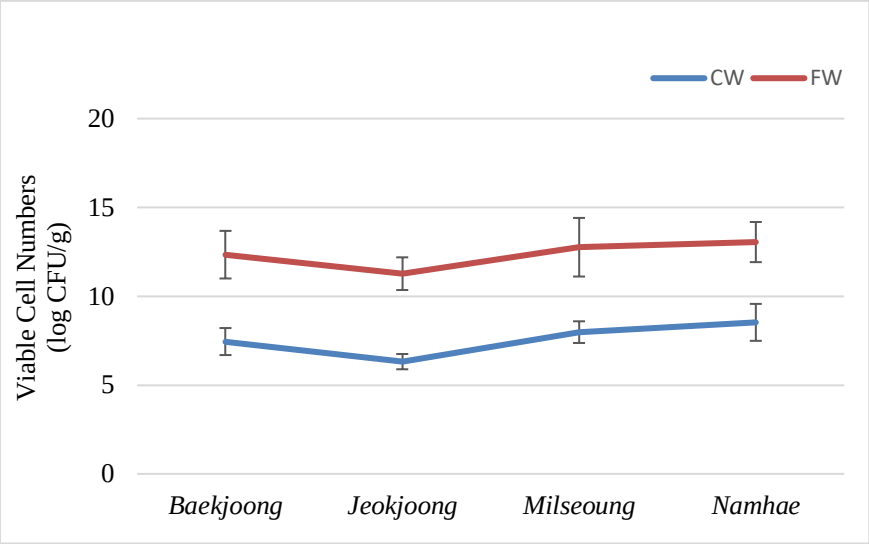


**Fig. 1.** Viable cell numbers of cooked wheat and fermented wheat. Error bars (mean ± SD) represent significant differences among the treatments. The data were analyzed with a one-way ANOVA followed by Duncan multiple range tests ($P < 0.05$). CW: cooked wheat, FW: fermented wheat

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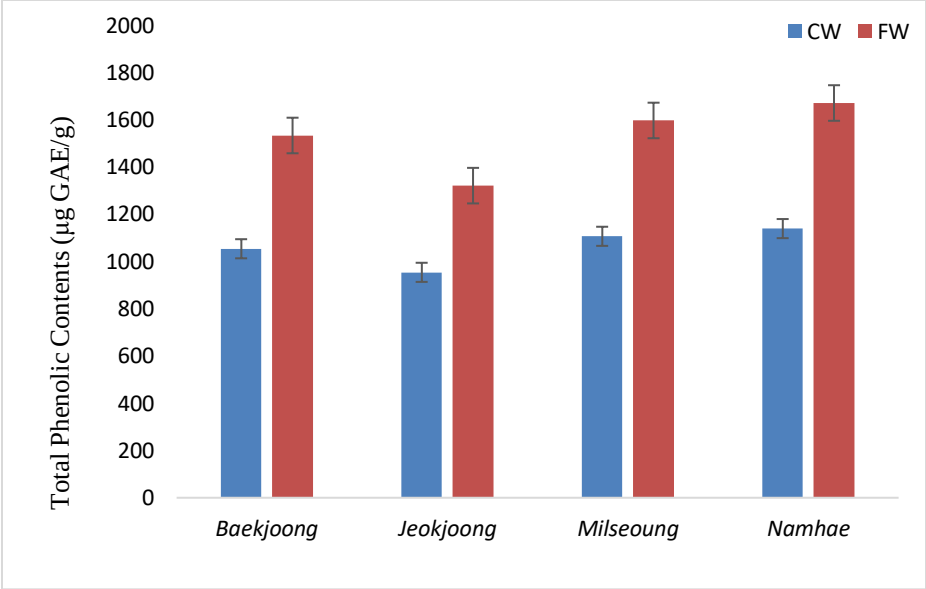


434

435 **Fig. 2.** Total phenolic contents of cooked wheat and fermented wheat. Error bars (mean ± SD) represent significant
436 differences among the treatments. The data were analyzed with a one-way ANOVA followed by Duncan multiple
437 range tests ($P < 0.05$). CW: cooked wheat, FW: fermented wheat

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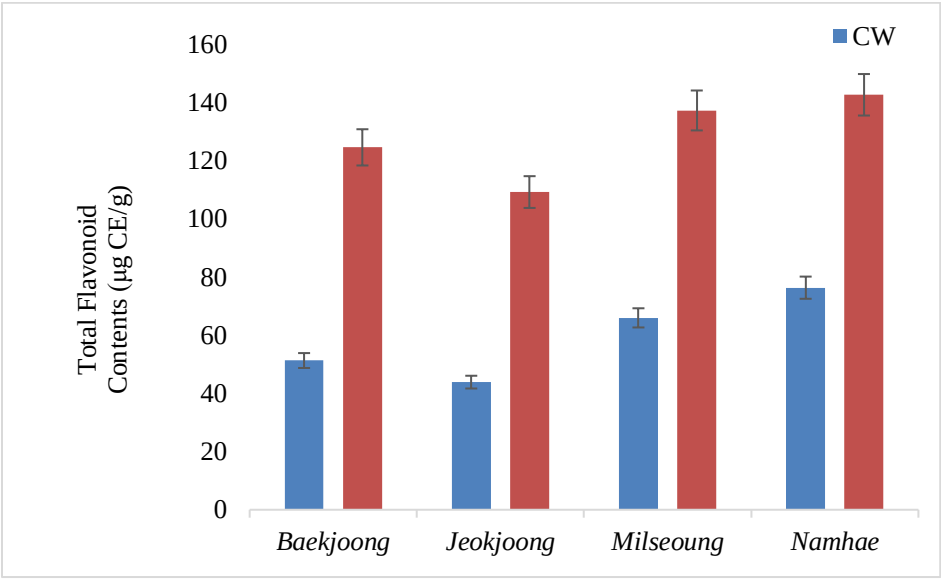


439

440 **Fig. 3.** Total flavonoid contents of cooked wheat and fermented wheat. Error bars (mean ± SD) represent significant
441 differences among the treatments. The data were analyzed with a one-way ANOVA followed by Duncan multiple
442 range tests ($P < 0.05$). CW: cooked wheat, FW: fermented wheat

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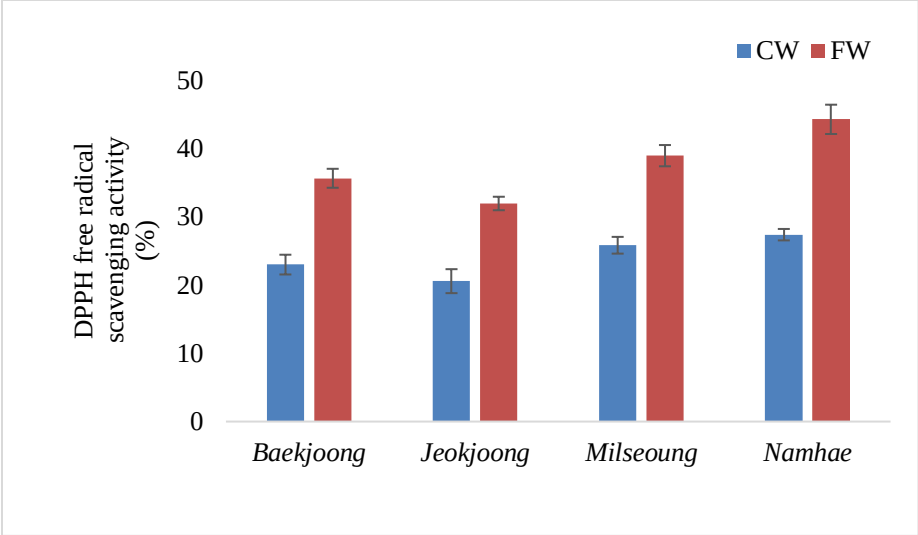


**Fig. 4.** DPPH free radical scavenging activity of cooked wheat and fermented wheat. Error bars (mean ± SD) represent significant differences among the treatments. The data were analyzed with a one-way ANOVA followed by Duncan multiple range tests ($P < 0.05$). CW: cooked wheat, FW: fermented wheat

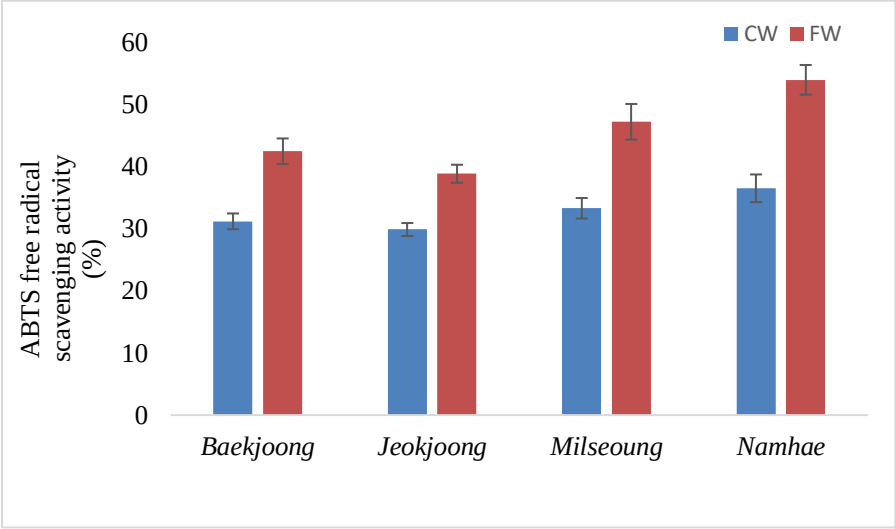


**Fig. 5.** ABTS free radical scavenging activity of cooked wheat and fermented wheat. Error bars (mean ± SD) represent significant differences among the treatments. The data were analyzed with a one-way ANOVA followed by Duncan multiple range tests ($P < 0.05$). CW: cooked wheat, FW: fermented wheat