COMPARATIVE STUDY OF THE NUTRITIONAL QUALITY OF POTATO–WHEAT STEAMED AND BAKED BREADS MADE WITH FOUR POTATO FLOUR CULTIVARS

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ABSTRACT
We investigated the nutritional quality of steamed and baked breads containing 35% potato flour from four potato cultivars. Compared with traditional wheat varieties, potato–wheat steamed and baked breads contained higher dietary fiber (1.87-fold), K (2.68-fold), vitamin C (28.56-fold), and total polyphenol (1.90-fold) contents and greater antioxidant activity (1.23-fold). Moreover, the estimated glycemic index of potato–wheat breads ranged from 61.20 (Hongmei-wheat baked bread) to 67.36 (Atlantic-wheat steamed bread), which was lower than that of wheat steamed bread (70.22) and baked bread (70.62). In terms of nutritional value, Hongmei was the optimum cultivar, followed by Blue Congo, Shepody, and Atlantic. For the same cultivar, the nutritional value of steamed bread was higher than that of baked bread. In conclusion, potato flour is a potential wheat flour supplement that improves the nutritional and functional properties of breads.

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Introduction
Bread, which represents a traditional staple food in Western and Eastern countries, varies by ingredients and heating methods. Baked bread is popular in Western countries, while steamed bread, a type of fermented and steamed wheat-based food of Chinese origin, represents ~40% of the wheat consumption in China. Steamed bread, which has been gaining considerable popularity in Asian countries and Asian communities outside Asia, is cooked by steaming, as opposed to baking (Zhu 2014). However, most traditional steamed and baked breads are made from simple ingredients (e.g., wheat flour, water, and yeast) and lack essential nutrients, including lysine, dietary fiber, vitamins, and minerals (Choo & Aziz 2010). Regardless of the steamed and baked breads, bread contains a high amount of rapidly digestible starch and has a high glycemic index (GI). A large number of studies have shown a negative association between the consumption of high GI foods and the prevention of obesity, diabetes, and cardiovascular diseases (Brand-Miller et al. 2009; Thomas & Pfeiffer 2012). Therefore, healthy flours or ingredients should be incorporated into steamed and baked breads to increase their nutrient content (Borneo & Aguirre 2008).

Potato is the fourth most important food crop in the world after rice, wheat, and maize (FAOSTA 2016). Potato protein has a balanced amino acid composition, which is superior to that of cereal proteins (Bártová et al. 2015). Additionally, total vitamin and mineral levels in potato flour are higher than in wheat flour. Chun et al. (2005) reported that potatoes represent the third most important source of phenolics (especially chorogenic acid) after apples and oranges (Malenberg & Theander 1985). Furthermore, potatoes contain other phytochemicals such as flavonoids, polyamines, and carotenoids, which are highly desirable in the diet due to their beneficial effects on human health (Ezekiel et al. 2013). Therefore, the incorporation of potato flour into steamed and baked breads would enhance their nutritional and functional qualities.

Researchers have attempted to improve the nutritional value of steamed and baked breads by adding rice, legume flour, tuberous root, oat, corn, wheat germ, barely, fiber, and polyphenols (Sun et al. 2015; Villarino et al. 2015; Sui et al. 2016; Zhu et al. 2016).
Zhu et al. (2016) reported that black tea addition increased the antioxidant activity of wheat bread, and Sui et al. (2016) concluded that bread containing 4% anthocyanin-rich black rice extract powder had higher antioxidant activity and lower starch digestion rates (−20.5%) compared with those of wheat bread. Lau et al. (2015) observed that processing conditions had pronounced effects on starch digestibility in bread, and that steamed bread was healthier in terms of glycemic response. Ragae et al. (2011) reported that the GI of wheat bread was not significantly affected by the addition of whole grain flour or fiber. Moreover, different flour cultivars may affect the chemical and amino acid composition and nutritional value of steamed and baked breads (Angioloni & Collar 2012; Farooq & Boye 2011; Sivaramakrishnan et al. 2014; Villarino et al. 2015). Therefore, different flour or functional components have different effects on the nutritional value and starch digestibility of bread.

For the preparation of bread, up to 20% potato flour can be mixed with wheat flour (Anjum et al. 2008; Ijah et al. 2014). In a previous study, where we used different potato flour concentrations (0–100%) for the preparation of potato–wheat bread, we observed that specific volume decreased and hardness increased with increasing potato flour addition and that 35% potato flour results in potato–wheat bread of appropriate specific volume and hardness. The aim of this study was to investigate the effect of 35% potato flour addition from four potato cultivars on the proximate composition, mineral and vitamin content, index of nutritional quality (INQ), amino acid composition, antioxidant activity, in vitro starch digestibility, and glycemic response of steamed and baked breads. Additionally, the comprehensive nutritional value was determined through gray relation analysis. Our study findings will provide useful information on the potential applications of potato flour in the manufacture of steamed and baked breads.

**Materials and methods**

**Materials**

Wheat flour was purchased from Qijian Food Industry Co. Ltd. (Beijing, China). Four different potato cultivars (Atlantic, Shepody, Hongmei, and Blue Congo) were kindly provided by the Institute of Vegetables and Flowers of the Chinese Academy of Agricultural Sciences (Beijing, China). Vital protein and trehalose were obtained from Henan Zhongxing Chemical Co., Ltd. (Zhengzhou, Henan, P.R. of China), and yeast was acquired from Angel Yeast Co., Ltd. (Hubei, China).

**Preparation of potato flour**

Fresh potatoes were peeled, washed with tap water, steamed at 100°C for 30 min, drum dried at 170–200°C, milled with a hammer mill, and sieved through a 100 μm screen to obtain flour of uniform particle size. All samples were stored at 20°C in sealed aluminum bags. Wheat flour was mixed with 35% potato flour from different cultivars and labeled as Atlantic-Wheat (AW), Shepody-Wheat (SW), Hongmei-Wheat (HW), and Blue Congo-Wheat (BCW).

**Steamed and baked bread making**

Steamed bread was made according to the report by Ma et al. (2014) with slight modifications. Batches of 100 g mixed flour, 0.5 g trehalose, 1 g active dry yeast, 2 g vital protein (except for wheat steamed bread), and water (51.5 g for wheat; 61.5 g for AW; 62.3 g for SW; 63.4 g for HW; and 65.7 g for BCW) were mixed at low speed (80 rpm) for 8 min in a Hobart mixer A-120 (The Hobart Manufacturing Company, Tory, OH). Baked bread was made with batches of 100 g mixed flour, 0.5 g trehalose, 2 g active dry yeast, 2 g vital protein (except for wheat steamed bread), and water (61.8 g for wheat; 73.8 g for AW; 74.8 g for SW; 76.1 g for HW; and 78.8 g for BCW) that were first mixed at low speed (80 rpm) for 3 min and subsequently at high speed (120 rpm) for 5 min in the Hobart mixer.

Dough was leavened in a fermenting box at 35°C for 40 min at 75% relative humidity. Following fermentation, the dough was mixed at low speed (80 rpm) for 3.5 min and divided into 100 g round-shaped pieces. Finally, the dough pieces were placed in a steam cooker (Supor Co., Ltd., Hangzhou, Zhejiang, China), covered with a lid, and steamed over boiling water for 30 min under atmospheric pressure. Baked bread was baked in an electrical oven (ACA model ATO-CF24B, ACA Inc., Zhuhai, China) at 160°C for 35 min. All steamed and baked breads were freeze-dried, ground in a commercial grinder, and stored at 25°C in sealed aluminum bags.

**Proximate composition and gross energy**

Ash, crude fat, and crude protein contents were determined by AOAC methods (AOAC 2000). Ash content (g/100 g DW) was determined by weighing bread samples before and after heat treatment (550°C for 12 h). Crude fat (g/100 g DW) content was determined by AOAC method 960.39. Crude protein (g/100 g DW) was assessed by the micro-kjeldahl method (AOAC method 976.05). Crude fiber (g/100 g DW) was determined by AOAC method 976.08.
determined by ISO method 5498:1981. Carbohydrate content (g/100 g DW) was calculated by subtracting the sum of ash, crude fat, crude protein, and crude fiber contents from 100. Gross energy (kcal/100 g DW) was determined in a bomb calorimeter according to ISO method 9831 (ISO 1998).

**Mineral content**

Steamed and baked bread samples were digested in concentrated HNO₃ (AOAC 2000). The digest was transferred to a 25 ml volumetric flask, and the volume was adjusted to 25 ml with deionized water. A blank digest was prepared in a similar manner. Mineral content, expressed as mg mineral/100 g DW, was determined by inductively coupled plasma atomic emission spectrometry (ICAP6000, Thermo Fisher Scientific, Waltham, MA).

**Vitamin content**

Vitamin C, vitamin B₁, vitamin B₂ (riboflavin), and vitamin B₃ (nicotinamide) were extracted and determined by a slightly modified HPLC method previously reported (Gratacós-Cubarsi et al. 2011; Sánchez-Mata et al. 2000). Briefly, 1 g of sample was mixed with 9 ml of 0.1 M HCl or ethanol and maintained at 100 °C for 30 min in a water bath. After cooling, 6 ml of 2.5 M sodium acetate and 1 ml of 10% (w/v) taka-diastase solution were added. Samples were incubated overnight at 37 °C and centrifuged at 500 g for 5 min at 4 °C. The resulting supernatant was adjusted to 20 ml with ultrapure water. An aliquot (5 ml) was purified using an Oasis MCX cartridge (6 cc–150 mg, Waters Corp., Manchester, UK) for the simultaneous determination of vitamins C, B₁, B₂, and B₃. HPLC-DAD/FLD analyses were performed in a Waters System (Waters Corp., Manchester, UK) equipped with a 717 Plus autosampler, a 1525 binary HPLC pump, a 2996 photodiode array detector, and a 2475 fluorescence detector (Waters Corp., Milford, MA). A linear gradient elution between solvent A (0.2% H₃PO₄ in 20% w/v MeOH) and solvent B (0.2% H₃PO₄ in 20% w/v water) was carried out by varying B from 100% to 30% from 5 to 10 min. The linear regression equations of vitamins C, B₁, B₂, and B₃ were $y = 66, 108x - 3548.7$ ($R^2 = 0.9994$), $y = 64,112x - 6724.8$ ($R^2 = 0.9993$), $y = 42,804x - 95.194$ ($R^2 = 0.9997$), and $y = 41,831x - 2600.9$ ($R^2 = 0.9995$), respectively. Vitamin contents were expressed as milligram of vitamins C, B₁, B₂, and B₃ per 100 milligram bread powder on a DW basis.

**Index of nutritional quality**

The index of nutritional quality (INQ) was calculated according to the following equation (Sun et al. 2014; Venom 2013):

$$\text{INQ} = \left( \frac{\text{Nutrient amount in 100g DW 35% potato—wheat baked bread or steamed bread/Chinese NRV}}{\text{Calories in 100g DW 35% potato bread or steamed bread/Average energy intake}} \right)$$

where the Chinese nutrient reference value (NRV) for protein, fat, carbohydrate, and fiber are 60 g, ≤60 g, 300 g, and 25 g, respectively; the NRV for calcium (Ca), phosphorus (P), potassium (K), sodium (Na), magnesium (Mg), iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn) are 800 mg, 700 mg, 2000 mg, 2,000 mg, 300 mg, 15 mg, 15 mg, 1.5 mg, and 3 mg, respectively; the NRV for vitamins C, B₁, B₂, and B₃ are 100 mg, 1.4 mg, 1.4 mg, and 5 mg, respectively; and the average energy intake is 2000 kcal (Chinese Nutrient Reference Value).

**Amino acid composition**

The amino acid composition of both steamed and baked breads were obtained using the Biochrom 3.1 amino acid analyzer (Amersham, Britain) according to the method by Bártová et al. (2015). Briefly, 10 ml of 6 N HCl was added to 100 mg sample in a test tube. The test tube was vacuated with nitrogen, sealed, heated in an oven at 110 °C for 24 h, and allowed to cool to room temperature. The hydrolysate was filtered to remove visible sediments and evaporated to dryness under vacuum at 60 °C. The hydrolysate was dissolved in 1 ml of 0.02 N HCl. An aliquot (20 μl) was injected into the amino acid analyzer (tryptophan could not be determined by this method).

Initial oxidation to methionine sulfone and cysteic acid was performed with a performic acid/phenol mixture before hydrolysis for the determination of methionine and cysteine. Protein quality was determined by amino acid score (AAS). The amino acid composition appropriate for adult human consumption (FAO/WHO 1991) was used for the estimation of AAS. The recommended levels of individual amino acids were the following: valine, 5 g/100 g; methionine + cystine, 3.5 g/100 g; isoleucine, 4 g/100 g; leucine, 7 g/100 g; phenylalanine + tyrosine, 6 g/100 g; lysine,
5.5 g/100 g; threonine, 4 g/100; and tryptophan, 1 g/100 g.

**Total polyphenol content (TPC) and antioxidant activity**

TPC was measured by the Folin–Ciocalteu method with a slight modification (Singleton et al. 1999). Polyphenols of steamed and baked bread samples were extracted according to the method of Sun et al. (2014). A calibration curve was generated with chlorogenic acid standards (Sigma-Aldrich, Inc., St. Louis, MO), ranging from 0.02 to 0.10 mg/ml. The linear regression equation was $y = 8.7671x + 0.0068$ and $R^2 = 0.9994$. TPC was expressed as milligram chlorogenic acid equivalents (CAE) per gram bread powder on a DW basis. TPC of breads was calculated according to the following equation:

$$TPC = \frac{(A - 0.0068)}{8.7671} \times \frac{V}{M}$$

where $A$ is the absorbance, $V$ is the volume of the crude extract diluent (ml), and $M$ is the mass of the tested bread powder on a DW basis (g).

Antioxidant activity of the bread samples was determined with the oxygen radical absorption capacity (ORAC) assay (Sun et al. 2014). ORAC values were expressed as micrograms Trolox equivalents (TE) per 100 mg bread powder on a DW basis.

**In vitro starch digestibility and expected glycemic index**

In vitro starch digestibility of breads was measured according to the modified method reported by Englyst and Hudson (1996) and applied by Ronda et al. (2012). Hydrolyzed glucose at 20 min (G20) and 120 min (G120) and total glucose (TG) were determined by the glucose oxidase/peroxidase colorimetric method. Free sugar glucose (FGS) was determined by the method reported by Englyst et al. (2003). Based on the results, rapidly digested starch (RDS) = 0.9 × (G20 – FGS), slowly digestible starch (SDS) = 0.9 × (G120 – G20), and resistant starch (RS) = 0.9 × (TG – G120).

Hydrolysis index (HI) was obtained by dividing the area under the sample hydrolysis curve (0–180 min) by the area of a standard material (glucose) over the same time period. The estimated glycemic index (eGI) was calculated using the following equation (Granfeldt et al. 1992):

$$eGI = 8.198 + 0.862HI$$

**Comprehensive nutritional value**

Nutrient level represents an important index of the nutritional value of breads. However, it is not comprehensive to evaluate the nutritional value of breads according to a single nutrient. Gray relation analysis is a technique of system theory that is used to evaluate the comprehensive nutritional value of breads. Gray system theory was first introduced by Deng (1989). The fundamental definition of grayness is that the information is incomplete or unknown; therefore, an element of the incomplete message is a gray element. Gray relation analysis measures the correlation among gray elements.

In this study, the steamed and baked breads represent a gray system; each baked or steamed bread is a factor in the system. The nutritional value correlation between the samples and an ideal sample was determined. Based on the aim of this study, the ideal sample was selected by combining the upper or lower nutritional contents. Crude protein, dietary fiber, K, Ca, vitamin C, total polyphenol content, antioxidant activity, etc., which are positively correlated with nutritional content and low GI, utilized 5% of the maximum value of the tested breads. However, crude fat, carbohydrate, eGI, etc., which are negatively correlated with the nutritional content and low GI, utilized 5% of the minimum value of the tested breads. A high correlation coefficient is indicative that the degree of similarity between the sample and the ideal sample is high. The correlation coefficient was calculated according to the method reported by Kadier et al. (2015). Assuming that the ideal list was $X_0$, the compared list was $X_i$, $i = 1, 2, 3, \ldots$, and $X_0 = \{X_0(1), X_0(2), X_0(3) \ldots X_0(k), X_i \{X_i(1), X_i(2), X_i(3) \ldots X_i(k), k = 1, 2, 3 \ldots \}$. The correlation coefficient between the samples and ideal sample at the $k$ point was calculated using the following equation:

$$\zeta_{i(k)} = \frac{\min\{\Delta i(k)\} + \rho \max\{\Delta i(k)\}}{|\Delta i(k)| + \rho \max\{\Delta i(k)\}}$$

where $\Delta i(k) = |X_0(k) - X_i(k)|$, $\min|\Delta i(k)|$ is the minimum value of the first level, $\min\{\Delta i(k)\}$ is the minimum value of the second level, $\max|\Delta i(k)|$ is the maximum value of the first level, and $\max\{\Delta i(k)\}$ is the maximum value of the second level. In Equation (4), $\rho$ ($0 \leq \rho \leq 1$) is the distinguishing coefficient. The smaller the value $\rho$, the higher its distinguishability. In this study, $\rho$ was set to 0.5, because this value offers moderate distinguishing effects and good stability.

The average gray relational coefficient at the $k$ point was determined using the following equation:
The weight at the $k$ point was calculated with the following equation:

$$W_k = \frac{\gamma_k}{\sum_1^M \gamma_k}$$

The gray relational degree was determined by the following equation:

$$G_i = \sum_{k=1}^M \varepsilon_i(k) W_k$$

Statistical analysis

All the experiments were carried out in triplicate. Statistical analyses were performed using the Statistical Analysis System version 8.1 software (SAS Institute, Cary, NC). Statistical significance was set to $p < 0.05$.

Results and discussion

Proximate composition

The proximate and total dietary fiber compositions of potato–wheat steamed and baked breads are provided in Table 1. Protein content ranged from 13.15 (AW) to 15.40 (HW) g/100 g DW in potato–wheat baked breads and from 12.55 (AW) to 15.08 (HW) g/100 g DW in potato–wheat steamed breads. There was a significant difference in protein content between potato–wheat steamed and baked breads, because the protein content of potato flour is different among cultivars (see Table S1). Moreover, the protein content of HW steamed and baked breads were significantly higher than that of traditional wheat steamed and baked breads.

The ash content of potato–wheat steamed and baked breads, which ranged from 1.78 (HW steamed bread) to 3.83 (SW baked bread) g/100 g DW, were significantly higher than that of wheat breads at the same heating method. The carbohydrate content and gross energy of breads ranged, respectively, from 79.46 (HW baked bread) to 84.11 (wheat steamed bread) g/100 g DW and from 385.34 (BCW baked bread) to 402.10 (wheat steamed bread) KJ/100 g DW (Table 1). Additionally, carbohydrate and gross energy of steamed breads was higher than that of baked breads, which may be due to the higher baking temperatures that caused the loss of substances that produce energy.

Table 1. Proximate composition and gross energy of the potato–wheat steamed and baked breads (g/100 g DW).

| Samples             | Crude protein | Crude fat | Crude fiber | Ash | Dietary fiber | Carbohydrate | Gross energy*a |
|---------------------|---------------|-----------|-------------|-----|---------------|--------------|----------------|
| Steamed bread       |               |           |             |     |               |              |                |
| Wheat               | 13.49 ± 0.05  | 1.30 ± 0.03 | 0.30 ± 0.01  | 0.00 ± 0.01 | 2.65 ± 0.08  | 84.11 ± 0.28  | 402.10 ± 0.11  |
| AW                  | 12.55 ± 0.08  | 1.06 ± 0.01 | 0.60 ± 0.01  | 1.91 ± 0.01 | 4.55 ± 0.18  | 83.80 ± 0.12  | 395.26 ± 0.30  |
| HW                  | 13.04 ± 0.11  | 1.03 ± 0.02 | 0.75 ± 0.02  | 2.00 ± 0.01 | 3.86 ± 0.12  | 83.18 ± 0.09  | 394.5 ± 0.08   |
| SW                  | 12.13 ± 0.12  | 0.62 ± 0.01 | 0.58 ± 0.01  | 1.92 ± 0.01 | 6.33 ± 0.14  | 83.68 ± 0.08  | 393.5 ± 0.22   |
| BCW                 | 15.08 ± 0.08  | 1.02 ± 0.02 | 0.52 ± 0.01  | 1.78 ± 0.02 | 5.18 ± 0.07  | 81.60 ± 0.16  | 395.89 ± 0.12  |
| Baked bread         |               |           |             |     |               |              |                |
| Wheat               | 13.47 ± 0.02  | 1.30 ± 0.03 | 0.28 ± 0.01  | 2.19 ± 0.02 | 3.93 ± 0.12  | 82.76 ± 0.20  | 387.46 ± 0.50  |
| AW                  | 13.15 ± 0.09  | 1.01 ± 0.01 | 0.60 ± 0.01  | 3.64 ± 0.01 | 3.14 ± 0.14  | 81.60 ± 0.21  | 386.09 ± 0.83  |
| SW                  | 13.95 ± 0.12  | 0.99 ± 0.02 | 0.78 ± 0.01  | 3.61 ± 0.01 | 6.72 ± 0.11  | 80.73 ± 0.13  | 386.79 ± 0.43  |
| BCW                 | 14.16 ± 0.09  | 0.58 ± 0.02 | 0.78 ± 0.01  | 3.61 ± 0.01 | 6.72 ± 0.11  | 80.73 ± 0.13  | 386.79 ± 0.43  |
| HW                  | 15.40 ± 0.15  | 1.00 ± 0.01 | 0.62 ± 0.01  | 3.52 ± 0.02 | 5.43 ± 0.09  | 79.46 ± 0.23  | 384.84 ± 0.14  |
| Average             |               |           |             |     |               |              |                |
| Wheat breads        | 13.48 ± 0.02  | 1.30 ± 0.03 | 0.29 ± 0.01  | 1.91 ± 0.09 | 2.51 ± 0.10  | 83.43 ± 0.24  | 399.36 ± 0.52  |
| Average             | 13.79 ± 0.10  | 0.91 ± 0.02 | 0.64 ± 0.01  | 2.96 ± 1.08 | 4.71 ± 0.56  | 81.86 ± 0.16  | 390.88 ± 0.38  |

*aGross energy was expressed in kcal/100 g DW. AW: Atlantic potato flour–wheat flour; SW: Shepody potato flour–wheat flour; BCW: Blue Congo potato flour–wheat flour; HW: Hongmei potato flour–wheat flour.

Values followed by different superscript letters in each column indicate significant differences ($p < .05$)
Table 2 shows the mineral content of potato–wheat steamed and baked breads. Minerals are classified into two groups: macroelements (Ca, K, P, Mg, and Na) and microelements (Fe, Mn, Zn, and Cu). In this study, Ca ranged from 24.21 (wheat baked bread) to 61.63 (HW baked bread) mg/100 g DW; K ranged from 342.73 (wheat steamed bread) to 1150 (HW steamed bread) mg/100 g DW; P ranged from 108.63 (wheat baked bread) to 183.47 (HW steamed bread) mg/100 g DW; Mg ranged from 25.03 (wheat baked bread) to 62.54 (HW steamed bread) mg/100 g DW; and Na ranged from 8.27 (wheat steamed bread) to 16.59 (SW baked bread) mg/100 g DW. The addition of potato flour significantly increased the content of macroelements. Moreover, breads made with the Hongmei potato cultivar had the highest content of macroelements, with the exception of Na.

The most abundant macroelement was K, followed by P, Mg, Ca, and Na. The K/Na ratios determined in AW steamed bread (53.44), SW steamed bread (81.94), BCW steamed bread (89.67), HW steamed bread (100.70), AW baked bread (60.60), SW baked bread (62.95), BCW baked bread (80.46), and HW baked bread (89.09) were higher than those in wheat steamed bread (41.44) and wheat baked bread (44.50). Low K/Na ratios are associated with hypokalemia, high blood pressure, diabetes, and cancer (Li et al. 2015); therefore, potato–wheat steamed and baked breads may be healthier options for individuals with these conditions. Amongst all potato cultivars, Hongmei had the highest K/Na ratio.

The average Mg content of potato–wheat steamed and baked breads was 53.70 mg/100 g DW, which was higher than that of wheat steamed bread (33.29 mg/100 g DW) and baked bread (25.03 mg/100 g DW).

Moreover, there was a considerable difference in Mg content based on potato cultivar. As a result of its interaction with phosphate, Mg is essential in nucleic acid synthesis. Low Mg levels have been associated with several diseases including asthma, diabetes, and osteoporosis (Dalen & Jacobson 1974).

Fe ranged from 1.29 (wheat steamed bread) to 3.58 (BCW steamed bread) mg/100 g DW, Mn ranged from 0.39 (AW baked bread) to 0.63 (BCW baked bread) mg/100 g DW, Zn ranged from 0.68 (wheat baked bread) to 1.07 (BCW steamed bread) mg/100 g DW, and Cu ranged from 0.09 (wheat and AW bread) to 0.17 (SW steamed bread) mg/100 g DW. The most abundant microelement was Fe, followed by Mn, Zn, and Cu. Even though heme iron from meat is more bioavailable than non-heme iron from potato, the intake of heme Fe/hemoglobin from red meat may increase the risk of colorectal cancer (Wang & Farid 2015). The microelement content of potato–wheat steamed and baked breads was significantly higher than that of wheat steamed and baked breads.

### Vitamin content

The vitamin content of steamed and baked breads is presented in Table 2. Vitamin B1 ranged from 0.17 (BCW and HW steamed bread) to 0.26 (SW steamed bread) mg/100 g DW, vitamin B2 ranged from 0.09 (BCW steamed bread) to 0.30 (wheat steamed bread) mg/100 g DW, vitamin B3 ranged from 1.53 (wheat steamed bread) to 5.76 (HW steamed bread) mg/100 g DW, and vitamin C ranged from 1.75 (wheat baked bread) to 87.87 (HW baked bread) mg/100 g DW. Therefore, potato flour addition, especially from Blue Congo and Hongmei potato cultivars, significantly increased vitamin B2 and vitamin C contents of steamed and baked breads. The average vitamin C

### Table 2. Mineral element composition and vitamin content of potato–wheat steamed and baked breads (mg/100 g DW).

|                  | Steamed bread | Baked bread | Average |
|------------------|--------------|-------------|---------|
|                  | Wheat AW SW BCW HW | Wheat AW SW BCW HW | Wheat breads Potato–wheat breads |
| Na               | 8.27 16.59 11.67 11.11 11.42 8.28 15.78 11.71 11.92 11.13 | 8.28 12.67 |
| Ca               | 31.21 48.97 56.23 52.73 59.29 24.21 47.43 46.82 48.72 61.63 | 27.71 52.73 |
| K                | 342.73 886.59 956.23 966.24 1150.00 368.44 956.23 737.11 959.11 991.60 | 355.59 954.14 |
| P                | 125.79 168.92 172.53 180.95 183.47 108.63 158.24 176.76 182.17 172.74 | 117.21 174.47 |
| Fe               | 1.29 2.54 2.15 3.58 2.65 2.38 2.23 2.38 3.12 3.23 | 1.84 2.74 |
| Zn               | 0.90 1.01 0.98 1.07 0.93 0.68 0.94 0.68 0.92 1.01 | 0.79 0.94 |
| Cu               | 0.12 0.11 0.17 0.16 0.14 0.09 0.09 0.14 0.13 0.14 | 0.11 0.14 |
| Mn               | 0.43 0.52 0.52 0.63 0.61 0.55 0.49 0.39 0.57 0.62 | 0.49 0.54 |
| Mg               | 33.29 49.23 57.54 53.51 60.89 25.03 48.52 47.63 49.72 62.54 | 29.16 53.70 |
| Vitamin B1       | 0.21 0.21 0.26 0.17 0.17 0.22 0.21 0.23 0.18 0.18 | 0.22 0.20 |
| Vitamin B2       | 0.09 0.11 0.12 0.30 0.19 0.11 0.15 0.17 0.26 0.28 | 0.10 0.20 |
| Vitamin B3       | 2.16 2.75 3.87 4.51 5.76 1.53 2.91 3.85 5.41 4.20 | 1.85 4.16 |
| Vitamin C        | 1.82 16.15 35.27 72.04 83.87 1.75 18.72 22.95 76.70 87.87 | 1.79 51.73 |

Note: AW: Atlantic potato flour–wheat flour; SW: Shepody potato flour–wheat flour; BCW: Blue Congo potato flour–wheat flour; HW: Hongmei potato flour–wheat flour.

Values followed by different superscript letters in each column indicate significant differences (p < .05).
content of HW steamed and baked breads was approximately 45-fold higher than that of wheat bread. Vitamin C is important in wound healing and in the prevention of scurvy. Additionally, vitamin C is an antioxidant that minimizes oxidative stress (Padayatty et al. 2003).

**Amino acid composition**

The amino acid composition of potato–wheat steamed and baked breads (mg/g protein) is shown in Table 3. Glutamic acid was the most predominant amino acid of wheat, potato–wheat steamed, and baked breads, which was confirmed by the results of Jiang et al. (2008), who observed that the main amino acid of wheat flour was glutamic acid. Lysine and threonine are the first and second most limiting amino acids, respectively, in wheat, barley, and rye (Shewry 2006). Potato flour addition increased lysine content, but it did not reach the FAO/WHO standard. Lysine score of limited amino acids (adult) was shown to be 37.45, 45.20, 45.94, 37.45, 45.14, and 45.94, respectively, in wheat, barley, and rye (Shewry 2008), which was confirmed by the results of Jiang et al. (2013). The AAS of steamed breads was higher than that of baked breads, probably because low steaming temperatures (100 °C) may retain more essential amino acids and nutrients (Zhu 2014).

**INQ**

INQ is a measure of the relationship between the amount of a nutrient in single foods, meals, and diets and the NRV. A food item with an INQ value of 2–6 is considered to be a good source of that specific nutrient, and a food item with an INQ value >6 is considered to be an excellent source of that particular nutrient (Venom 2013). In this study, potato–wheat steamed and baked breads were good sources of K, which is important for the maintenance of fluid and electrolyte balance in body cells. Insufficient intakes of K lead to hypokalemia, which contributes to life-threatening conditions such as cardiac arrhythmias and acute respiratory failure (Li et al. 2015). Potato–wheat steamed and baked breads from all potato cultivars with the exception of Atlantic were good sources of vitamin C (Table 4). The INQs revealed that vitamin B3, P, and dietary fiber of potato–wheat steamed and baked breads were significantly higher than those of wheat steamed and baked breads.

**TPC and antioxidant activity**

The TPC of potato–wheat steamed and baked breads is shown in Figure 1(a). HW steamed and baked breads had the highest TPC (4.99 and 4.89 mg CAE/g DW, respectively), whereas wheat steamed bread had the lowest TPC (1.58 mg CAE/g DW). There were significant differences (p < 0.05) in TPC between steamed and baked breads made with different potato cultivars, which was probably attributed to differences in polyphenol oxidase activity, genotype, storage conditions, and nutrient composition (Dale et al. 2003). Potato–wheat steamed and baked breads contain higher bioactive polyphenols than wheat steamed and baked breads.

### Table 3. Amino acid compositions of different potato–wheat steamed and baked breads (mg/g protein).

| Non-essential amino acids (NEAA) | Steamed bread | Baked bread | Steamed bread | Baked bread |
|----------------------------------|---------------|-------------|---------------|-------------|
| Total amino acids (TAA)          | Wheat | AW | SW | BCW | HW | Wheat | AW | SW | BCW | HW |
| Aspartic acid                   | 33.63f | 59.16f | 64.74c | 64.29f | 77.99a | 39.92 | 63.54c | 63.95c | 56.70g | 73.87b |
| Glutamic acid                   | 30.93f | 291.23f | 296.30d | 328.64f | 281.40g | 31.96b | 298.98c | 284.19d | 276.37b | 276.34f |
| Serine                           | 39.67f | 37.63a | 37.62a | 35.84f | 35.24f | 39.12c | 31.98c | 37.41f | 32.27f | 33.75f |
| Glycine                          | 33.91f | 31.12d | 32.50c | 32.43d | 29.95a | 32.30e | 34.97b | 30.99g | 28.32f | 29.32f |
| Arginine                         | 30.81f | 29.14c | 31.53d | 36.35a | 36.46a | 30.91f | 31.26c | 30.60g | 29.92f | 34.72f |
| Alanine                          | 30.51f | 36.59 | 42.25c | 41.92d | 39.15c | 41.07e | 47.20b | 37.01d | 39.84f | 39.36f |
| Essential and semi-essential amino acids (EAA and SEAA) | | | | | | | | | | |
| Threonine                        | 23.03f | 24.84d | 25.05c | 25.43 | 23.65 | 23.04f | 26.84e | 24.57f | 21.83f | 23.65c |
| Cysteine                         | 42.01f | 23.26 | 31.59c | 30.15 | 24.27f | 28.43b | 37.98c | 24.12f | 29.28f | 30.60f |
| Valine                           | 46.73f | 39.39 | 42.75a | 53.11 | 40.97f | 49.21d | 60.53e | 44.16 | 40.23f | 52.43f |
| Methionine                       | 11.81f | 9.95 | 11.16a | 20.34 | 8.23 | 14.34d | 19.76b | 13.59f | 10.88f | 16.35f |
| Isoleucine                       | 31.30f | 30.08a | 31.84b | 31.34 | 29.65 | 30.88f | 32.65e | 30.24 | 26.94f | 28.33c |
| Leucine                          | 61.26f | 57.20 | 60.23c | 57.14 | 53.69a | 60.63f | 60.82c | 56.45f | 47.46f | 51.12c |
| Tyrosine                         | 34.10f | 19.75 | 35.18c | 32.57 | 29.06f | 32.38b | 28.64e | 31.32f | 23.99f | 26.28c |
| Phenylalanine                    | 46.35f | 42.69 | 48.44a | 40.46 | 39.11f | 45.20b | 43.76c | 43.44f | 33.61f | 37.05c |
| Lysine                           | 20.60f | 24.86 | 24.54a | 26.10 | 25.22f | 18.38b | 24.83c | 25.27f | 20.90f | 22.30f |
| Histidine                        | 18.51f | 17.21 | 14.11 | 17.79 | 16.16 | 18.94e | 17.57e | 15.86f | 15.19b | 16.17e |
| Tryptophan                       | 7.68f | 7.51f | 7.38a | 7.74f | 6.95f | 6.88g | 5.97e | 6.08f | 6.08b | 7.55c |

**Note:** AW: Atlantic potato flour–wheat flour; SW: Shepody potato flour–wheat flour; BCW: Blue Congo potato flour–wheat flour; HW: Hongmei potato flour–wheat flour.

Values followed by different superscript letters in each column indicate significant differences (p < .05)
baked breads, which may have significant health-promoting and medicinal effects.

Antioxidant activity was determined by the ORAC method, and the results are shown in Figure 1(b). HW baked bread had the highest antioxidant activity (2385.01 μg TE/g DW), whereas wheat steamed bread had the lowest antioxidant activity (1542.48 μg TE/g DW), which was in agreement with the results of Yousif et al. (2012), who reported that sorghum addition increased the antioxidant capacity of flat bread. Moreover, the antioxidant activity of steamed bread was similar to that of baked bread for the same cultivar except for wheat and Hongmei breads. Furthermore, there was no significant difference in antioxidant activity between HW and BCW baked breads. However, TPC was significantly different between these two cultivars, suggesting that polyphenols from different potato cultivars might contain different phenolic constituents, and even if the phenolic constituents were similar, the proportions of different phenolic constituents and other antioxidants (vitamin C, flavonoids, and carotenoids) might be different (Al-Saikhan et al. 1995). In addition, these two potato cultivars might contain components with either synergistic or antagonistic effects on the antioxidant activity of polyphenols. There were significant differences in antioxidant activity between steamed and baked breads of different potato cultivars, which were probably attributed to different TPC, polyphenol types, and nutrient composition. The correlations between antioxidant activity and crude protein, crude fat, crude fiber, carbohydrate, and TPC are shown in Figure 1(c–g). The correlation coefficient between antioxidant activity and TPC ($R^2 = 0.8544$) was the highest, followed by the correlation between antioxidant activity and crude protein content ($R^2 = 0.4862$). There were negative correlation coefficients between antioxidant activity and crude fat, and carbohydrate contents. Therefore, polyphenols are considered to be the most important antioxidants in steamed and baked breads. Polyphenols are the most important natural antioxidants, which play significant roles in the organoleptic and nutritional qualities of cereals, tubers, fruits, and vegetables. Interestingly, there was a positive correlation between antioxidant activity and protein content. This result could be attributed to the antioxidant activity of proteins and polypeptides resulting from fermentation. Michalska et al. (2008) moreover reported that Maillard reaction products generated during cooking have antioxidant activity.

### In vitro starch digestibility and eGI

Potato–wheat and wheat steamed and baked breads were subjected to in vitro enzymatic digestion under controlled conditions to quantify the amount of RDS, SDS, and RS (Table 5). In a previous study, RDS was correlated with in vitro glycemic response and could be a proxy indicator of GI value (Englyst 2003). RDS varied from 17.65% (AW baked bread) to 27.61% (HW steamed bread). Steamed breads had higher RDS contents than baked breads of the same cultivar, indicating that processing condition had a marked effect on in vitro starch digestibility. This result could be attributed to higher specific volume (see Figure S1) and porosity of steamed breads, resulting in increased accessibility of amylases to starch granules, rendering starch more susceptible to hydrolysis. SDS ranged from 46.54% (BCW steamed bread) to 58.02% (wheat baked bread). SDS content was higher in baked breads than in steamed breads with the exception of Hongmei, possibly due to the rapid evaporation of water from the outermost region of dough in the presence of dry heat that resulted in incomplete gelatinization of starch granules (Primo-Martin et al. 2007). Digestibility decreased with decreasing gelatinization, because swelling and hydration decreases the chemical reactivity between starch granules and amylases (Parada & Aguilera 2011). The use of moist heat during steaming did not result in crust formation.
accounting for the significantly lower SDS content. SDS is slowly digested in the small intestine and induces a gradual increase of postprandial plasma glucose and insulin levels (Englyst & Hudson 1996). RS comprises the sum of intact starch and retrograded starch that passes into the large intestine. In this study, RS

Figure 1. (a) Total polyphenol content of potato steamed and baked breads. (b) Antioxidant activity of potato steamed and baked breads. (c) Correlation coefficient between crude protein content and antioxidant activity of potato steamed and baked breads. (d) Correlation coefficient between total polyphenol content and antioxidant activity of potato steamed and baked breads. (e) Correlation coefficient between crude fat content and antioxidant activity of potato steamed and baked breads. (f) Correlation coefficient between crude fiber content and antioxidant activity of potato steamed and baked breads. (g) Correlation coefficient between carbohydrate content and antioxidant activity of potato steamed and baked breads. Note: AW: Atlantic potato flour-wheat flour; SW: Shepody potato flour-wheat flour; BCW: Blue Congo potato flour-wheat flour; HW: Hongmei potato flour-wheat flour.
Table 5. *In vitro* starch digestibility and estimated glycemic index of different potato–wheat steamed and baked breads.

| Samples            | RDS (%) | SDS (%) | RS (%) | eGI     |
|--------------------|---------|---------|--------|---------|
| Steamed bread      |         |         |        |         |
| Wheat              | 25.14 ± 0.78b | 52.67 ± 0.09d | 70.22 ± 0.85a |         |
| AW                 | 24.03 ± 0.23c  | 50.28 ± 0.11f  | 67.36 ± 0.48b |         |
| SW                 | 24.39 ± 0.14c  | 55.87 ± 0.28g  | 65.73 ± 0.54a |         |
| BCW                | 22.23 ± 0.52a  | 46.34 ± 0.31h  | 61.78 ± 0.24d |         |
| HW                 | 27.61 ± 0.36a  | 49.04 ± 0.21i  | 65.35 ± 0.13f |         |
| Baked bread        |         |         |        |         |
| Wheat              | 23.03 ± 0.48d  | 58.02 ± 0.11j  | 70.62 ± 0.56a |         |
| AW                 | 17.65 ± 0.12h  | 51.24 ± 0.08e  | 63.95 ± 0.23a |         |
| SW                 | 17.68 ± 0.31h  | 46.63 ± 0.02h  | 64.24 ± 0.19g |         |
| BCW                | 19.87 ± 0.24f  | 50.10 ± 0.04f  | 63.11 ± 0.11g |         |
| HW                 | 18.75 ± 0.20g  | 52.97 ± 0.21i  | 61.20 ± 0.08f |         |

Note: AW: Atlantic potato flour–wheat flour; SW: Shepody potato flour–wheat flour; BCW: Blue Congo potato flour–wheat flour; HW: Hongmei potato flour–wheat flour; RDS: rapidly digestible starch; SDS: slowly digestible starch; RS: resistant starch; eGI: estimated glycemic index.

Values followed by different superscript letters in each column indicate significant differences (p < .05)

varied from 18.95% (wheat baked bread) to 35.68% (SW baked bread). RS content was higher in potato–wheat breads than in wheat breads with the exception of SW steamed bread, which could be attributed to differences in starch particle size. It has been reported that the α-amylase affinity for native starches is dependent on the particle size of starch, due to the enzyme feasibility for binding/adsorption (Tahir et al. 2010). The first step in enzymatic hydrolysis is enzyme binding and adsorption, which is limited due to the lower surface area of potato–wheat breads compared with that of wheat breads. It has reported that the particle size of potato starch is larger than that of wheat starch. As particle size increases, the surface area exposed to digestive enzymes decreases, leading to reduced digestion rates (Pi-Sunyer 2002).

The values of eGI ranged from 61.20 (HW baked bread) to 70.62 (wheat baked bread). eGI was lower in potato–wheat baked breads than in steamed breads of the same cultivar, which could be due to the fact that water loss in baked breads was more substantial than that in steam breads. Starch gelatinization of baked breads was limited because of their low moisture content (Roder et al. 2009). Additionally, the baking process resulted in a compacted bread crust, which limited hydrolysis by α-amylase and affected eGI (Fardet et al. 2006). Furthermore, eGI was lower in potato–wheat breads than in wheat breads, possibly due to the lower specific volume, larger starch particle size, higher dietary fiber content, and higher total polyphenol content of potato–wheat breads, which decreased eGI (Pi-Sunyer 2002; Roder et al. 2009; Sui et al. 2016). Moreover, eGIs of HW breads were lower than those of AW and SW breads, probably due to differences in TPC content, which was in accordance with the results of Goh et al. (2015), who reported that green tea extract reduced the glyemic potential of both steamed and baked breads. Similarly, Sui et al. (2016) observed that anthocyanin addition reduced the digestion rate of wheat bread. Therefore, the lower eGI of potato–wheat mixed breads (especially HW and BCW breads) compared with that of wheat breads is more suitable for the prevention of obesity, diabetes, and cardiovascular diseases.

**Comprehensive nutritional value**

The content of one specific nutrient is not indicative of overall quality. Therefore, it is important to perform a comprehensive nutritional analysis. In this study, gray relational analysis was performed to assess the comprehensive nutritional value of 10 different steamed and baked breads (Table S2). The results revealed that potato varieties significantly affected nutritional values (Table S3). In decreasing order of gray relational grade values, the breads were HW steamed bread (0.8625) > BCW steamed bread (0.8585) > HW baked bread (0.8465) > BCW baked bread (0.8412) > SW steamed bread (0.7846) > SW baked bread (0.7433) > AW steamed bread (0.7278) > AW baked bread (0.7251) > wheat steamed bread (0.6483) > wheat baked bread (0.6301). In general, the gray relational grade value of steamed breads was higher than that of baked breads of the same cultivar. The bread-making temperature of baked breads (160°C) was higher than that of steamed breads (100°C), which resulted in the reduction of essential amino acids, vitamins, and total polyphenols. Moreover, the comprehensive nutritional value of Hongmei and Blue Congo was higher than that of Shepody and Atlantic. Hongmei and Blue Congo contain more nutritional and functional components, especially polyphenols, which have antioxidant, anticancer, and anti-hypertensive effects. Compared with wheat bread, potato–wheat bread had a higher comprehensive nutritional value. Based on comprehensive nutritional values, Hongmei was the optimum potato...
cultivar, followed by Blue Congo, Shepody, and Atlantic.

Conclusions
The findings revealed that potato cultivar had significant effects on the nutritional properties of potato–wheat steamed and baked breads. Potato–wheat steamed and baked breads had higher dietary fiber, minerals, vitamins, essential AAS, and antioxidative activity than wheat steamed and baked breads. The INQ results revealed that potato–wheat steamed and baked breads were good sources of K and vitamin C. Gray relational analysis showed that potato flour addition increased the comprehensive nutritional value of steamed and baked breads. For the same cultivar, the comprehensive nutritional value of steamed breads was higher than that of baked breads. Moreover, Hongmei was the optimal potato cultivar for bread making, followed by Blue Congo, Shepody, and Atlantic. In conclusion, potato flour, which contains several nutrients and bioactive compounds, could be added into steamed and baked breads in an attempt to reduce malnutrition.

Disclosure statement
The authors have no conflicts of interest to declare.

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