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Protein characteristics of Chinese black-grained wheat

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Abstract

Protein properties of black-grained wheat (BGW) were compared with those of five carefully selected wheat controls (Taifen 1, Klasic, Yecora Rojo, Glenlea and Anza) in order to find potential uses for BGW. Protein content, mixing properties, gluten index and amino acid composition were measured. BGW whole meal had a higher protein content (17.71%) than was found in controls. Gluten index of BGW flour (69.74) was generally low compared to controls. Mid-line peak times determined using mixograph were significantly longer ($p < 0.05$) for most controls (5.41–6.27 min) in comparison to BGW flour (<3.00 min). Dough stickiness (223.76 g) of BGW was somewhat stronger than that of Klasic and CES flours. Total essential amino acid and total amino acid contents in whole meal were 4.45% and 15.74%, respectively, for BGW. The amino acid composition was relatively stable after high-temperature drying of wet BGW gluten. In vitro protein digestibility of BGW wheat meal was the lowest.

Keywords: Wheat; Black wheat; Protein; Digestibility; Gluten index; Mixing properties; Amino acid composition; Electrophoresis

1. Introduction

Black-coloured foods have a special place in Chinese food culture and enjoy wide acceptance in the marketplace. Many studies on black-seeded cereals have proven them to be associated with health and improved nutrition, and therefore form the basis for high value products, for instance, popular food products from black-grained rice and black-grained soybean (Lai, 1995; Lai & Zhang, 1995). In fact, since the 1970s the Wheat Biotechnology Laboratory of the Institute of Crop Genetics, Shanxi Academy of Agricultural Science, has been engaged in research leading to the development of black-grained wheat from previously existing blue and purple lines (Sun et al., 1996, 1999). After over 20 years effort a new black-grained wheat variety (BGW) has been developed and it is now available for utilization as a new raw food material for value-added products (Bai et al., 2000, 2002; Li, Sun, & Ren, 2004; Yang, Li, Chu, & Sun, 2001). Elemental Se content of BGW was high up to 1.04 mg/kg in comparison with 0.26 mg/kg of common wheat (Bai et al., 2000). Seed colour of BGW is visually black and the grain size is comparable to that of the controls chosen in the current investigation. The colour of wheat, usually white or red (although purple is known), is related to pigments in the seed coat. Basic wheat pigments include carotenes, xanthophylls and phenolic compounds (Beta, Nam, Dexter, & Sapirstein, 2005; Kruger & Reed, 1988). The main pigment component of BGW seed was an anthocyanin phenolic compound (Sun, Sun, & Wang, 2004). Anthocyanins are known to exhibit good antioxidant activity

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(Awika, Rooney, & Waniska, 2004). While there are several chemical components in wheat, traditional nutrients of major importance include starch and proteins. Among cereals, only wheat has the ability to form a strong, cohesive dough due to the uniqueness of its proteins.

Evaluation of protein properties includes determination of amino acid composition, molecular weights, protein digestibility, and gluten strength. Protein digestibility is essentially a measure of the rate of in vitro protein hydrolysis by digestive enzymes. It is also a factor most likely to affect amino acid availability since proteolysis is influenced both by the linear amino acid sequence and the tertiary structure of a protein (Gopal, Monteiro, Virupaksha, & Ramachandra, 1988). Gluten properties are associated with the end use of wheat flour. The total gluten content and composition in the wheat flour protein is of interest in the nutritional evaluation of the wheat accessions (Abdel-Aal, Hucl, & Sosulski, 1995). The gluten index is used as a measure of gluten strength. The objective of the study was to determine the protein properties of BGW and compare them to five carefully selected wheat controls. The results will be used to identify potential uses for BGW as raw material for food production.

2. Materials and methods

2.1. Materials

The samples used for the study comprised one black-grained wheat and five carefully selected commercial wheat reference samples used in bread and noodle production. Chinese black-grained wheat (BGW) and one commercial reference Taifen 1 wheat (TW) samples were obtained from Institute of Crop Genetics, Shanxi Academy of Agricultural Science, Taiyuan, China. Three commercial US cultivars, Anza wheat (AW, California), Klasic wheat (KW, California) and Yecora Rojo wheat (YRW, California), were supplied by the University of California, Davis. One commercial Canadian extra strong wheat Glenlea (GW, Manitoba) sample was obtained from Canada. All reference wheat samples with the exception of TW and AW are used for bread-making. TW and AW are used for noodle production.

Wheat flour was obtained by milling grain with a Quadrumat Junior laboratory mill (Brabender OHG, Duisberg, Germany). After separating wheat bran, wheat flour extraction rate ranged from 70% to 80%. Wheat whole meal was prepared by milling wheat grain with a Cyclone sample mill (Udy Corp., Fort Collins, Colorado, USA). Wheat whole meal included flour and bran.

Freeze-dried (FD) gluten was obtained by hand-washing the flour dough according to the method of Qiu (1998). Wet gluten was immediately frozen in liquid nitrogen and freeze-dried. Main steps during hand-washing were: first making 100 g flour to dough by adding adequate water (25–35 mL depending on the flour), resting the dough in a covered container for 2 h, and finally washing dough in 2000 mL water for 15 min at room temperature to remove starch. The washings were repeated three times.

Wet gluten yield and gluten index were determined by the machine washing Method 38-12 of the AACC (1995). Preparation of wet gluten was according to the method of Perten (1990). Briefly 10 g flour was mixed for 20 s with 4.8 mL of 2% NaCl solution, followed by washing for 5 min with 2% NaCl solution at a flow rate of 50–60 mL/min on a special 88-μm sieve using a Perten Glutomatic Gluten Index machine (Perten Instruments AB, S-141 05 Huddinge, Sweden). Afterwards, the wet gluten piece was centrifuged at 6000 rpm for 1 min on a special 600-μm metallic sieve using a Perten Centrifuge 2015 machine (Perten Instruments AB, S-141 05 Huddinge, Sweden). Wet gluten samples obtained from both sides of the sieve after centrifugation were dried at 150 °C (high temperature drying) using a special Perten Glutork 2020 dryer. The gluten that remained on top of the sieve after centrifugation was labeled as high temperature-dried (HTD) gluten 1. The gluten that passed through the sieve was labeled as HTD gluten 2. Total protein content of the above samples was analyzed by the AACC Method 46-11A (1995).

2.2. In vitro protein digestibility

Pepsin (Pepsin porcine gastric mucosa, 800–2500 units/mg protein, Sigma Chemical Co., St. Louis, USA) and trypsin (Trypsin from bovine pancreas, $\geqslant$10,000 BAEE units/mg protein, Sigma Chemical Co., St. Louis, USA) were used for in vitro protein digestibility (IVPD) studies. IVPD was determined by an improved method of Ramachandra, Virupaksha, and Shadaksharaswamy (1977) and Gopal et al. (1988). For pepsin, 50 mg of whole meal or dry gluten samples were weighed into a series of test tubes and 5.0 mL of 0.075 N HCl and 0.5 mL of pepsin solution (2.0 mg/mL) in 0.075 N HCl were added to each tube. The tubes were incubated at 37 °C and enzyme action was stopped at 30, 60 min and 24 h by addition of 5 mL of 10% (w/v) trichloroacetic acid (TCA). The reaction mixture was filtered through Whatman No. 1 filter paper, and the residue on the filter was washed with warm water. Nitrogen in the residue was estimated by the micro-Kjeldahl procedure (AACC Method 46-11A, 1995). For trypsin, the same IVPD procedure was conducted essentially as described for pepsin, except that incubation was in 0.1 M phosphate buffer, pH 7.6. IVPD was obtained by calculating the

difference between the amount of total nitrogen in the sample before and after in vitro digestion with pepsin or trypsin. Kjeldahl nitrogen was multiplied by the factor 5.7 to obtain total protein.

2.3. Amino acid analysis

Amino acid composition of flour, whole meal and gluten samples of black-grained wheat and the four wheat references was determined using a Hitachi Amino Acid Analyser, Model Hitachi 835-50 (Tokyo, Japan). Preparation of hydrolyzate was according to the method of Anjuma, Ahmada, Butta, Sheikhb, and Pasha (2005). Sample (0.1 g) was hydrolyzed with 10 mL of 6 N HCl at 110 °C for 22 h. The hydrolyzate was evaporated under vacuum at 60 °C to remove HCl. Then the hydrolyzate was dissolved in 5 mL of 0.02 N HCl, centrifuged at 1000 rpm and filtered to remove the visible sediments. The supernatant (20 μL) was injected into the amino acid analyzer for the determination of the amino acid composition of each sample. The amino acids were separated on a cation exchanger resin column (150 mm × 2.6 mm i.d., No. 2619 resin) using sodium citric acid buffer at pH 2.2, a column temperature of 53 °C, a flow rate of 0.225 mL min^{-1} and a postcolumn reaction with ninhydrin (0.3 mL min^{-1} ninhydrin flow rate) followed by a photometric detection at 570 nm according to the procedure of Llames and Fontaine (1994). Amino acid standard (AAS18, Sigma Chemical Co., St. Louis, USA) was used for calibration. Tryptophan was not determined. Sample results were expressed as percentage amino acid composition on dry weight basis.

2.4. Dough stickiness determination

To measure dough stickiness, three doughs were prepared from individual flours and the mean taken as the stickiness value. A dough prepared from flour (2 g, 14% moisture basis) and measured water was mixed to its optimum in a 2g mixograph instrument (TMCO, Lincoln, NE) and transferred into the Stickiness Cell (SMS/KSU, Haslemere, England). Dough stickiness data was determined using a TA-XT2 texture analyser (Stable Micro Systems, Godalming, UK) following the procedures described by Chen and Hoseney (1995). Parameter selection of texture analyzer was as follows: No. 3 TA-XT2 library program, 40 g compression force, plexiglass probe of 25 mm diameter, 5 g trigger force, probe compression travel speed of 2 mm/s, probe reversing speed of 10 mm/s, holding time of 0.1 s, and probe travel distance of 4 mm depth. Four determinations were performed per dough. The stickiness readings were averaged as dough stickiness.

2.5. Electrophoresis

Laemmli's (1970) discontinuous high resolution sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) system, as modified by Fullington, Cole, and Kasarda (1983) for wheat seed storage proteins, was used to extract and fractionate total proteins in 10% (w/v) polyacrylamide gels. Electrophoresis was overnight at 8 mA constant current until the tracking dye, pyronin Y had reached the bottom of the gel. Molecular weight markers (205, 116, 66 and 39.8 kDa) were purchased from Sigma (F3526, Sigma Chemical Co., St. Louis, USA).

2.6. Mixograph analysis

Mixograph characteristics were determined using a National 2g Mixograph Instrument (TMCO, Lincoln, NE) following the procedures described by Rath, Gras, Wrigley, and Walker (1990) and in the AACC Method 54-40A (1995).

2.7. Statistical analysis

All samples were analyzed at least in duplicate determinations and the results were presented as averages. Data were subjected to analysis of variance (ANOVA). Means were separated using Fisher's protected least significant difference (LSD) test at $p = 0.05$. Quantitative results were expressed on a dry weight basis (dwb), unless stated.

3. Results and discussion

3.1. Gluten index and gluten content

A high gluten index value indicated that limited wet gluten could pass through the special gluten index sieve after centrifugation. Taifen 1 wheat had the lowest gluten index value in five wheat cultivars (Table 1). Gluten index of Chinese black-grained wheat (BGW) was 69.74, a significantly higher value ($p < 0.05$) than that of Taifen 1 (TW), but significantly lower ($p < 0.05$) than that of Klasic (KW), Yecora Rojo (YRW) and Glenlea wheat (GW). Low gluten index value also showed poor strength of wet gluten dough as observed in BGW flour in comparison to that of KW, YRW and GW flours. For breadmaking, the optimum gluten index range is between 60 and 90 (Perten, 1990). The results were consistent with our earlier experiment on SDS sedimentation values (SDS-SV) of their whole meals. The order of their SDS-sedimentation test results was as follows: GW 16.9 mL/g > KW 16.5 mL/g > YRW 15.0 mL/g > BGW 13.3 mL/g > TW 9.9 mL/g (Li, Corke, & Sun, 1998). SDS-sedimentation value is often used to screen

Table 1
Gluten index, wet gluten and dry gluten content of wheat flours

	GI%	WG% (14%mb)	DG% (dwb)
BGW-flour	69.74b	41.96b	17.15b
TW-flour	50.09c	54.20a	23.35a
KW-flour	98.66a	29.45dc	11.93c
YRW-flour	98.88a	26.82d	10.84c
GW-flour	99.37a	31.23c	12.66c
LSD	8.9814	7.5264	3.6354

GI, gluten index; WG, wet gluten; DG, dry gluten; LSD, least significance difference at $p < 0.05$ level of probability. Mean values for flour samples having similar letters in the same column are not significantly different.

for gluten strength in wheat cultivars and has a positive correlation with gluten strength (Dick & Quick, 1983). Statistical analysis showed that there was a positive correlation coefficient of about 0.9606 between gluten index and SDS-sedimentation value (Li et al., 1998). Yields (%) of wet and dry gluten in BGW flour were 41.96% and 17.15% respectively, significantly lower ($p < 0.05$) than that of TW flour, but higher ($p < 0.05$) in comparison to that of GW, KW and YRW flours (Table 1).

3.2. In vitro protein digestibility

IVPD results of five wheat flours or their FD gluten using pepsin and trypsin are shown in Table 2. IVPD was increased with increase in incubation time. With pepsin, IVPD of BGW whole meal was the lowest at each incubation time compared to the other four whole meals. At 30 and 60 min, BGW whole meal had significantly lower IVPD ($p < 0.05$) than the rest of the wheat whole meal. At 24 h, only KW whole meal had significantly higher IVPD ($p < 0.05$) than BGW and other whole meal.

IVPD of BGW FD gluten was significantly the highest ($p < 0.05$) at 30 min and 24 h incubation time (71.5% at 30 min, 96.4% at 24 h). There were significant differences ($p < 0.05$) in IVPD of wheat FD gluten among some wheat cultivars at 30 and 60 min, with the highest variation of 22.0% observed between BGW and GW FD gluten at 30 min. But there were no significant differences ($p < 0.05$) in IVPD of wheat FD gluten among wheat cultivars at 24 h.

In the case of trypsin, most of the digestion took place in 60 min for wheat whole meal. Further incubation up to 24 h resulted only in a slight increase in hydrolysis. Differences in IVPD among wheat cultivars were not significant after 1–24-h periods of incubation. IVPD of BGW after 30 min incubation was significantly different ($p < 0.05$) from YRW and GW whole meal. However, incubation periods longer than 30 min resulted in similar IVPD among the whole meals. Results obtained for the IVPD of FD gluten showed significant differences ($p < 0.05$) among wheat cultivars. At 30 min incubation, IVPD of BGW FD gluten was significantly higher ($p < 0.05$) than all the glutens with the exception of YRW gluten. At 60 min and 24 h incubation, IVPD for BGW FD gluten was significantly higher ($p < 0.05$) compared to the four wheat gluten controls.

3.3. Amino acid compositions

Amino acid values and protein contents for black-grained wheat and its controls are given in Table 3 (a–d). Generally similar levels of each type of amino acid were observed in BGW whole meal and its flour when compared to the four wheat controls. However, Val and Glu were slightly higher in BGW whole meal. A significant correlation between grain protein percentage and amino acid values has been reported (Acouistucci, Degidio, & Vallega, 1995). A positive correlation between protein content and their total amino acid was also found. Total essential amino acid (TEAA) and total amino acid (TAA) contents were 4.45% and 15.74%,

Table 2
In vitro protein digestibility (IVPD): comparison of wheat whole meals and their FD gluten

Items	IVPD (%) for pepsin			IVPD (%) for trypsin		
	30 (min)	60 (min)	24 (h)	30 (min)	60 (min)	24 (h)
BGW-whole meal	64.72b	67.65b	82.42b	60.16a	66.42a	66.87a
TW-whole meal	66.90a	71.07a	82.94b	60.70a	64.93a	66.62a
KW-whole meal	66.16a	71.11a	87.23a	59.86ba	63.31a	67.15a
YRW-whole meal	69.50a	71.22a	84.43b	57.81b	66.60a	67.84a
GW-whole meal	67.75a	69.59ab	84.04b	58.04b	64.10a	65.93a
LSD	3.4208	2.8920	2.5518	2.0649	3.7229	3.4873
BGW-FD-gluten	71.52a	78.15b	96.45a	24.99b	45.30a	58.58a
TW-FD-gluten	51.50d	59.80d	95.07a	6.71d	7.12e	53.69b
KW-FD-gluten	66.75b	81.17a	95.42a	17.43c	17.78d	36.55e
YRW-FD-gluten	64.01c	62.53c	95.37a	35.22a	39.40b	44.28d
GW-FD-gluten	49.47d	61.90dc	94.62a	15.73c	20.55c	47.02c
LSD	2.1415	2.516	2.1989	2.0913	2.3542	2.27217

LSD, least significance difference at $p < 0.05$ level of probability. Mean values for meal or gluten samples having similar letters in the same column are not significantly different.

Table 3a
Amino acid comparison of whole meals (WM) (%, dwb)

Items	BGW-WM	TW-WM	KW-WM	YRW-WM	GW-WM	LSD
ASP (%)	0.70a	0.67a	0.52a	0.51a	0.55a	0.2135
THR[a] (%)	0.39a	0.32a	0.28a	0.28a	0.31a	0.1382
SER (%)	0.67a	0.58a	0.51a	0.51a	0.55a	0.1841
GLU (%)	6.16a	5.67ba	4.78b	4.70b	4.85b	0.9851
GLY (%)	0.61a	0.56a	0.49a	0.49a	0.50a	0.1526
ALA (%)	0.52a	0.48a	0.39a	0.39a	0.42a	0.1369
CYS (%)	0.13a	0.07a	0.09a	0.09a	0.11a	0.0841
VAL[a] (%)	0.68a	0.60ba	0.49b	0.49b	0.52b	0.1491
MET (%)	0.19a	0.11ba	0.12ba	0.06b	0.14ba	0.1036
ILE[a] (%)	0.49a	0.43a	0.34a	0.35a	0.36a	0.1572
LEU[a] (%)	1.01a	0.99a	0.81a	0.81a	0.84a	0.5021
TYR[a] (%)	0.37a	0.17a	0.29a	0.31a	0.28a	0.2613
PHE[a] (%)	0.70a	0.63a	0.53a	0.53a	0.54a	0.3382
LYS[a] (%)	0.40a	0.36a	0.30a	0.30a	0.32a	0.2085
HIS[a] (%)	0.32a	0.30a	0.24a	0.24a	0.25a	0.1066
ARG (%)	0.77a	0.54a	0.56a	0.58a	0.59a	0.2692
PRO (%)	1.55a	1.44a	1.19a	1.18a	1.18a	0.4381
TEAAs[b] (%)	4.45a	3.80ba	3.28b	3.31b	3.42b	0.9014
TAAs[c] (%)	15.74a	13.91b	11.94c	11.81c	12.28c	1.4721
Protein (%)	17.71a	17.00a	14.07b	13.67b	14.52b	1.6035

LSD, least significance difference at $p < 0.05$ level of probability. Mean values for amino acid or protein content having similar letters in the same row are not significantly different.
[a] Essential amino acid.
[b] Total essential amino acid.
[c] Total amino acid.

respectively, for BGW whole meal. The TEAA and TAA of BGW whole meal were significantly higher ($p < 0.05$) than that of TW, KW, YRW and GW. BGW flour contained 4.23% of TEAA and 15.54% of TAA (Table 4). The TEAA of BGW flour was 21.55%, 36.45%, 54.38% and 29.75% higher than that of TW, KW, YRW and GW, respectively. Similarly the TAA of BGW flour was 18.35%, 33.62%, 52.80% and 28.43% higher than that of TW, KW, YRW and GW, respectively.

Table 3b
Amino acid comparison of flours (%, dwb)

Items	BGW-flour	TW-flour	KW-flour	YRW-flour	GW-flour	LSD
ASP (%)	0.55a	0.47a	0.43a	0.34a	0.44a	0.2514
THR[a] (%)	0.36a	0.28a	0.26a	0.22a	0.28a	0.1836
SER (%)	0.65a	0.54a	0.49a	0.42a	0.52a	0.2671
GLU (%)	6.72a	5.87ba	5.08b	4.55cb	5.21b	1.1323
GLY (%)	0.50a	0.41a	0.41a	0.33a	0.43a	0.1826
ALA (%)	0.41a	0.32a	0.30a	0.25a	0.33a	0.1725
CYS (%)	0.16a	0.11a	0.08a	0.06a	0.11a	0.1051
VAL[a] (%)	0.63a	0.51a	0.46a	0.40a	0.48a	0.2471
MET (%)	0.19a	0.14a	0.14a	0.11a	0.15a	0.1024
ILE[a] (%)	0.49a	0.40a	0.33a	0.29a	0.36a	0.2252
LEU[a] (%)	1.07a	0.88ba	0.76ba	0.69b	0.81ba	0.3456
TYR[a] (%)	0.39a	0.33a	0.30a	0.26a	0.32a	0.1458
PHE[a] (%)	0.70a	0.60a	0.53a	0.48a	0.54a	0.2581
LYS[a] (%)	0.29a	0.24a	0.24a	0.20a	0.25a	0.1106
HIS[a] (%)	0.30a	0.24a	0.22a	0.20a	0.22a	0.1257
ARG (%)	0.64a	0.49a	0.48a	0.39a	0.49a	0.2891
PRO (%)	1.52a	1.32a	1.11a	0.99b	1.17a	0.4713
TEAAs[b] (%)	4.23a	3.48b	3.10b	2.74b	3.26b	0.7016
TAAs[c] (%)	15.54a	13.13ba	11.63b	10.17c	12.10b	2.1359
Protein (%)	18.26a	15.94ba	13.76b	12.59c	14.23b	2.6801

LSD, least significance difference at $p < 0.05$ level of probability. Mean values for amino acid or protein content having similar letters in the same row are not significantly different.
[a] Essential amino acid.
[b] Total essential amino acid.
[c] Total amino acid.

Table 3c
Amino acid comparison of freeze-dried gluten (FDG) (%, dwb)

Items	BGW-FDG	TW-FDG	KW-FDG	YRW-FDG	GW-FDG	LSD
ASP (%)	2.51a	2.43a	2.07a	2.38a	2.44a	0.5013
THR[a] (%)	1.91a	1.78a	1.72a	1.87a	1.91a	0.3152
SER (%)	3.70a	3.58a	3.40a	3.72a	3.83a	0.6051
GLU (%)	37.01a	36.92a	33.18b	37.10a	37.73a	3.4018
GLY (%)	2.65a	2.51a	2.47a	2.82a	2.50a	0.5043
ALA (%)	2.00a	1.93a	1.77a	1.96a	1.89a	0.3815
CYS (%)	1.15a	1.10a	1.01a	1.01a	1.20a	0.2306
VAL[a] (%)	2.97a	2.90a	2.57a	2.84a	2.95a	0.5611
MET (%)	1.00a	1.02a	0.95a	1.02a	1.10a	0.3029
ILE[a] (%)	2.68a	2.61a	2.28a	2.53a	2.60a	0.5851
LEU[a] (%)	5.64a	5.57a	4.94a	5.47a	5.50a	1.5025
TYR[a] (%)	2.08a	2.30a	2.07a	2.34a	2.42a	0.4705
PHE[a] (%)	3.91a	3.78a	3.26a	3.82a	3.98a	0.8012
LYS[a] (%)	1.35a	1.26a	1.08a	1.30a	1.22a	0.3476
HIS[a] (%)	1.40a	1.39a	1.21a	1.40a	1.41a	0.3239
ARG (%)	2.98a	2.80a	2.69a	3.03a	3.10a	0.5317
PRO (%)	9.32a	9.24a	7.86b	9.04ba	9.26a	1.3602
TEAAs[b] (%)	21.94a	21.59a	19.13b	21.57a	21.99a	2.3058
TAAs[c] (%)	84.28a	83.12a	74.53b	83.66a	85.04a	4.5605
Protein (%)	86.44a	84.69a	77.94b	86.31a	86.89a	4.2062

LSD, least significance difference at $p < 0.05$ level of probability. Mean values for amino acid or protein content having similar letters in the same row are not significantly different.
[a] Essential amino acid.
[b] Total essential amino acid.
[c] Total amino acid.

Amino acid composition of FD and HTD gluten was also compared (Tables 3c and 3d). HTD gluten was the sample obtained by drying with Perten Glutork 2020 dryer at 150 °C. The amino acid composition between FD and HTD glutens was useful in understanding the effect of temperature on gluten quality. The TEAA, TAA and protein contents ranged from 19.93% to 21.99%, 74.53% to 85.04% and 77.94% to 86.89%, respectively, for the five FD gluten samples. High temperature drying of gluten resulted in significant

Table 3d
Amino acid comparison of high temperature dried gluten (HTDG) (%, dwb)

Items	BGW-HTDG 1	BGW-HTDG 2	TW-HTDG 1	TW-HTDG 2	KW-HTDG	YRW-HTDG	GW-HTDG	LSD
ASP (%)	2.29a	2.24a	1.59b	1.43c	0.91dc	2.22a	2.03ba	0.5516
THR[a] (%)	1.93a	1.88a	1.22b	1.08b	0.78b	1.95a	1.90a	0.5425
SER (%)	3.83a	3.74a	2.60b	2.31b	1.75b	4.13a	4.09a	0.8613
GLU (%)	38.64a	37.46a	26.42a	23.68a	18.60b	41.11a	38.95a	3.0572
GLY (%)	2.30a	2.24a	1.61b	1.45b	0.29c	2.41a	2.39a	0.5081
ALA (%)	1.89a	1.85a	1.34b	1.22b	0.86c	1.73a	1.73a	0.3503
CYS (%)	1.03a	1.10a	0.71b	0.64b	0.44cb	0.86ba	0.79ba	0.3133
VAL[a] (%)	3.01a	2.91a	2.07b	1.88cb	1.36c	2.77a	2.65a	0.5601
MET (%)	1.17a	1.13a	0.74b	0.65b	0.21c	1.13a	1.10a	0.2813
ILE[a] (%)	2.70a	2.61a	1.86b	1.68b	1.22c	2.46a	2.37a	0.4561
LEU[a] (%)	5.68a	5.52a	3.92b	3.56cb	2.61c	5.55a	5.29a	1.0581
TYR[a] (%)	2.38a	2.29a	1.64b	1.50b	1.04c	2.26a	2.17a	0.4501
PHE[a] (%)	3.98a	3.87a	2.78bc	2.50bc	1.93c	4.32a	3.82a	0.8625
LYS[a] (%)	1.02a	1.00a	0.72cb	0.63cb	0.48c	1.09a	0.97a	0.2458
HIS[a] (%)	1.43a	1.37a	0.97b	0.87cb	0.66c	1.43a	1.29a	0.2281
ARG (%)	2.90a	2.82a	1.91b	1.72cb	1.25c	2.90a	2.80a	0.6012
PRO (%)	9.82a	9.44a	6.56b	5.95b	4.43c	10.38a	9.95a	1.5305
TEAAs[b] (%)	22.13a	21.45a	15.18b	13.70b	10.08c	21.83a	20.46a	2.8135
TAAs[c] (%)	86.00a	83.47a	58.65b	52.75c	38.82d	88.72a	84.29a	5.0126
Protein (%)	88.47a	88.47a	85.32a	85.32a	78.25b	90.81a	86.73a	5.8206

LSD, least significance difference at $p < 0.05$ level of probability. Mean values for amino acid or protein content having similar letters in the same row are not significantly different. (*Note*. KW, YRW, GW yielded only gluten 1).
[a] Essential amino acid.
[b] Total essential amino acid.
[c] Total amino acid.

decreases ($p < 0.05$) in amino acid content of some cultivars. For example, TAA content of KW HTD gluten was only 38.82% in comparison with 74.53% of KW FD gluten. Since the level of protein in the gluten dough for KW was also low when compared to other cultivars (Tables 3c and 3d), this indicated the presence of non-protein components. At 150 °C it may be possible that protein got bound or reacted with these non-protein components and did not get fully hydrolyzed under the conditions provided for hydrolysis to release amino acids (6 N HCl, 110 °C, 22 h). New high molecular weight components were formed after preheating whey protein isolate at 120 °C for 3 h, and β-lactoglobulin was more sensitive to preheating than α-lactalbumin, and preheating also resulted in the conformational changes of proteins of whey protein isolate (Fujino et al., 1995). After high temperature drying, the order of TAA content was as follows: YRW HTD gluten > BGW HTD gluten 1 > GW HTD gluten > TW HTD gluten 1 > KW HTD gluten (Table 3d). The TEAA and TAA content of HTD gluten 2 was similar or slightly lower in comparison with its HTD gluten 1 counterpart, however, the TAA content between TW HTD gluten 1 and TW HTD gluten 2 was significant ($p < 0.05$). The results also indicated that high temperature drying significantly ($p < 0.05$) reduced the TEAA and TAA amino acid contents of wet gluten dough for TW and KW (Tables 3c and 3d).

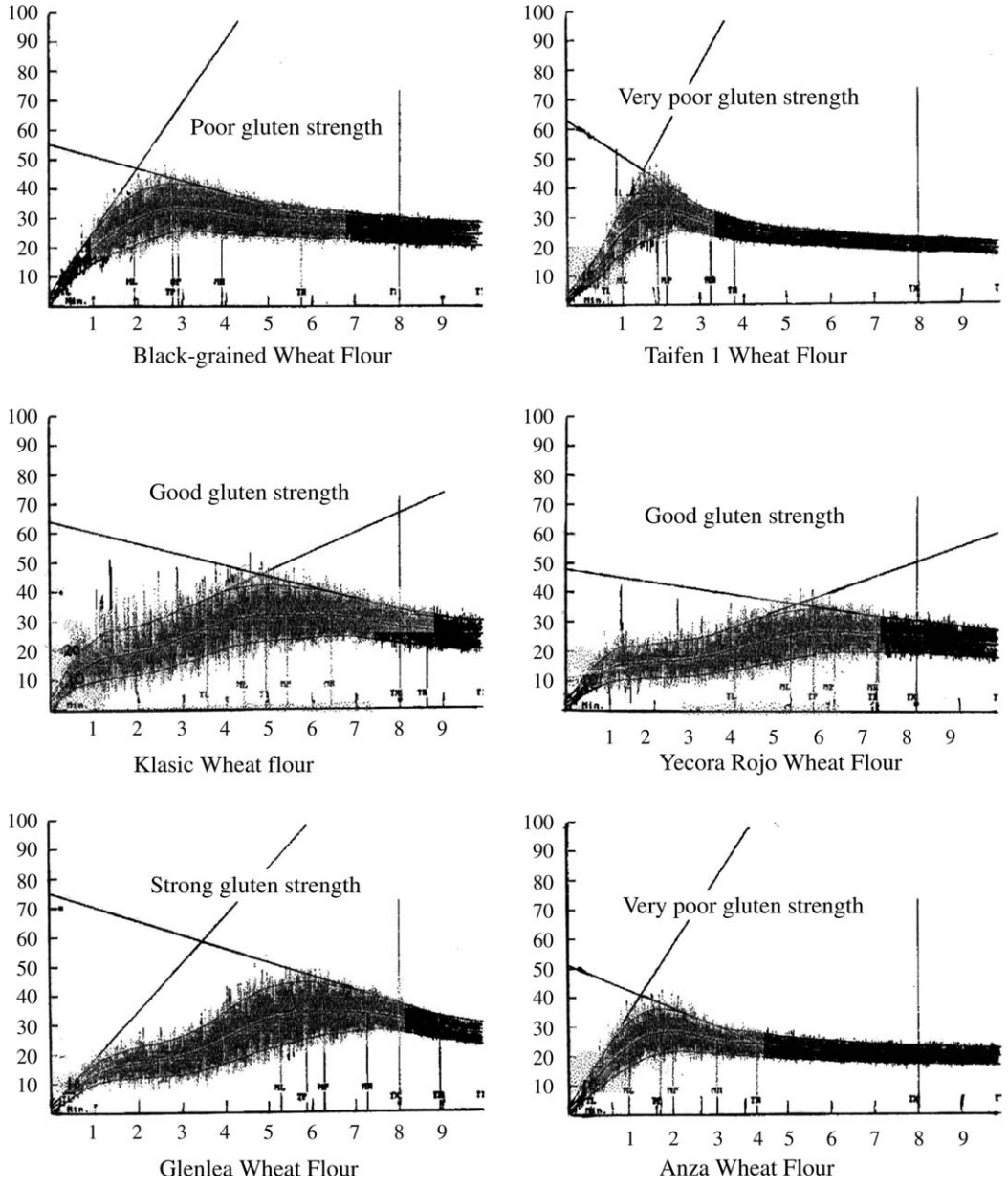

Fig. 1. Mixograph curves of Chinese black-grained wheat flour compared to its controls in water.

3.4. Mixograph characteristics

The mixograph results for black-grained wheat flour and its controls are summarized in Fig. 1 and Table 4. Difference in mixograph curves also indicated good or poor gluten strength of different wheat cultivars (Fig. 1). Gluten 'strength' could be estimated from mixograph curve and some of the mixograph parameters. Of the mixograph parameters, midline peak time (MPT) and integral at MPT (MPTI) showed the best correlation with gluten strength. Height value at MPT (MPTH) showed the best relationship with baking performance of gluten and with loaf volume for unfractionated and reconstituted flours. Khatkar and Schofield (1996) indicated that MPTH (also known as peak dough resistance) was the most useful mixograph parameters for assessing gluten bread making quality, and the 2g mixograph proved to be a simple and rapid instrument for studying mixing properties and for evaluating the baking potential of gluten. Weak gluten developed quickly and needed a shorter time to mix to MPTH. A longer time period to mix to MPTH indicated better gluten strength in the wheat flour. A longer duration was also consistent with its relatively high MPTI value. In water, gluten strength for BGW flour (MPT 2.93 min) was weak when compared to KW, YRW and GW flours (MPT > 5.00 min). Salt and sucrose had significant effects ($p < 0.05$) on gluten strength of BGW flour and the controls (Table 7). He, Roach, and Hoseney (1992) reported that neutral salts could change the hydrophobic interaction among gluten proteins and that the gluten proteins from poor-quality flour were less hydrophobic than those from the good-quality flour. Our results indicated that the effect of salt on gluten was associated with its strength. For relatively strong glutens, as found in BGW, KW, YRW and GW flours, adding salt further increased the strength. However, for TW flour containing weak gluten, adding salt did not affect its gluten strength. The effect of sucrose on gluten was also associated with gluten strength. For BGW flour, a 1% (w/v) sucrose solution significantly increased MPT ($p < 0.05$). In contrast, the same solution decreased MPT by 0.37 min in TW flour. The effects of salt and sucrose on MPTH value (used in predicting loaf volume) varied with different wheat cultivars and require further studies.

Table 4
Comparison of mixograph characteristics of wheat flours

	MPT (min)	MPTI (%Tq*min)	MPTH (MU)	MPTW (MU)
BGW-flour				
In water	2.93e	67.1d	33.6b	16.1b
In 1% NaCl	4.37b	106.8b	36.4b	19.0b
In 2% NaCl	3.90c	106.6b	41.8a	26.2a
In 3% NaCl	5.03a	148.9a	42.4a	27.4a
In 1% Sucrose	3.52d	92.5c	37.2b	19.7b
LSD	0.4561	11.0315	4.2358	3.7351
TW-flour				
In water	2.26ab	46.2b	32.3b	15.8b
In 1% NaCl	2.13bc	48.3b	35.3b	19.7a
In 2% NaCl	2.44a	64.8a	40.3a	22.0a
In 3% NaCl	2.26ab	45.5b	32.6b	22.6a
In 1% Sucrose	1.89c	40.9b	32.2b	14.8b
LSD	0.3016	7.936	4.1052	3.5813
KW-flour				
In water	5.41d	122.1d	32.3c	17.9c
In 1% NaCl	5.93c	135.4c	36.6b	23.3b
In 2% NaCl	6.76b	170.5b	42.4a	28.8a
In 3% NaCl	7.76a	211.8a	42.4a	25.9ab
In 1% Sucrose	5.53d	142.8c	37.8b	20.0c
LSD	0.3952	12.3841	4.2762	3.2018
YRW-flour				
In water	6.11d	115.3c	27.2b	14.2c
In 1% NaCl	6.69c	130.7b	30.0b	18.7b
In 2% NaCl	7.21ba	151.6a	34.8a	21.5ba
In 3% NaCl	7.57a	155.7a	36.5a	24.7a
In 1% Sucrose	7.06cb	122.7cb	27.7b	13.3c
LSD	0.4361	13.8255	4.6139	3.8013
GW-flour				
In water	6.27b	134.8b	34.4bc	17.5b
In 1% NaCl	5.63c	101.5c	30.2d	18.4b
In 2% NaCl	6.97a	139.6b	37.9ba	24.7a
In 3% NaCl	7.22a	150.4a	40.6a	26.1a
In 1% Sucrose	6.30b	126.4b	31.9dc	17.0b
LSD	0.5137	13.6913	3.9016	3.3306

MPT – M, mid line; P, peak; *T*, time (min). MPTI – integral (%Tq*min) at MPT. MPTH – height value (MU) at MPT. MPTW – width (MU) at MPT. LSD – least significance difference at $p < 0.05$ level of probability. Mean values for individual flours having similar letters in the same column are not significantly different.

3.5. Dough stickiness

Results on dough stickiness of six wheat flour samples are listed in Table 5. Dough stickiness decreased in flours as follows: AW > TW > YRW > BGW > KW > GW. Dough stickiness has an important effect on bakery characteristics because sticky doughs present problems during baking (Chen & Hoseney, 1995). Hence bakery characteristics of Chinese black-grained wheat flour were significantly better ($p < 0.05$) than that of YRW, TW and AW flours, but somewhat stickier in comparison with KW and GW flours.

3.6. Electrophoretic storage-protein profile

The SDS-PAGE electrophoregrams of total storage protein extracted from the BGW, TW, KW, YRW, GW and AW are presented in Fig. 2. In the high-molecular-weight glutenin (HMW-glu) region, bands (genotype code) appearing at the top for YRW and AW and GW have been reported before. Genes have, respectively, coded the 1, 17 + 18 and 5 + 10 subunits in Yecora Roji (Mansur, Qualset, Kasarda, & Morris, 1990; Martin & Carrio, 1999), the 2 + 12 and 7 + 8

Table 5
Dough stickiness characteristics

Name	BGW-flour	TW-flour	KW-flour	YRW-flour	GW-flour	AW-flour	LSD
Stickiness (g)	223.76d	392.75b	186.01e	313.05c	182.67e	414.49a	21.3041

LSD, least significance difference at $p < 0.05$ level of probability. Mean values for flour samples having similar letters in the same row are not significantly different.

subunits in Anza (Carrillo, Rousset, Qualset, & Kasarda, 1990; Mansur et al., 1990) and the 2*, 7 + 8, and 5 + 10 subunits in Glenlea (Hussain, Lukow, & McKenzie, 1998; Kim & Bushuk, 1995). Our results showed that bands for Taifen 1 wheat were similar to those of Anza wheat whereas bands for Klasic wheat were similar to those of Yecora Rojo wheat in the HMW-glu region. Bands appearing at the top for the Chinese black-grained wheat were similar to those of Glenlea wheat. Results also showed that some bands for BGW and GW were similar to those of KW and YRW whereas other bands for BGW and GW were similar to those of TW and AW. Many studies (Bournouf & Bouriouet, 1980; Moonen, Kescheepstra, & Graveland, 1982; Payne, Holt, Harinder, McCartney, & Lawrence, 1987) have proved that baking quality is strongly correlated with the presence or absence of HMW-glu subunits 1 and 2* and subunits 5 and 10. Poor baking quality is usually associated with subunits 2 and 12. SDS-PAGE result of BGW was useful for predicting its potential use. Band 2* subunit for BGW and GW was not very clear under this electrophoretic conditions and further studies are needed.

Fig. 2. 10% SDS gel electrophoresis profile of black-grained wheat protein compared to five wheat controls. *Notes*. M – molecular weight marker ((a) 205 kDa; (b) 116 kDa; (c) 66 kDa; (d) 39.8 kDa); 1 – Anza wheat (genotype code: 2, 7, 8, 12); 2 – Taifen 1 wheat (genotype code: same as Anza); 3 – Klasic wheat (genotype code: same as Yecora Rojo); 4 – Yecora Rojo wheat (genotype code: 1, 5, 17, 18, 10); 5 – Black-grained wheat (genotype code: same as Glenlea); 6 – Glenlea wheat (genotype code: 2*, 5, 7, 8, 10).

4. Conclusions

Protein properties of Chinese black-grained wheat were evaluated in comparison with five specially selected wheat controls. Gluten index and mixograph data indicated that gluten strength in Chinese black-grained wheat flour was better than Taifen 1, but significantly poorer ($p < 0.05$) when compared to Klasic, Yecora Rojo and Glenlea wheat flours. Dough stickiness data showed that Chinese black-grained flour was significantly weaker ($p < 0.05$) than that of Anza, Taifen 1 and Yecora Rojo, but stronger ($p < 0.05$) in comparison to Klasic and Glenlea wheat flours. Because of the high protein content in Chinese black-grained wheat whole meal and its flour, the total essential amino acid and total amino acid contents were also higher than levels found in controls. After high temperature drying of wet gluten, amino acid composition of Chinese black-grained wheat gluten was heat stable with minimal loss in comparison with Taifen 1 and Klasic wheat gluten. With a high content of elemental Se and protein and possibly phenolic compounds, Chinese black-grained wheat is potentially a raw material for the development of functional foods. It appears as a suitable candidate for noodle production and in bread-making if used in combination with strong flours. Its gluten strength can be further improved through breeding.

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