Study of the protein, antioxidant activity, and starch during in vitro simulated digestion of green wheat and wheat cooked flours

Kangyi Zhang, Yun Zhang, Ning Xu, Xue Yang, Guozhi Zhang, Yu Zhang & Qinghao Liu

To cite this article: Kangyi Zhang, Yun Zhang, Ning Xu, Xue Yang, Guozhi Zhang, Yu Zhang & Qinghao Liu (2020) Study of the protein, antioxidant activity, and starch during in vitro simulated digestion of green wheat and wheat cooked flours, International Journal of Food Properties, 23:1, 722-735, DOI: 10.1080/10942912.2020.1754234

To link to this article: https://doi.org/10.1080/10942912.2020.1754234

© 2020 The Author(s). Published with license by Taylor & Francis Group, LLC.

Published online: 28 Apr 2020.

Article views: 656

View related articles

View Crossmark data

Citing articles: 2 View citing articles
Study of the protein, antioxidant activity, and starch during *in vitro* simulated digestion of green wheat and wheat cooked flours

Kangyi Zhang\(^a\), Yun Zhang\(^{a,b}\), Ning Xu\(^b\), Xue Yang\(^b\), Guozhi Zhang\(^{a,b}\), Yu Zhang\(^{a,b}\), and Qinghao Liu\(^c\)

\(^a\)Center of Agricultural Products Processing, Henan Academy of Agricultural Sciences, Zhengzhou, China; \(^b\)College of Food Science and Technology, Henan University of Technology, Zhengzhou, China; \(^c\)School of Chemical Engineering and Technology, North University of China, Taiyuan, China

**ABSTRACT**

The aim of this study was to investigate the changes in protein, antioxidant activity, and starch of two varieties of green wheat and wheat *in vitro* simulated digestion. The experiment was used a new *in vitro* simulated digestion process which was closer to the body’s digestive system. The protein utilization of green wheat in the beginning of digestion was lower than that of wheat. However, in the intestinal stage, it was slightly higher than wheat to reach. The total phenolic, flavonoid content and DPPH radical scavenging power of green wheat were about twice that of wheat. The antioxidant activity of green wheat was stronger than that of wheat. Although the glucose content of green wheat was higher than wheat, the GI of green wheat (52–54) was always much lower than wheat (65–71). Therefore, the appropriate replacement of wheat with green wheat as the main food is more conducive to the absorption of nutrients, the use of antioxidant activity and the regulation of blood glucose.

**ARTICLE HISTORY**

Received 10 January 2020
Revised 25 March 2020
Accepted 28 March 2020

**KEYWORDS**

Antioxidant; Green wheat; *In vitro* simulated digestion; Protein; Starch

**Introduction**

Green wheat is a traditional whole grain food in China with a history of more than 1,000 years. It has a unique flavor and is rich in nutrients. Green wheat is wheat at the end of milky stage. Our previous researches have shown that green wheat contains 14.90% protein, 12.88% dietary fiber, 14.44 mg/g ferulic acid, and a large amount of vitamins and minerals, while starch and fat are only 45.17% and 1.32%. Its nutritional value is much higher than wheat, and it also has great advantages in antioxidants. In Middle Eastern countries and other countries, roasted green wheat (also called frikeh, frekeh, or freekeh) is becoming an increasingly popular food.\(^{1–5}\) However, roasted green wheat will cause huge losses to the nutrition of green wheat. Therefore, a new preservation method should be used to maintain the original characteristics and shelf life of the green wheat. And it is more suitable for diabetes with its low starch content.

Protein is an important indicator of evaluating the nutritional value of food. In the study of proteins, studies on the extent of their digestion and absorption are often overlooked. Protein digestibility refers to the ratio of nitrogen absorbed by proteins in the body to nitrogen intake, reflecting the extent to which proteins are decomposed and absorbed by digestive enzymes.\(^{6}\) Digestible protein has better nutritional value than non-digestible protein.\(^{7}\) Hydrolysis of proteins may affect the utilization of amino acids, as the hydrolysis process is affected by linear amino acid sequences and tertiary structure of proteins.\(^{8}\) Proteins can be divided into albumin, globulin, prolamin, and gluten by their solubility. Albumin and globulin in wheat have high nutritional value. First, owing to the water-soluble and salt-soluble environment present in the human body,
albumin, and globulin may be more easily digested and absorbed by the body. Second, albumin and globulin have a more reasonable amino acid composition, including the lysine, threonine, and tryptophan required by the human body. Therefore, the protein digestibility should be measured and analyzed, which is of great significance for the comprehensive evaluation of food nutrition.

Free radicals are intermediate metabolites produced by the body’s metabolism. Under normal physiological conditions, the free radical production system and the scavenging system are in dynamic equilibrium in the human body, protecting the cells and tissues from the free radical damage. Experimental studies and epidemiology have shown that cereals contain a large amount of antioxidant active substances, and sufficient amounts of cereal foods intake can effectively reduce the risk of chronic degenerative diseases such as diabetes, cardiovascular diseases, and tumors. At present, the research shows that natural phenolic substances mainly include phenolic acids and flavonoids, and most of them are combined with other substances (including proteins, monosaccharides, organic acids, etc.) in the form of ester bonds, glycosidic bonds, ether glycosidic bonds, etc. It is decomposed by the upper digestive enzymes but can be also released by enzymatic hydrolysis of an enzyme (such as glycosidase) secreted by microorganisms to exert an antioxidant effect in the colon in vitro. There have been many reports on in vitro chemistry for the evaluation of antioxidant activity in cereals. Baublis et al. found that the total antioxidant activity of cereals was significantly improved under the action of gastric acid and gastrointestinal digestive enzymes. Serrano et al. also believed that the use of in vitro chemical evaluation to evaluate the antioxidant activity of grains may lead to underestimated risks. It is considered that the gastrointestinal environment should be considered when evaluating the antioxidant activity of cereals. By studying the traditional Brazilian food feijoada, Faller et al. believed that phenolic substances reach the colon after ingestion and were highly utilized. Chandrasekara and Shahidi investigated the antioxidant analysis of millet. Guo et al. also analyzed the phenolic substances and antioxidant capacity of tartary buckwheat. Hidalgo et al. studied carotenoids, total phenols, phenolic acids, and antioxidant capacity of water bread (WB) in white bread, white bread, and white bread. These results highlighted the potential health benefits of underutilized crops. Pastoriza et al. used a global antioxidant response (GAR) method to simulate digestion through the gastrointestinal tract. The GAR method not only showed higher antioxidant activity but also measured antioxidant activity in insoluble parts. Delgado-Andrade et al. proposed a new method to measure the global antioxidant response (GAR) of cereal derivatives, which can avoid underestimating the antioxidant activity of cereal derivatives. Lafarga et al. evaluated the bioaccessibility of polyphenols and antioxidant activity in cooked pulses and to study the effect of cooking on their total phenolic content and antioxidant capacity. The release of phenolics from cooked legumes was mainly achieved during the intestinal phase. Adom et al. showed the distribution of antioxidants in different fractions of wheat flour and pointed out that the total phenolic content of the bran/germ fraction was 15–18 times higher than the respective endosperm fractions. Meanwhile, they also studied the difference in antioxidant activity of 11 different varieties of wheat and found that total phenolic content, total antioxidant activity, and total flavonoid content were not significantly different among 11 wheat varieties. In contrast, there was a large difference in carotenoid content, which was related to different genotypes. Liyanapathirana and Shahidi extracted phenolic compounds from soft wheat and its milling fractions and their in vitro antioxidant activity were evaluated. However, reports on the evaluation of the antioxidant activity of wheat flour using simulated gastrointestinal digestive processes are still rare, except for bran and whole-grain foods.

The protein and antioxidant activities of green wheat in vitro digestion have not been studied. At present, a shift in healthy opinion has occurred in favor of cutting starch with the aim of controlling the blood glucose level. Therefore, researchers have increasingly attracted interest in the digestive process of starch and changes in blood glucose in the body. Tang et al. researched the in vitro digestibility of starch in naked oats and derived the effect of non-starch components on the low digestibility of naked oats. Senay et al. investigated the starch composition and digestibility of hard
red spring wheat cultivars. In 1981, Jenkins et al. proposed the concept of glycemic index (GI). The digestibility of starch is closely related to the value of GI. To evaluate the glycemic index, Bustos et al. performed in vitro digestion and sensory analysis of pasta. The results showed that by using insoluble fiber, the nutritional quality of pasta can be improved and a lower glycemic index can be obtained. Zabidi et al. found that the addition of Chempedak seed flour reduced the estimated glycemic index (EGI). Green wheat has a higher content of dietary fiber and a lower content of starch than wheat. It is meaningful to study the digestion process of starch and changes in blood glucose of green wheat. The changes of protein, antioxidant activity, and starch in the digestion process of green wheat and wheat were studied by in vitro simulated digestion experiments.

Materials and methods

Materials

Two different varieties of green wheat and wheat Zhengmai No. 7698 and Bainong No. 207 (GWZ, GWB, WZ, and WB) were provided by the Henan Academy of Agricultural Sciences, Zhengzhou, P. R. China. Green wheat was wheat at the end of milky stage. And they were planted in the experimental field of Henan Academy of Agricultural Sciences and harvested in 2019.

Preparation of samples

The green wheat flour or wheat flour and water were mixed in the ratio of 1:10 and had a boiling water bath for 10 min. The gelatinized flour was vacuum freeze-dried and grounded into powder for further determination.

In vitro simulated digestive fluid

Materials: Salivary α-amylase (A-3176-500KU, 10 U/mg, Sigma-Aldrich Corp., St. Louis, Mo., U.S. A.); Pepsin (P6887-5 G, 3706 U/mg, Sigma-Aldrich Corp., St. Louis, Mo., U.S.A.); Pancreatin (P1750-100 G, Sigma-Aldrich Corp., St. Louis, Mo., U.S.A.); Amyloglucosidase (A7420, 31.2 U/mg, Sigma-Aldrich Corp., St. Louis, Mo., U.S.A.); Buffer 1 (1.21 g NaHCO3, 1.57 g KCl, 1.59 ml 1.59 mmol/L CaCl2, 0.41 ml 0.2 mmol/L MgCl2, and ultrapure water); Buffer 2 (175 μl 0.02 mol/L HCl and ultrapure water); Buffer 3 (11.8 ml 14.4 mmol/L CH3COOH, 4 ml 1.0 mmol/L CaCl2, 100 μl 4.9 mmol/L MgCl2, 90 ml 2.0 mmol/L NaOH and ultrapure water); Buffer 4 (202 mg NaOH and 250 ml ultrapure water).

Method: To prepare the simulated saliva (SS), 1.125 g salivary α-amylase was dissolved in a mixture of 45 ml buffer 1. Adjust pH value to 7 ± 0.2 by adding HCl or NaOH. Simulated gastric fluid contains 105 ml buffer 2 and 105 mg pepsin. The pH value adjusted to 2 (0.5 g/L citric acid, 0.5 g/L maleic acid, 420 μL lactic acid, and 500 mL/L acetic acid). Pancreatin (113.75 mg) and amyloglucosidase (0.492 ml) were added to 104.508 ml buffer 3 to make a simulated intestinal fluid (SIF). Buffer 4 was used to adjust the pH, including 202 mg NaOH and 250 ml ultrapure water.

In vitro digestion

In vitro simulated digestion of green wheat and wheat-cooked flours were performed as described by the past reports, with slight modifications. The process of in vitro simulated digestion was done using simulated digestive system GI20 (NI Ltd., Australia). During the oral stage, 0.5 g sample was blended with 2 ml SS and added a rotor to simulate peristaltic digestion. The process was maintained for 5 min and then entered the stomach stage for 1.5 h. At this point, 5 ml of simulated gastric fluid was added. At the gastrointestinal stage, further 5 ml of buffer 4 and 25 ml of buffer 3 were added to simulate the pH in the intestinal fluid. Then, 5 ml of simulate intestinal fluid was injected. The intestinal stage lasted for 4
The entire digestion process was constant at 37°C. Take out the required samples at each time point and the samples were taken every 0.5 h in the stomach stage and every 1 h in the intestinal stage (the abbreviations of OA, S1, S2, S3, I1, I2, I3, and I4 represent sampling points in the oral cavity, gastric stage, and intestinal stage, respectively). The enzyme was deactivated in a water bath at 70°C for 10 min and centrifuged at 4500 rmp for 20 min. The supernatant was collected and filtered through a 0.45 μm filter for detection. The extracts were kept at 4°C until analysis.

**In vitro protein digestibility**

A digestion solution (4 mL) was obtained, and an equal volume of 15% trichloroacetic acid solution was added to precipitate protein. Then, it was centrifuged at 10,000 r/min for 15 min, and the supernatant was collected. Ten milliliters of the supernatant was tested with a fully automated Kjeldahl analyzer (K1100, Jinan Hanon Instruments Co., Ltd, China). The protein digestibility was calculated according to [Eq. (1)]:

\[
\text{Protein digestibility} = \frac{\text{Supernatant protein content}}{\text{Total protein content of the sample}} \times 100\% 
\]

**Total phenolic content**

Total phenolic content was determined by the Folin–Ciocalteu (FC) colorimetric method and improvement according to the protocols described by Singleton and Rossi. Accurately weigh 0.1 g of gallic acid, and dissolve it in distilled water to 100 ml. A standard stock solution of gallic acid having a mass concentration of 1000 mg/L was obtained and a series of standard solutions were prepared. Standards or samples (1 ml) were mixed with 50 ml distilled water, and then added 1 ml of FC developer and 3 ml of 20% Na₂CO₃. After the solution was mixed and protected from light in a water bath at 50°C for 30 min, the regression equation between the absorbance and the concentration of the gallic acid standard solution was measured at a wavelength of 765 nm, and the total phenol content was calculated. The total phenolic content is expressed in milligrams of the equivalent of gallic acid contained in 100 g of dry weight (mg GA/100 g DW). In order to better understand the bioavailability of active substances in the digestion process, the following formula was defined [Eq. (2)]:

\[
\text{Bioavailability of active substances (BASx)} = \frac{C_x}{C_0}
\]

where \(C_x\) is the concentration of the active substance in each digestion stage (\(X = 1\), oral digestion stage; \(X = 2\), gastric digestion stage; \(X = 3\), intestinal digestion stage); \(C_0\) is the active substance concentration of the solid sample.

**Total flavonoid content**

The total flavonoids content was determined by NaNO₂-Al(NO)₃ method. Samples (0.1 ml) were mixed completely with 0.2 mL of 5% NaNO₂ solution. After placing at room temperature for 6 min, 0.2 mL of 10% AlCl₃ was added and then allowed to stand for 6 min again. Then, 2 mL of 1 mol/L NaOH solution was added and finally dilute to 5 mL with distilled water. After 15 min of balancing, the absorbance of the mixture was measured at a wavelength of 510 nm. The flavonoid content is expressed in milligrams of the equivalent of rutin contained in 100 g of dry weight (mg Rutin/100 g DW).

**Analysis of antioxidant activity**

The DPPH radical scavenging power assay was carried out according to the protocols described by Brand-Williams et al. with slight modifications. DPPH (8.0 mg) was dissolved in absolute ethanol
and the solution was made up to a 200 ml brown volumetric flask to keep away from light. Five grams of green wheat flour and wheat flour sample was soaked twice with 50 ml of absolute ethanol for 24 h, and the two extracts were combined. After centrifugation at 4000 r/min for 20 min, the supernatant was concentrated. In 1 ml of the sample and the supernatant, 3 ml of DPPH solution was separately added. The reaction was carried out in the dark at room temperature for 30 min, and the absorbance was measured at 517 nm with anhydrous ethanol as a blank. The DPPH radical scavenging activity (RSA) was calculated according to Eq. (3):

$$\text{RSA} \% = \frac{A_0 - (A_S - A_C)}{A_0} \times 100\%$$  (3)

where $A_0$ was the absorbance of 1 ml of distilled water and 3 ml of DPPH solution; $A_S$ was the absorbance of 1 ml of sample solution and 3 ml of DPPH solution; $A_C$ was the absorbance of 1 ml of sample solution and 3 ml of absolute ethanol. The IC$_{50}$ was recorded as the sample concentration when the RSA was 50%.

**In vitro starch digestibility**

A sample (0.4 mL) was obtained at different times, and the enzymes were inactivated by adding an equal volume of absolute ethanol. Subsequently, the glucose content was analyzed by the method of Tang et al. [34] The change of Glycemic Index (GI) at each stage was determined by GI20.

**Statistical Analysis**

Values were analyzed using SPSS (version 20.0, Chicago, USA). Origin Pro 8.6 program (Origin Lab Inc., USA) was also used for statistical analyses. Datum was exhibited as means ± standard deviations of at least triplicate determinations. GI was processed by GraphPad prism 8.

**Results and Discussion**

**Proximate composition**

According to the standard curve equation ($Y = 0.0214X + 0.0007$, $R^2 = 0.9996$, where $Y$ is the absorbance value, and $X$ is the Gallic acid concentration), the total phenolic content of green wheat and wheat was determined at 265 nm by using the spectrophotometer (Figure 1). The total phenolic content of green wheat is more than twice that of wheat. The respiration and accumulation of nutrients in wheat during maturation may cause the total phenolic content to be consumed slowly. The total flavonoid content was calculated according to the standard curve equation ($Y = 0.0425X + 0.0008$, $R^2 = 0.999$, where $Y$ is the absorbance value, and $X$ is the Gallic acid concentration). The flavonoid content of green wheat and wheat is still 2 times different. Its high content of flavonoids has significant preventive and adjuvant treatment effects on chronic degenerative diseases caused by oxidative stress, such as hypertension, coronary heart disease, diabetes, and tumors. [47] The antioxidant capacity of the digestive after oral, gastric, and gastrointestinal digestion was assessed using the DPPH assays. The DPPH radical scavenging power of green wheat and wheat-cooked flours is shown in Figure 1. The DPPH radical scavenging power of green wheat is more than four times that of wheat. It depends on the presence of more antioxidants in the green wheat, such as flavonoids, vitamins, and ferulic acid. The antioxidant capacity of Zhengmai No.7698 is higher than that of Bainong No.207. However, according to previous researches, there is no significant difference in protein between green wheat and wheat. [5]
In vitro protein digestibility of green wheat and wheat

Compared to wheat, green wheat is significantly different in the in vitro digestion process of the gastric (Figure 2). In the first stage of the gastric, the digestibility of green wheat protein is much lower than that of wheat. The reason may be caused by the fact that the green wheat is not fully mature and some proteins are not easily digested in the gastric. The glutenin content in green wheat is extremely low. However, in the final stage of the gastric and in the intestine stage, the protein digestibility of green wheat tends to be normal, even slightly higher than wheat. There were no significant differences in these changes between the different varieties. Jenkins et al. investigated the effects of protein and starch interactions on the processing of in vitro simulated digestion. Green wheat is different from wheat in starch, disaccharide, and oligosaccharides. This may also be the cause of the difference in protein digestibility. Želmira et al. found that some proteins of wheat were completely resistant to hydrolysis throughout the simulated gastric digestion process and some of them throughout the simulated duodenal digestion. Most of the polypeptides during subsequent incubation with enzymes resulted in gradual digestion, with only a trace of the parent proteins remaining after gastric digestion, whose majority disappeared after complete gastro-duodenal digestion. The protein digestibility of the green wheat and wheat varieties of Bainong No. 207 is slightly higher than that of Zhengmai No. 7698. The protein digestibility of both green wheat and wheat in the gastric stage is close to 80%. In addition, the protein digestibility of the gastrointestinal stage of the green wheat increased by 5.95% and 7.10% compared with the gastric stage. However, wheat is 9.65% and 9.11%, which is higher than green wheat. This result is different from the 24.87% increase in protein digestibility, possibly due to different forms of digested samples. Meanwhile, a large amount of dietary fiber in green wheat would also reduce the digestibility of protein.

Total phenolic content of green wheat and wheat after in vitro simulated digestion

During the in vitro simulated digestion process, the total phenolic content of the gastric state will slightly increase (Table 1). At the end of the intestinal state, the total phenolic content is essentially the same, and
the content of wheat is slightly higher. The total phenolic content of green wheat and wheat decreased by 90.31%, 88.49% and 80.92%, 82.95% in the oral to the intestinal stage. From the oral to gastric stage, the total phenol content in green wheat was significantly reduced, but there was no significant difference in the stomach. However, the total phenol content of wheat decreased significantly until the intestinal stage. The total phenol bioavailability of green wheat in the oral and gastric stages was higher than that of wheat. However, in the intestine, it has an opposite result. Both of them begin to reduce the total phenolic content at the beginning of the intestinal stage. The bioavailability of total phenolics in the two varieties of green wheat and wheat was basically the same, with no significant difference. Gawlik-Dziki et al.\[51\] studied the total phenolic content and antioxidant activity of buckwheat extract added to bread. Despite the digestion stage, the highest antiradical activity, reducing power and ability to inhibition of lipid peroxidation was observed with a 5% buckwheat extract addition in the samples. Their tendency to change in in vitro digests is similar to that of green wheat and wheat. The BAS values of green wheat and wheat are also different. In the oral stage, the BAS value of green wheat is higher than that of wheat, but the opposite is true in the intestinal stage. The phenomenon is explained by the change in phenolic substances in the ripening process of green wheat.
Total flavonoid content of green wheat and wheat after in vitro simulated digestion

From the third stage of the gastric, the green wheat can not be detected the flavonoid content in the digestive fluid (Table 2). However, the digestive fluid of wheat is not detected from the second stage of the gastric. From the oral cavity to the first stage of the gastric, the flavonoid content rapidly drops, which may be due to the digestive fluid of the gastric acting on the flavonoids. Among them, green wheat decreased by 98.24% and 98.44%, while wheat decreased by 96.98% and 97.88%. Therefore, the total flavonoid concentration peaks decrease after gastric digestion and are less absorbed in the oral cavity. It is the same conclusion that flavonoids can be absorbed in the stomach and small intestine. Both flavonoids from green wheat and wheat were absorbed in the stomach. The bioavailability of flavonoids showed that the total flavonoid content of the green wheat extract was high, but the content in the digestive environment of the human body was very low, that is, the bioavailability of total flavonoids in food was very low, and the flavonoids that could be absorbed were more small. Hao et al. studied red-grain wheat flavonoids and used them for in vitro simulated digestion to obtain similar results.

Effects of in vitro simulated digestion on DPPH· radical scavenging power of green wheat and wheat

The values of the DPPH radical scavenging power of the samples treated by digestion in vitro are presented in Table 3. From the oral cavity to the gastrointestinal stage, the DPPH· radical scavenging power of the green wheat gradually decreased. There was no significant difference in the DPPH radical scavenging ability of all samples in the last two stages of the stomach. However, wheat has a small rise in the gastric stage. It is similar to the results of previous studies of wheat-steamed bun. This may be related to high levels of flavonoids and total phenolics in green wheat. There was no significant decrease after I2 in all samples, except for wheat of Bainong No.207. There is a high
content of rutin and phenolic acids in the green wheat, and there is a significant correlation between the improvement of these substances and the enhancement of DPPH radical scavenging ability.\(^53\) Meanwhile, the antioxidants in the green wheat also showed strong activity in the emulsified lipid system, which is the same as the results of previous studies.\(^54\) Xu and Chang\(^55\) have used ultrasonically treated aqueous ethanol extracts, and this method was widely used to extract antioxidants from wheat bran. Since the green wheat is not fully mature, the wheat bran and the wheat kernel are tightly fitted. It is also the reason for the high antioxidant capacity of green wheat. However, from the oral to the gastric stage, the DPPH radical scavenging power of the green wheat decreased more than wheat. Appropriate consumption of green wheat instead of wheat can give people more antioxidant capacity.

In vitro starch digestibility and GI of green wheat and wheat

It can be known from Figure 3 that during the entire in vitro simulated digestion process, the trend of the glucose content in the digestive fluid of green wheat and wheat is basically the same. From the oral cavity to the stomach, the glucose content increases sharply, because the salivary amylase in the oral cavity breaks down starch and the maltose content increases, thereby increasing the glucose content. However, the glucose content of green wheat at this stage is slightly higher than that of wheat, which may be due to the higher broken starch content of green wheat. In the three stages of the stomach, the glucose content is slowly decreasing. This may be the reducing hydroxyl or aldehyde group of glucose, which is affected in acidic gastric fluid, resulting in a decrease in the measured glucose content. After entering the intestine, the glucose content begins to increase gradually, which is due to the action of pancreatin and amyloglucosidase, which converts starch into glucose. In the intestine stage, the glucose content of GWZ is higher than that of WZ. The glucose content of GWB and WB also shows the same results. In a word, after several stages of digestion, the glucose content of green wheat is slightly higher than that of wheat, and the increase in glucose before and after digestion is greater than wheat, so green wheat can provide the energy needed by the human body like wheat.

It can be seen from Figure 4 that from 10 min to 300 min of digestion, the color of the two types of green wheat changed from light green at first to a darker green or black. Under the same conditions, the color of wheat was changed from the original light green to red. This means that the GI of green wheat is significantly lower than wheat. The dark green color of green wheat when digested to 300 min is similar to the color of wheat when it is digested to 60 min, indicating that the blood sugar rises much faster after digesting wheat than green wheat. Between two different green wheat varieties, the color of GWB during digestion was always darker than that of GWZ. The result for wheat is the opposite. Although the glucose content of green wheat is higher than wheat, the GI of green wheat is always much lower than wheat. Due to its resistant starch content, dietary fiber, etc., content are higher than that of wheat.

### Table 3. DPPH· radical scavenging power (IC\(_{50}\) mg/mL) in digestive fluid at various stages of green wheat and wheat.

| Stage | Zhengmai No.7698 | Wheat | Bainong No.207 | Wheat |
|-------|------------------|-------|----------------|-------|
| OA    | 6.02 ± 0.21a     | 1.21 ± 0.08a | 6.13 ± 0.29a | 1.25 ± 0.11a |
| S1    | 4.01 ± 0.13b     | 1.03 ± 0.07ab | 3.99 ± 0.21b | 1.19 ± 0.10a |
| S2    | 2.28 ± 0.11c     | 0.98 ± 0.02ab | 2.23 ± 0.15c | 0.99 ± 0.06b |
| S3    | 2.15 ± 0.10c     | 1.07 ± 0.05bc | 2.19 ± 0.12c | 1.05 ± 0.09b |
| I1    | 1.02 ± 0.05d     | 0.78 ± 0.03c  | 1.69 ± 0.01d | 0.69 ± 0.02c |
| I2    | 0.73 ± 0.02e     | 0.56 ± 0.02c  | 1.21 ± 0.10e | 0.56 ± 0.03d |
| I3    | 0.68 ± 0.01e     | 0.20 ± 0.01d  | 0.79 ± 0.04f | 0.28 ± 0.02e |
| I4    | 0.55 ± 0.03e     | 0.05 ± 0.00d  | 0.61 ± 0.03f | 0.15 ± 0.01f |

Notes: The abbreviations of OA, S1, S2, S3, I1, I2, I3, and I4 represent sampling points in the oral cavity, gastric stage, and intestinal stage, respectively.
Both varieties of green wheat have a GI of 52–54, which is a low GI food, while wheat has a GI of 65–71, which is a high GI food\textsuperscript{[56]} (Figure 5). After eating green wheat, the blood sugar rises slowly and does not cause sharp fluctuations in blood sugar. Therefore, green wheat is more suitable as food for people with diabetes than wheat. It can be concluded that GWZ is most suitable for patients with diabetes and coronary heart disease and can be processed into green health food.

**Conclusion**

This study used a new in vitro simulation method to investigate the bioavailability of protein, antioxidant activity, and starch in wheat and wheat. The total protein bioavailability of green wheat and wheat is almost the same, but the protein bioavailability of wheat in the initial stage of the gastric is higher than that of green wheat. It was found that the antioxidant activity of green wheat was higher than that of wheat. In addition, the total phenol, flavonoids content, and DPPH radical scavenging power bioavailability of green wheat are higher than that of wheat. Green wheat digestive fluid has higher glucose content than wheat, but GI has opposite results. Therefore, green

*Figure 3. Glucose content of green wheat and wheat starch (GWZ: green wheat of Zhengmai No. 7698; GWB: green wheat of Bainong No. 207; WZ: wheat of Zhengmai No. 7698; WB: wheat of Bainong No. 207) during in vitro simulated digestion. OA, S1, S2, S3, I1, I2, I3, and I4 represent sampling points in the oral cavity, gastric stage, and intestinal stage, respectively.*
wheat has better protein digestibility and antioxidant activity than wheat, and more suitable for people with diabetes. The appropriate replacement of wheat with green wheat as the main food is more conducive to the human body’s nutrient absorption and utilization of antioxidant activity.

**Acknowledgments**

Yu Zhang, Guozhi Zhang, and Kangyi Zhang designed the experiment; Yu Zhang and Yun Zhang conducted the experiment; Yu Zhang, Yun Zhang, Ning Xu, and Xue Yang analyzed the results. All authors reviewed the manuscript. The authors declare that there is no conflict of interests regarding the publication of this paper.
Funding

This study was financially supported by Major Science and Technology projects of Henan Province (grant number: 151100111300); National Engineering Laboratory for Wheat & Corn Further Processing (grant number: NL2015003) and Engineering Technology Research Center for Grain & Oil Food, State Administration of Grain, (grant number: JGSG2016001).

ORCID

Guozhi Zhang http://orcid.org/0000-0002-1268-0037
Yu Zhang http://orcid.org/0000-0002-9499-6157

References

[1] Al-Mahasneh M. A.; Rababah T. M.; Bani-Amer M. M.; Al-Omari N. M. Fuzzy and Conventional Modeling of Open Sun Drying Kinetics for Roasted Green Wheat. Int. J. Food Prop. 2013, 16(1), 70–80. DOI: 10.1080/10942912.2010.528108.

[2] Al-Mahasneh, M. A.; Bani Amer, M. M.; Rababah, T. M. Modeling Moisture Sorption Isotherms in Roasted Green Wheat Using Least Square Regression and Neural-fuzzy Techniques. Food Bioprod. Process. 2012, 90(2), 165–170. DOI: 10.1016/j.fbp.2011.02.007.

[3] Al-Mahasneh, M. A.; Rababah, T. M. Effect of Moisture Content on Some Physical Properties of Roasted Green Wheat. J. Food Eng. 2007, 79(4), 1467–1473. DOI: 10.1016/j.jfoodeng.2006.04.045.

[4] Rosner, A.; Roasting Green Wheat in Galilee. Gastronomica. 2011, 11(2), 66–68. DOI: 10.1525/glc.2011.11.2.66.

[5] Zhang, Y.; Zhang, G. Z. Starch Content and Physicochemical Properties of Green Wheat Starch. Int. J. Food Prop. 2019, 22(1), 1463–1474. DOI: 10.1080/10492912.2019.1651739.

[6] Vilcacundo, R.; Miralles, B.; Carrillo, W.; Hernández-Ledesma, B. In Vitro, Chemopreventive Properties of Peptides Released from Quinoa (Chenopodium Quinoa, Willd.) Protein under Simulated Gastrointestinal Digestion. Food Res. Int. 2018, 105, 403–411. DOI: 10.1016/j.foodres.2017.11.036.

[7] Duodu, K. G.; Taylor, J. R. N.; Belton, P. S.; Hamaker, B. R. Factors Affecting Sorghum Protein Digestibility. J. Cereal Sci. 2003, 38(2), 117–131. DOI: 10.1016/S0733-5210(03)00016-X.

[8] Gopal, D. H.; Monteiro, P. V.; Virupaksha, T. K.; Ramachandra, G. Protein Concentrates from Italian Millet (Setaria Italica) and Their Enzymatic Hydrolysis. Food Chem. 1988, 29(2), 97–108. DOI: 10.1016/0308-8146(88)90092-1.

[9] Osborne, T. B.; The Scientific Results of the Ziegler Polar Expedition. (Scientific Books: The Proteins of the Wheat Kernel). Science. 1907, 26(677), 864–865. DOI: 10.1126/science.26.677.864.

[10] Wieser, H.; Seilmeier, W.; Belitz, H. D. Vergleichende untersuchungen über partielle aminosäuresequenzen von prolaminen und glutelinen verschiedener getreidearten. I. proteinfraktionierung nach osborne. Zeitschrift für Lebensmittel-Untersuchung und -Forschung. 1980, 170, 17–26. DOI: 10.1002/j.fodres.1980.0000016.

[11] Hung, P. V.; Morita, N. Distribution of Phenolic Compounds in the Graded Flours Milled from Whole Buckwheat Grains and Their Antioxidant Capacities. Food Chem. 2008, 109(2), 325–331. DOI: 10.1016/j.foodchem.2007.12.060.

[12] Wang, Q.; Shao, H.; Zhang, Z.; Yan, S. S. Phenolic Profile and Antioxidant Properties of Sand Rice (Agriophyllum Squarrosum) as Affected by Cooking and in Vitro Digestion. J. Sci. Food Agric. 2019, 99(8), 3871–3878. DOI: 10.1002/jsfa.9609.

[13] Wang, H.; Liu, J.; Liu, Z. Effect of Enzymatic Digestion, Chemical and Boiled Water Extraction Techniques on Apparent Antioxidant Bioactivities of Apple Peel. J. Food Meas. Charact. 2018, 13(2), 959–966. DOI: 10.1007/s11694-018-0010-3.

[14] Wilcox, J. K.; Ash, S. L.; Catignani, G. L. Antioxidants and Prevention of Chronic Disease. Crit. Rev. Food Sci. Nutr. 2004, 44(4), 275–295. DOI: 10.1080/10408690490468489.

[15] Okarter, N.; Liu, R. H. Health Benefits of Whole Grain Phytochemicals. Crit. Rev. Food Sci. Nutr. 2010, 50(3), 193–208. DOI: 10.1080/10408390802248734.

[16] Okarter, N.; C S. L.; Sorrells, M. E.; Liu, R. H. Phytochemical Content and Antioxidant Activity of Six Diverse Varieties of Whole Wheat. Food Chem. 2010, 119(1), 249–257. DOI: 10.1016/j.foodchem.2009.06.021.

[17] Adom, K. K.; Liu, R. H. Antioxidant Activity of Grains. J. Agric. Food Chem. 2002, 54(21), 4696–4704.

[18] Dykes. Phenolic Compounds in Cereal Grains and Their Health Benefits. Cereal Foods World. 2007, 52(3), 105–111.

[19] Liu, G.; Ying, D. Y.; Guo, B.; Cheng, L. J.; May, B.; Bird, T.; Sanguansri, L.; Cao, Y.; Augustin, M. Extrusion of Apple Pomace Increases Antioxidant Activity upon in Vitro Digestion. Food Funct. 2019, 10(2), 951–963. DOI: 10.1039/C8FO01083H.
[20] Andreasen, M. F.; Kroon, P. A.; Williamson, G.; Garcia-Conesa, M. T. Intestinal Release and Uptake of Phenolic Antioxidant Diferulic Acids. *Free Radical Biol. Med.* **2001**, *31*(3), 304–314. DOI: 10.1016/S0891-5849(01)00585-8.

[21] Baublis, A. J.; Lu, C.; Clydesdale, F. M.; Decker, E. A. Potential of Wheat-based Breakfast Cereals as a Source of Dietary Antioxidants. *J. Am. Coll. Nutr.* **2000**, *19*(3), 308S–311S. DOI: 10.1080/07315724.2000.10718965.

[22] Serrano, J.; Goni, I.; Saura-Calixto, F. Food Antioxidant Capacity Determined by Chemical Methods Underestimate the Physiological Antioxidant Capacity. *Food Res. Int.* **2007**, *40*(1), 0–21. DOI: 10.1016/j.foodres.2006.07.010.

[23] Faller, A. L. K.; Fialho, E.; Liu, R. H. Cellular Antioxidant Activity of Feijoada Whole Meal Coupled with an in Vitro Digestion. *J. Agric. Food Chem.* **2012**, 60(19), 4826–4832. DOI: 10.1021/jf300602w.

[24] Pastoriza, S.; Delgado-Andrade, C.; Haro, A. José Ángel Rufián Henares. A Physiologic Approach to Test the Global Antioxidant Response of Foods. The GAR Method. *Food Chem.* **2011**, *129*(4), 1926–1932. DOI: 10.1016/j.foodchem.2011.06.009.

[25] Delgado-Andrade, C.; Conde-Aguilera, J. A.; Haro, A.; De La Cueva, S. P. A Combined Procedure to Evaluate the Global Antioxidant Response of Bread. *J. Cereal Sci.* **2010**, 52(2), 239–246. DOI: 10.1016/j.jcs.2010.05.013.

[26] Guo, X. D.; Ma, Y. J.; Parry, J.; Gao, J. M.; Yu, L. L.; Wang, M. Phenolics Content and Antioxidant Activity of Tarty Buckwheat from Different Locations. *Molecules.* **2011**, 16(12), 9850–9867. DOI: 10.3390/molecules16129850.

[27] Hidalgo, A.; Ferraretto, A.; De Noni, I.; Bottani, M. Bioactive Compounds and Antioxidant Properties of Pseudocereals-enriched Water Biscuits and Their in Vitro Digestates. *Food Chem.* **2018**, 240, 799–807. Feb.1 DOI: 10.1016/j.foodchem.2017.08.014.

[28] Pastoriza, S.; Delgado-Andrade, C.; Haro, A. José Ángel Rufián Henares. A Physiologic Approach to Test the Global Antioxidant Response of Foods. The GAR Method. *Food Chem.* **2011**, 129(4), 1926–1932. DOI: 10.1016/j.foodchem.2011.06.009.

[29] Lafarga, T.; Villaró, S.; Bobo, G.; Simó, J.; Aguiló-Aguayo, I. Bioaccessibility and Antioxidant Activity of Phenolic Compounds in Cooked Pulses. *Int. J. Food Sci. Technol.* **2019**, 54(5), 1816–1823. DOI: 10.1111/ijfs.14082.

[30] Liyanapathirana, C.; Shahidi, F. Antioxidant Activity of Wheat Extracts as Affected by in Vitro Digestion. *Food Chem.* **2005**, 93(6), 2297–2306. DOI: 10.1016/j.foodchem.2004.05.013.

[31] Senay, S.; Bilge, B.; Catherine, S. S.; Ovando-Martinez, M. Starch Digestibility Properties of Bread from Hard Red Spring Wheat Cultivars Released in the Last 100 Years. *Cereal Chem.* **2009**, 97(1), 138–148.

[32] Jenkins, D. J.; Wolever, T. M.; Taylor, R. H.; Fielden, H.; Baldwin, J. M.; Bowling, A. C.; Newman, H. C.; Jenkins, A. L.; Goff, D. V. Glycemic Index of Foods: A Physiological Basis for Carbohydrate Exchange. *Am. J. Clin. Nutr.* **1981**, 34(3), 362–366. DOI: 10.1093/ajcn/34.3.362.

[33] Sugiyama, M.; Tang, A. C.; Wakaki, Y.; Koyama, W. Glycemic Index of Single and Mixed Meal Foods among Common Japanese Foods with White Rice as a Reference Food. *Eur. J. Clin. Nutr.* **2003**, 57(6), 743–752. DOI: 10.1038/sj.ejcn.1601606.

[34] Goni, I.; Garcia-Alonso, A.; Saura-Calixto, F. A Starch Hydrolysis Procedure to Estimate Glycemic Index. *Nutr. Res.* **1997**, 17(3), 403–437. DOI: 10.1016/0271-5317(97)00010-9.

[35] Bustos, M. C.; Perez, G. T.; León, A. E. Sensory and Nutritional Attributes of Fibre-enriched Pasta. *LWT Food Sci. Technol.* **2011**, 44(6), 0–1434. DOI: 10.1016/j.lwt.2011.02.002.

[36] Goni, I.; Goñi, I.; Saura-Calixto, F. Food Antioxidant Capacity Determined by Chemical Methods Underestimate the Physiological Antioxidant Capacity. *Food Res. Int.* **2007**, *40*(1), 0–21. DOI: 10.1016/j.foodres.2006.07.010.

[37] Falla, A. L. K.; Fialho, E.; Liu, R. H. Cellular Antioxidant Activity of Feijoada Whole Meal Coupled with an in Vitro Digestion. *J. Agric. Food Chem.* **2012**, 60(19), 4826–4832. DOI: 10.1021/jf300602w.

[38] Chandrasekara, A.; Shahidi, F. Bioaccessibility and Antioxidant Potential of Millet Grain Phenolics as Affected by Simulated in Vitro Digestion and Microbial Fermentation. *J. Funct. Foods.* **2012**, 4(1), 226–237. DOI: 10.1016/j.jff.2011.11.001.

[39] Guar, J.; Goñi, I.; Saura-Calixto, F. Food Antioxidant Capacity Determined by Chemical Methods May Underestimate the Physiological Antioxidant Capacity. *Food Res. Int.* **2007**, *40*(1), 0–21. DOI: 10.1016/j.foodres.2006.07.010.

[40] Adom, K. K.; Sorrells, M. E.; Liu, R. H. Phytochemicals and Antioxidant Activity of Milled Fractions of Different Wheat Varieties. *J. Agric. Food Chem.* **2005**, 53(6), 2297–2306. DOI: 10.1021/jf048456d.

[41] Adom, K. K.; Sorrells, M. E.; Liu, R. H. Phytochemical Profiles and Antioxidant Activity of Wheat Varieties. *J. Agric. Food Chem.* **2003**, 51(26), 7825–7834. DOI: 10.1021/jf030404l.

[42] Tang, M. Y.; Wang, L. Y.; Cheng, X. X.; Wu, Y. W.; Jie, O. Y. Non-starch Constituents Influence the in Vitro Digestibility of Naked Oat (Avena Nuda L.)starch[]. *Food Chem.* **2019**, 297(UNSP), 124953. DOI: 10.1016/j.foodchem.2019.124953.

[43] Senay, S.; Bilge, B.; Catherine, S. S.; Ovando-Martinez, M. Starch Digestibility Properties of Bread from Hard Red Spring Wheat Cultivars Released in the Last 100 Years. *Cereal Chem.* **2003**, 97(1), 138–148.

[44] Jenkins, D. J.; Wolever, T. M.; Taylor, R. H.; Barker, H.; Fielden, H.; Baldwin, J. M.; Bowling, A. C.; Newman, H. C.; Jenkins, A. L.; Goff, D. V. Glycemic Index of Foods: A Physiological Basis for Carbohydrate Exchange. *Am. J. Clin. Nutr.* **1981**, 34(3), 362–366. DOI: 10.1093/ajcn/34.3.362.

[45] Goni, I.; Goñi, I.; Saura-Calixto, F. Food Antioxidant Capacity Determined by Chemical Methods Underestimate the Physiological Antioxidant Capacity. *Food Res. Int.* **2007**, *40*(1), 0–21. DOI: 10.1016/j.foodres.2006.07.010.

[46] Intawongse, M.; Dean, J. R. Use of the Physiologically-based Extraction Test to Assess the Oral Bioaccessibility of Metals in Vegetable Plants Grown in Contaminated Soil. *Environ. Pollut.* **2008**, 152(1), 0–72. DOI: 10.1016/j.envpol.2007.05.022.

[47] Zhuang, P.; Zhang, C.; Li, Y.; Zou, B.; Mo, H.; K J, W.; J T, W.; Li, Z. Assessment of Influences of Cooking on Cadmium and Arsenic Bioaccessibility in Rice, Using an in Vitro Physiologically-based Extraction Test. *Food Chem.* **2016**, 213, 206–214. DOI: 10.1016/j.foodchem.2016.06.066.
INTERNATIONAL JOURNAL OF FOOD PROPERTIES

Nagah, A. M.; Seal, C. J. In Vitro Procedure to Predict Apparent Antioxidant Release from Wholegrain Foods Measured Using Three Different Analytical Methods. J. Sci. Food Agric. 2005, 85(7), 1177–1185. DOI: 10.1002/jsfa.2106.

Singleton, V.; Rossi, J. A. Colorimetry of Total Phenolics with Phosphomolybdic-phosphotungstic Acid Reagents. Am. J. Enol. Vitic. 1964, 16(3), 144–158.

Hou, F. L.; Hu, K.; Gong, Y. S.; Xu, J. R.; Wu, Y. X.; Zhang, M. W. Effects of in Vitro Simulated Digestion on the Flavonoid Content and Antioxidant Activity of Aged and Fresh Dried Tangerine Peel. J. Food Process. Preserv. 2017, 42(3), e13532. DOI: 10.1111/jfpp.13532.

Brand-Williams, W.; Cuvelier, M. E.; Berset, C. Use of Free Radical Method to Evaluate Antioxidant Activity. LWT Food Sci. Technol. 1995, 28(1), 25–30. DOI: 10.1016/S0023-6438(95)80008-5.

Yao, L. H.; Jiang, Y. M.; Shi, J.; Tomás-Barberánn, F. A.; Dattar, N.; Singanusong, R.; Chen, S. S. Flavonoids in Food and Their Health Benefits. Plant Foods Human Nutr. 2004, 59(3), 113–122. DOI: 10.1007/s11130-004-0049-7.

Jenkins, D. J. A.; Thorne, M. J.; Wolever, T. M.; Jenkins, A. L.; Rao, A. V.; Thompson, L. U. The Effect of Starch-protein Interaction in Wheat on the Glycemic Response and Rate of in Vitro Digestion. Am. J. Clin. Nutr. 1987, 45(5), 946–951. DOI: 10.1093/ajcn/45.5.946.

Gálová, Z.; Chňapek, M.; Palenčárová, E.; Balážová, Z. In Vitro Gastro - Intestinal Digestion of Wheat Coeliac Active Proteins. J. Microbiol. Biotechnol. Food Sci. 2012, 1(Special issue), 601–609.

Căpriță, R.; Căpriță, A.; Crețescu, I.; Nicu, V. In Vitro Determination of Wheat Dry Matter Solubility and Protein Digestibility. Lucrari Stiintifice Zootehnie Si Biotehnologii. 2012, 45, 2.

Gawlik-Dziki, U.; Dziki, D.; Baraniak, B.; Lin, R. F. The Effect of Simulated Digestion in Vitro on Bioactivity of Wheat Bread with Tartary Buckwheat Flavones Addition. LWT - Food Sci. Technol. 2009, 42(1), 0–143. DOI: 10.1016/j.lwt.2008.06.009.

Hao, J. M.; Li, Y.; Yang, Z. P.; Yang, H.; Zhu, Y. C.; Sun, M.; Gao, Z. Q. The Optimal Ethanol Extraction Process in Water Bath and Antioxidant Ability in Vitro of Red-grain Wheat Flavonoids. J. Chin. Cereal. Oils Assoc. 2015, 30(7), 12–18.

Sedej, I.; Sakač, M.; Mandić, A.; Misan, A.; Pestoric, M.; Simurina, O.; Canadanovic-Brunet, J. Quality Assessment of Gluten Free Crackers Based on Buckwheat Flour. LWT Food Sci. Technol. 2011, 44(3), 694–699. DOI: 10.1016/j.lwt.2010.11.010.

Zielinski, H.; Achremowicz, B.; Przygodzka, M. Antioxidants in Cereal Grains. Zynnows-Nauka Technologia Jakos. 2012, 19(1), 5–26.

Xu, B. J.; Chang, S. K. C. A Comparative Study on Phenolic Profiles and Antioxidant Activities of Legumes as Affected by Extraction Solvents. J. Food Sci. 2007, 72(2), S159–S166. DOI: 10.1111/j.1750-3841.2006.00260.x.

C J, C.; Taylor, A. Dietary Hyperglycemia, Glycemic Index and Metabolic Retinal Diseases. Pro. Retinal Eye Res. 2011, 30(1), 18–53. DOI: 10.1016/j.preteyeres.2010.09.001.