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Influences of cooking and storage on γ-aminobutyric acid (GABA) content and distribution in mung bean and its noodle products

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ABSTRACT

The γ-aminobutyric acid (GABA) content and distribution of mung bean (MB), heat and relative humidity treated MB (HRH-MB) under different cooking and storage conditions were investigated. The quality characteristics of noodles prepared by MB and HRH-MB were evaluated. Results showed that black MB varieties presented a higher average GABA content under HRH treatment than that of green MB varieties. Soaking significantly increased the GABA content of MB ($P < 0.05$), but, 58.78% of GABA in HRH-MB were distributed in the soaking solution. After cooking, although the GABA of HRH-MB had a slightly degradation, its content was higher than MB. With the increase of storage time, the GABA content in MB increased initially, followed by a decrease, whereas a slower reduction was observed on HRH-MB. Besides, compared with the mixed MB-wheat flour and noodles, the pasting properties of mixed HRH-MB-wheat flour, the color and texture properties of mixed HRH-MB-wheat noodles were improved and closer to wheat flour and noodles, respectively. Furthermore, the mixed HRH-MB-wheat noodles exhibited the highest GABA content (16.56 mg/100 g DW) after cooking. Therefore, HRH-MB may be an ideal material for enhancing GABA and improving the quality characteristic of MB-based foods.

1. Introduction

γ-aminobutyric acid (GABA), as an inhibitory neurotransmitter in animals and humans, is involved in the regulation of cardiovascular functions and has multiple health benefits, such as neurological disorder prevention (Hepsomali, Groeger, Nishihira, & Scholey, 2020), anti-diabetic (Rezazadeh, Sharifi, Sharifi, & Soltani, 2021), and anti-hypertensive effects (Nishimura et al., 2016; Diana, Quílez, & Rafecas, 2014). However, the content of GABA in the daily food, such as legumes and grains, are relatively low (Ma et al., 2021; Hou et al., 2021; Han et al., 2021). Thus, the enrichment of GABA in food has attracted the attention of numerous researchers. At present, the GABA was mainly accumulated via controlling the temperature (Morrison, Frégeau-Reid, & Cober, 2013), oxygen concentration (Komatsuzaki et al., 2007), microorganism (Hsueh et al., 2021) and additives (Guo, Yang, Chen, Song, & Gu, 2012) during the period of soaking, germination and fermentation. In addition, heat and relative humidity (HRH) treatment was a novel approach to enhance GABA content of legumes and grains seeds with low moisture content (Fukumori et al., 2013). However, the degradation of GABA in hydrothermally-treated GABA-containing sugar solutions system by Maillard reaction (MRs) has been demonstrated by Lamberts, Rombouts, and Delcour (2008). About 15.8–48.30% of GABA loss in cooked brown rice has also been reported by Yu et al. (2021). It is believed that the amount of GABA reduction of germinated parboiled rice during storage depended on the storage temperature and time (Klaykruayat, Mahayothee, Khuwijitjaru, Nagle, & Müllera, 2020). Therefore, in order to increase the daily intake of GABA and improve human health, it is vital to not only increase the content of GABA in the raw materials, but to clarify the effect of cooking and storage on the content and distribution of GABA in various products, so as to guide the process of GABA-enriched food products.

Mung bean (*Vigna radiata* L., MB) is one of the most important and popular pulses due to its multiple nutrients, including high protein and fiber content, medium starch, low fat and rich functional components

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(Ganesan & Xu, 2018). Thus, it can be used as a good raw material for developing healthy foods in view of its various potential health benefits (Nikmaram et al., 2017; Hou, Zhao, Yousaf, Xue, & Shen, 2020; Hou et al., 2019). In recent years, with an increasing interest on GABA health benefits, MB, as a potential raw material for GABA accumulation, has attracted more and more attentions (Nikmaram et al., 2017). It has been reported that germination (Tiansawang, Luangpituksa, Varanyanond, & Hansawasdi, 2016) and fermentation (Yeap et al., 2012) promoted the GABA enhancement in MB. Cooking process decreased the GABA content of germinated MB (Tiansawang et al., 2016). And the GABA accumulation capability of MB depended on varieties (Ma et al., 2021). Tiansawang et al. (2016) found that germinated MB had the highest GABA content compared with germinated soybean, black bean and sesame, but different cooking process included boiling, steaming, microwave cooking and open pan roasting had a varied reduction on their GABA content. Although Liu et al. (2018) presented the application of germinated MB flour on noodles, there is no data about the GABA content and distribution on noodles. Notably, HRH treatment enhanced GABA content of grains and legumes seeds without changing its morphology and consumption patterns (seeds, soup, congee and etc), and HRH treated MB (HRH-MB) showed the highest GABA content compared with other grains and legumes (Fukumori et al., 2013). However, to the best of our knowledge, the effect of traditional MB cooking process included steam, boiling, roast and microwave on the GABA content of MB and HRH-MB seeds has not been investigated. Moreover, the quality characteristics, the GABA content and distribution of noodles incorporated with MB and HRH-MB flour have not been reported.

Therefore, the present study was designed 1) to investigate the GABA content of different MB varieties under HRH treatment; 2) to study the GABA content and distributions of MB and HRH-MB under different cooking and storage conditions; 3) to explore the GABA content, color, cooking and texture properties of noodles prepared by MB and HRH-MB flour respectively. The results may provide useful information about how cooking and storage conditions affect the GABA content and distribution of MB and HRH-MB, and promote the GABA-enriched MB noodles development.

2. Materials and methods

2.1. Materials and reagents

Eighty kinds of green MB varieties and eleven kinds of black MB varieties were provided by Chinese Academy of Agricultural Sciences-Institute of Crop Science (Beijing, China). The GABA was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Dabsyl chloride was purchased from Med Chem Express LLC. (New Jersey, America). All other reagents and solvents used in the experiment were of analytical and/or HPLC grade.

2.2. HRH treatment

According to the method of Fukumori et al. (2013), the above MB varieties were treated by HRH method. Briefly, different MB varieties were treated at 70 °C, 95% RH for 4 h in a constant temperature and humidity test chamber respectively (KW-TH-49T, Dongguan KOWIN Testing Equipment Co., Ltd., Guangdong, China). Then the HRH-MB samples were obtained after drying overnight at 45 °C. Part of MB and HRH-MB were pulverized to flour by a laboratory mill (CT 410 Cyclotec TM laboratory mill from FOSS Scino Co. Ltd., Suzhou, China). Afterwards, samples were stored at room temperature until further analysis.

2.3. Cooking conditions

MB (50 g) and HRH-MB seeds (50 g) were separately cooked by the different cooking methods, including steaming (Steam, with 150 mL distilled water steamed at 100 °C for 1 h), high press steaming (H-Steam, with 150 mL distilled water steamed at pressure cooker for 35 min, Bear YLB-A20Q1, Bear Electric Appliance Co., Ltd., Guangdong, China), boiling (Boiling, with 400 mL distilled water boiled at 100 °C for 45 min), high press boiling (H-Boiling, with 250 mL distilled water boiled at pressure cooker for 25 min), roast (Roast, roasted at roaster 160–180 °C for 10 min, Gene Cafe CBR-101 Coffee Roaster, Speedwell Coffee Company, Inc., Plymouth, England) and microwave (Microwave, 900 W for 3 min, Galanz G90F23CSLV-PM, Galanz Group Co., Ltd., Guangdong, China); soaking (Soaking, with 250 mL distilled water soaked at room temperature for 5 h), steaming after soaking (S-Steam, with 100 mL distilled water steamed at 100 °C for 45 min), high press steaming after soaking (S–H-Steam, with 100 mL distilled water steamed at pressure cooker for 30 min), boiling after soaking (S-Boiling, with 300 mL distilled water boiled at 100 °C for 30 min), high press boiling after soaking (S–H-Boiling, with 300 mL distilled water boiled at pressure cooker for 20 min), roast after soaking (S-Roast, roast at 160–180 °C for 15 min) and microwave after soaking (S-Microwave, microwave at 900 W for 5 min). After that, these solid samples were freeze drying, pulverized to flour and stored at room temperature for further analysis. The liquid was stored at −20 °C for further analysis.

2.4. Storage conditions

The MB seeds, HRH-MB seeds, MB flour and HRH-MB flour were stored at 45 °C and room temperature for 5 months, respectively.

2.5. GABA content determination

The GABA content was analyzed by HPLC using a method as described in our previous study (Ma et al., 2021).

2.6. Pasting properties

The pasting properties of samples were investigated using Rapid Visco Analyzer (RVA-TechMaster, Perten, Sweden). Viscosity profiles of samples were recorded using flours suspensions (4.5 g flour: 20 g water). The temperature-time conditions were shown as follows: a heating step from 50 to 95 °C at 6 °C/min after an equilibration time of 1 min at 50 °C, a holding phase at 95 °C for 5 min, a cooling step from 95 to 50 °C at 6 °C/min and a holding phase at 50 °C for 2 min.

2.7. Noodle preparation

The noodles were elaborated with the method reported by Zhao et al. (2020). The ingredients in the control noodle (wheat flour) formulation consisted of 150 g wheat flour and 49.5 g distilled water. The flour of MB and HRH-MB were then to replace 20% of wheat flour in the control formulation respectively. The crumb dough was made using a dough-mixer for 5 min, and rested for 20 min at room temperature. The crumb dough was sheet from 2 mm to 1 mm at 0.2 mm at a time. Then, the sheet was cut in to noodles about 2 mm wide.

2.8. Color

The color of raw noodles (L*, a* and b* value) were measured by Digi Eve digital electronic eye (Shanghai Eutin International Trading Co., Ltd., Shanghai, China). A standard white and colour board were used to standardize the colourimeter separately.

2.9. The micro-structures determination

The internal morphology of MB and HRH-MB seeds and flour were observed by a Scanning Electron Microscopy (SEM, SU-8010, Hitachi, Tokyo, Japan). The samples were frozen in liquid nitrogen and then sublimated rapidly to ensure that no water was present in the samples.

The samples were coated with sputtered gold before being photographed.

2.10. Cooking properties

The optimal cooking time (OCT) for each formulation depended on the disappearance of the opaque line in the center of the noodles. Once the OCT was evaluated by triplicate, noodle samples were cooked. The breaking rate (BR) and cooking loss (CL) were measured by the AACC method 66-50 (AACC, 2000) with some modifications. Briefly, each group of noodle sample (about 30 g) was cooked in 500 mL of boiling water according to the OCT. Then, cooked noodle was washed by cold water for 30 s to prevent over boiling. After that, cooking water and rinse water were put into beaker and boiled until the volume of water was less than 50 mL. Then, the beaker was dried at 105 °C until constant weight. The residue was weighed and reported as percentage of the original noodle samples.

$$\mathrm{BR}\ (\%) = \mathrm{N}_1 \times 100/\mathrm{N}_0;$$

$$\mathrm{CL}\ (\%) = \mathrm{W}_1 \times 100\%/\mathrm{W}_0$$

N_1 and N_0 mean the number of broken noodles of cooked noodles and the total of fresh noodles respectively. W_1 and W_0 mean the weight of residues of cooked noodles and fresh noodles respectively.

2.11. Texture analysis

Texture properties of cooked noodles were evaluated by TA-XT plus Texture Analyzer (Texture Technology Corp., Scarsdale, NY, UAS). Noodles were cooked and washed according to 2.8. TPA was measured using the HDS/PES probe. The pretest speed, test speed, and post test speed were 2.0 mm/s, 0.80 mm/s, and 0.80 mm/s, respectively. The strain was set as 75%. The tensile properties were determined using the A/SPR probe. The pretest speed, test speed, and post-speed were 2.0 mm/s, 2.0 mm/s, and 10 mm/s, respectively. And the distance setting was 150 mm. Each sample was measured for 9 times.

2.12. Statistical analysis

The data of the experiments were replicated three times. The statistical analysis was conducted by using the SPSS version 11.5. An analysis of the variance (ANOVA) test and Duncan Multiple Range Test were performed to determine whether a significant difference ($P < 0.05$) occurred in each attribute.

3. Results and discussion

3.1. GABA content of different MB varieties under HRH treatment

In this study, 80 kinds of green MB varieties and 11 kinds of black MB varieties were collected and treated by HRH, and their GABA content is shown in Fig. 1. The GABA contents of different MB varieties were very low and had significant differences among different varieties ($P < 0.05$), ranging from 0.77 to 16.78 mg/100 g DW, which were similar to previous results (Ma et al., 2021; Tiansawang et al., 2016; Fukumori et al., 2013). Intriguingly, under HRH treatment, the GABA content of MB varieties increased to 24.41–113.15 mg/100 g DW. Furthermore, significant differences ($P < 0.05$) were observed between green MB varieties (24.41–87.71 mg/100 g DW) and black MB varieties (65.95–113.15 mg/100 g DW). Therefore, these results demonstrated that black MB varieties had a better potential for GABA accumulation under HRH treatment than green MB varieties.

From the perspective of HRH treatment conditions, moisture was one of the most important factor for GABA accumulation (Kim, Lee, Lim, & Han, 2015; Youn, Park, Jang, & Rhee, 2011). However, it is well known that different bean varieties had a different amount of water absorption capacity under the same treatment conditions, such as soaking (Morrison et al., 2013). In terms of the inherent factors for GABA accumulation in MB under HRH treatment, free amino acids including glutamic acid, serine, ornithine, arginine and glycine in MB had a significant positive correlation ($P < 0.05$) with GABA content in HRH-MB (Ma et al., 2021). Particularly, glutamic acid was the precursor substance of GABA and it can be directly converted into GABA by glutamate decarboxylase (GAD) (Zhao, Xie, Wang, & Li, 2017). Therefore, the differences of GABA accumulation capacity between green and black MB varieties might be attributed to the differences of water absorption capacity, free amino acids contents (especially glutamic acid), and the related enzyme activities (especially GAD).

3.2. GABA content and distribution of MB and HRH-MB under different cooking methods

Traditionally, the cooking methods of MB seeds are direct cooking and cooking after soaking. Less time was spent to cook for soaked MB seeds than non-soaked MB seeds. Fig. 2 shows the GABA content and distribution of MB and HRH-MB under different cooking methods.

In terms of direct cooking methods, such as steam, H-Steam, boiling, H-Boiling, roast and microwave, the GABA content of cooked MB had a slight increase (from 1.77 to 6.44 mg/100 g DW) in cooked MB as compared with the raw MB (1.59 mg/100 g DW). However, in previous study, the GABA content in commercial processed cereal-based products, such as rice, was found to be decreased because of MRs (García-Baños et al., 2004; Yu et al., 2021; Lamberts et al., 2008). In the present study, at the early stage of boiling, the dormant MB seeds might be partially activated, which stimulated the GABA accumulation. Furthermore, the accumulation efficiency of GABA in MB seeds was higher than its loss efficiency (Fukumori et al., 2013). Besides, the hypoxic conditions, which were provided by high pressure cooking, could also promote its accumulation efficiency of GABA (Ding et al., 2016). For HRH-MB seeds, although HRH treatment largely increased its GABA content (from 1.59 to 102.32 mg/100 g DW), the GABA content decreased by 3.96–14.35% under different cooking methods. A study by Lamberts et al. (2008) reported that GABA losses in GABA-containing sugar solutions depended on the heating time, the varieties of sugar and the composition of solution. Therefore, the reduction of GABA content in HRH-MB might be related to their cooking conditions, chemical composition and concentration. In addition, the results presented that 47.28–52.01% of GABA were distributed in the soup after boiling and H-boiling, which should be attributed to the water solubility of GABA.

From the perspective of cooking after soaking, our results showed that the GABA content in the soaked MB increased largely (from 1.59 to 32.33 mg/100 g DW), whereas, 7.00% of GABA were distributed in the soaked solution. Similar results were also found in other soaked cereals including rice, soybean and sesame (Tiansawang et al., 2016). That is because soaking leads to the water absorption of MB, which could activate the relevant enzymes in the germination process. For example, the glutamate decarboxylase enzyme can catalyze the conversion of glutamic acid to GABA (Komatsuzaki et al., 2007). Besides, boiling and H-boiling caused a significant increases in GABA content ($P < 0.05$). The production of GABA at the early stage of cooking exceeds the loss of GABA due to MRs during boiling. However, for HRH-MB, the majority of GABA (51.56%) was distributed in soaked solution after soaking, which might be because the combination of MB coat and cotyledon is relatively loose as compared with the raw MB coat, and GABA was highly soluble in water. In addition, the GABA content of soaked MB decreased by 0–17.50% under different cooking methods. Furthermore, about 51.76–62.75% of GABA of soaked samples were distributed in the soup after boiling and H-boiling.

Based on the above results, we demonstrated that soaking is a good way to increase the GABA content of MB and cooked MB. But, for HRH-MB, most of GABA could be lost in the soaking solution. The results showed that cooking could increase the GABA content of MB, but it reduced the GABA content of HRH-MB. Furthermore, most of GABA were distributed in the water during boiling because of its water solubility, and the distribution amount of GABA was closely related to the structure of samples. Therefore, appropriate cooking methods are very important to increase the GABA content of MB and decrease the GABA loss of HRH-MB.

3.3. *The changes of GABA content under different storage conditions*

The content of GABA in MB and HRH-MB under different storage conditions were presented in Fig. 3. The results showed that the GABA content of MB increased initially, followed by a decrease with the increasing storage time at 45 °C, while the GABA content of HRH-MB decreased continuously. However, the GABA content of HRH-MB under R-5 (storage at room temperature for 5 months) conditions is significantly higher than that under H-5 conditions (storage at high temperature - 45 °C for 5 months). In specific, the GABA content of MB seeds and flour continually increased from 1.59 to 3.89 and 3.50 mg/100 g DW within 3 and 2 months respectively under 45 °C storage conditions, but the value decreased to 2.73 and 2.40 mg/100 g DW at the end of 5 months respectively. For HRH-MB seeds under 45 °C storage conditions, the content of GABA remained unchanged within 1 month, then the value continually decreased to 88.98 mg/100 g DW at the end of 3 months. However, this value remained basically unchanged for the next two months. As for HRH-MB powder, under 45 °C storage conditions, the GABA content continually decreased to 89.58 mg/100 g DW within 2 months and then remained unchanged.

Parnsakhorn and Langkapin (2013) reported that the reduction of GABA content in germinated brown rice was 28.70% after storage for 8 months at 37 °C. Klaykruayat et al. (2020) reported that the GABA content of germinated parboiled rice decreased by 31.69–34.34% after 6 months at 30 °C. However, our results showed that the content of GABA in MB increased 1.42–2.44 times under different storage conditions. The GABA content of HRH-MB seeds and flour decreased by 14.25% and 13.16% after storage at 45 °C for 5 months, respectively. The GABA content of HRH-MB seeds and flour decreased by 6.39% and 5.82% after storage at room temperature for 5 months, respectively. It has been reported that the related enzyme of GABA synthesis and degradation of dormant seeds can be activated by stress (Podlešáková, Ugena, Spíchal, Doležal, & Diego, 2019). The changes of GABA in MB and HRH-MB should be related to the enzymes of its synthesis and degradation (Zhao et al., 2017). These results suggested that HRH-MB should be ideally stored under low temperature and for a short period of time to resist the GABA degradation.

3.4. *The pasting properties of flours*

The pasting curves of wheat flour, mixed MB-wheat flour and HRH-MB-wheat flour are presented in Supplement Fig. 1, and the related parameters are shown in Table 1. Theses RVA curve shapes of different samples were similar, showing a trend of an initial increase, followed by a decrease and an increase at last. As shown in Table 1, the pasting parameters, including peak viscosity (PV), trough viscosity (TV), breakdown viscosity (BDV), final viscosity (FV), setback viscosity (SBV) and peak time (Pt) decreased significantly ($P < 0.05$) with the addition of MB and HRH-MB flour, whereas, the pasting temperature (PT) value increased significantly ($P < 0.05$). It has been reported that a lower amylose content might associated with a higher PV and a lower PT; a higher resistant starch content was linked to a lower viscosity and Pt; a lower phosphorus content tended to exhibit lower PV and BDV (Zaidul, Norulaini, Omar, Yamauchi, & Noda, 2007; Blennow, Bay-Smidt, & Bauer, 2001; Fu, Tian, Sun, & Li, 2008; Noda et al., 2006). Generally, the starch content of MB is lower than that of wheat, but the content of amylose and resistant starch are higher than that of wheat (Li, Shu, Zhang, & Shen, 2011; Shevkani, Singh, Bajaj, & Kaur, 2016; Štěrbová

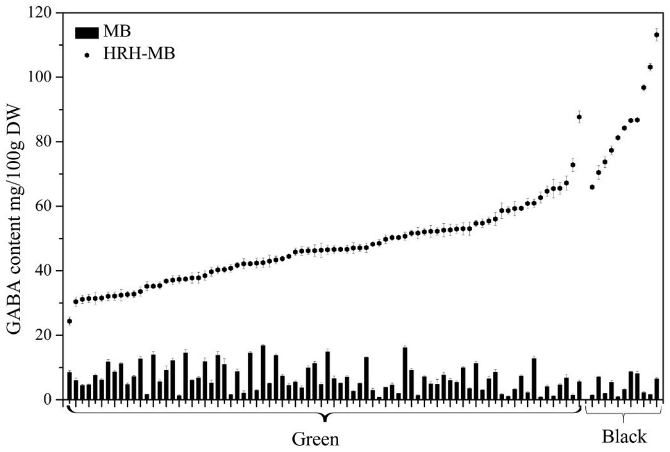

Fig. 1. GABA contents of mung bean (MB) varieties under heat and relative humidity (HRH) treatment.

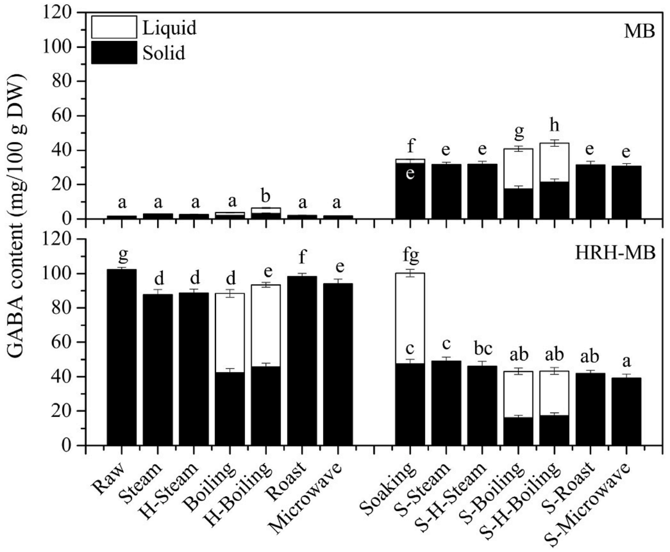

Fig. 2. GABA contents and distribution of mung bean (MB) under different cooking methods.

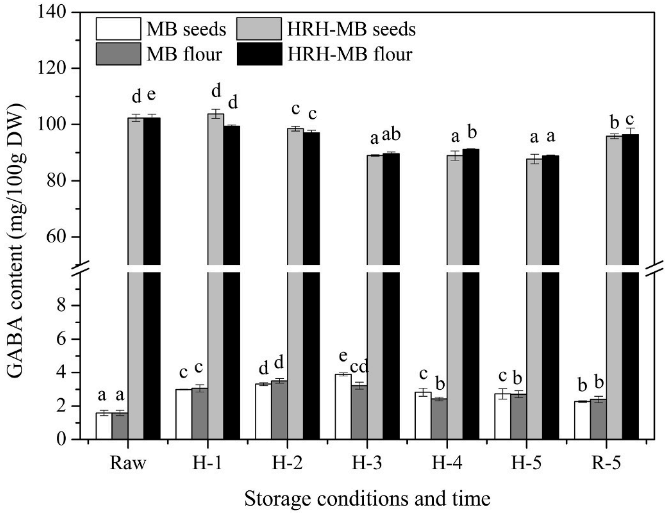

Fig. 3. GABA contents of mung bean (MB) under different storage conditions.

Table 1
Effect of mung bean (MB) and heat and relative humidity treated mung bean (HRH-MB) flour on pasting properties of wheat flour.

Name	Wheat flour	Mixed MB-wheat flour	Mixed HRH-MB-wheat flour
PV (cp)	5092 ± 41c	4116 ± 45a	4585 ± 9b
TV (cp)	2152 ± 22c	1719 ± 8a	1996 ± 8b
BDV (cp)	2940 ± 22c	2397 ± 41a	2589 ± 17b
FV (cp)	4843 ± 31c	3763 ± 59a	4449 ± 23b
SBV (cp)	2691 ± 9c	2045 ± 53a	2453 ± 16b
Pt (min)	9.09 ± 0.03b	8.67 ± 0.07a	8.78 ± 0.05a
PT (°C)	63.97 ± 0.02a	65.42 ± 0.19b	65.80 ± 0.39b

PV, peak viscosity; TV, trough viscosity; FV, final viscosity; BDV, breakdown viscosity; SBV, setback viscosity; Pt, peak time; PT, pasting temperature. Different letters represent significant differences among wheat flour, mixed MB-wheat flour and mixed HRH-MB-wheat flour ($P < 0.05$).

et al., 2016). Zaidul et al. (2007) presented that wheat flour had a high phosphorus content. Thus, the differences among wheat flour, mixed MB-wheat flour and mixed HRH-MB-wheat flour might relate to their starch profiles and phosphorus content. Furthermore, the results exhibited that all the values of pasting parameters of mixed HRH-MB-wheat flour, except for PT and Pt, were higher than mixed MB-wheat flour, and were close to wheat flour. Li et al (2011) reported that, under high hydrostatic pressure treatment, the PV, TV, FV and SBV of MB starch were significantly increased, while the starch granular structure will be converted from C-type to B-type-like pattern. Therefore, these alteration in the viscosity of mixed HRH-MB-wheat flour might be related to the changes of MB starch granular structures during the transformation and reorganization of crystalline structure under HRH treatment (Katopo, Song, & Jane, 2002; Li et al., 2011; Li et al., 2011).

Ekunseitan et al. (2016) reported that a high PT value of starch indicated that starch granules are highly associated with each other, possessing higher water-binding ability, gelatinization degree, and lower swelling ability. The increase of PT in wheat flour with the addition of MB and HRH-MB flour suggested the possibility of these changes. PV was related with the starch swelling by the starch granule rupturing and its disruption rate (Zhang et al., 2019). The decrease of PV value of wheat flour with the addition of MB and HRH-MB flour indicated that the decreased of starch granule swelling and breakage. TV reflected the ability of paste to resist cracking (Ekunseitan et al., 2016). The TV value of wheat flour decreased with the addition of MB and HRH-MB flour, which suggested that MB and HRH-MB flour decreased the ability of wheat flour paste to resist cracking. BDV was usually to measure the difficulty with disintegrating of starch (Collado, Mabesa, Oates, & Corke, 2001). The lower BDV values of wheat flour with the addition of MB and HRH-MB flour indicated that these paste were more stable during continuous heating and shearing process. SBV represented the gelling ability or retrogradation tendency of starchy flour. The reduction in SBV suggested that the pastes of wheat flour with the addition of MB and HRH-MB flour were not easy to aging.

3.5. *Color, morphology and microstructure of noodles*

As shown in Table 2, the value of L*, a* and b* of the noodles were significantly decreased ($P < 0.05$) after the addition of MB and HRH-MB flour into wheat flour, which indicated that the color of noodles changed from white to black, from red to green and from yellow to blue. These can also be partly identified by morphology of noodles (Figs. 1–4 and a-c). Similar findings were presented by Liu et al. (2018), where the L* values for germinated MB flour containing noodles decreased significantly as compared with wheat flour noodles. However, the value of L* and a* of HRH-MB-wheat noodles were significantly higher than MB-wheat noodles ($P < 0.05$), which suggested that HRH treatment diminished the differences in the color of noodles which caused by MB flour. Castañeda-Ovando, Pacheco-Hernández, Páez-Hernández, Rodríguez, and Galán-Vidal (2009) reviewed that the stability of anthocyanin was affected by several factors including temperature, oxygen chemical structure and etc. Therefore, these results might be related to the degradation of anthocyanins of MB under HRH treatment.

Table 2
The color, cooking properties and texture profiles of noodles.

Name	Wheat noodle	Mixed MB-wheat noodle	Mixed HRH-MB-wheat noodle
Color			
L*	87.62 ± 0.22c	55.62 ± 0.30a	57.68 ± 0.35b
a*	1.53 ± 0.09c	−0.72 ± 0.05a	0.14 ± 0.05b
b*	20.82 ± 0.22b	10.91 ± 0.08a	11.05 ± 0.17a
Cooking properties			
OCT (min)	4.00	5.00	5.00
CL (%)	5.70 ± 0.32a	8.30 ± 0.57b	9.60 ± 0.62c
Texture properties			
Hardness (g)	3740.24 ± 94.11a	5760.43 ± 132.65c	5020.04 ± 204.74b
Adhesiveness (g.s)	−80.68 ± 11.14b	−98.09 ± 9.93a	−91.91 ± 11.94 ab
Springiness (%)	94.22 ± 1.46a	94.08 ± 1.15a	93.06 ± 1.42a
Cohesiveness	0.59 ± 0.01a	0.61 ± 0.01b	0.62 ± 0.01b
Gumminess	2225.35 ± 68.14a	3515.73 ± 1102.53c	3115.24 ± 89.98b
Chewiness	2097.28 ± 87.30a	3307.71 ± 104.83c	2899.10 ± 99.90b
Resilience (%)	25.05 ± 1.44a	27.56 ± 0.64b	28.54 ± 1.02b
Tensile strength (g)	15.35 ± 1.76a	26.35 ± 1.67b	25.20 ± 2.01b
Breaking distance (mm)	127.63 ± 14.39b	93.98 ± 9.68a	94.15 ± 8.98a

OCT, optimal cooking time; CL, cook loss. Different letters represent significant differences among wheat noodle, mixed MB-wheat noodle and mixed HRH-MB-wheat noodle ($P < 0.05$).

The SEM images of noodles are shown in Fig. 4 (1-1, 2-1 and 3-1). The micro-structure of noodles showed that the mixed protein matrix was different from gluten network of wheat flour noodles after adding MB flour. Wheat flour noodles showed that the starch was uniformly encapsulated in the continuous gluten network. Noodles by adding MB and HRH-MB presented gluten network but not much an uniform and continuous structure, where the starch was only partly embedded in the mixed protein network and there were also pores in the mixed protein matrix, especially for MB noodles.

3.6. *Cooking and texture properties of noodles*

The cooking and texture properties of noodles incorporated with MB and HRH-MB flour are presented in Table 2. Compared with wheat flour noodles, noodles with the incorporation of MB and HRH-MB flour had a higher OCT value. Liu et al. (2018) also reported that, in comparison with noodles made with only wheat flour, the incorporation of germination MB flour exhibited a higher OCT value. Results of MB-wheat noodles and HRH-MB-wheat noodles had higher CL than that of wheat flour noodles, especially for HRH-MB-wheat noodles, which may be due to the higher cooking time and lower gluten content of MB noodles compared with wheat flour noodles. It is well-know that gluten plays a major role in determining the cooking quality and textural properties of the noodles (Gao et al., 2021; Yan & Lu, 2020). Therefore, on the one hand, long time cooking promoted the distribution of substrates in the water. On the other hand, lower protein gluten content could weaken the structure of gluten network, which also made the substrates in noodles easier to disperse in the water during cooking.

The TPA parameters of cooked noodles including hardness, cohesiveness, gumminess, chewiness and resilience were significantly increased with the addition of MB and HRH-MB flour ($P < 0.05$), while the adhesiveness was decreased and the springiness remained unchanged. High firmness of wheat noodles with the addition of MB and HRH-MB flour may be attributed to the high amylose content, which has

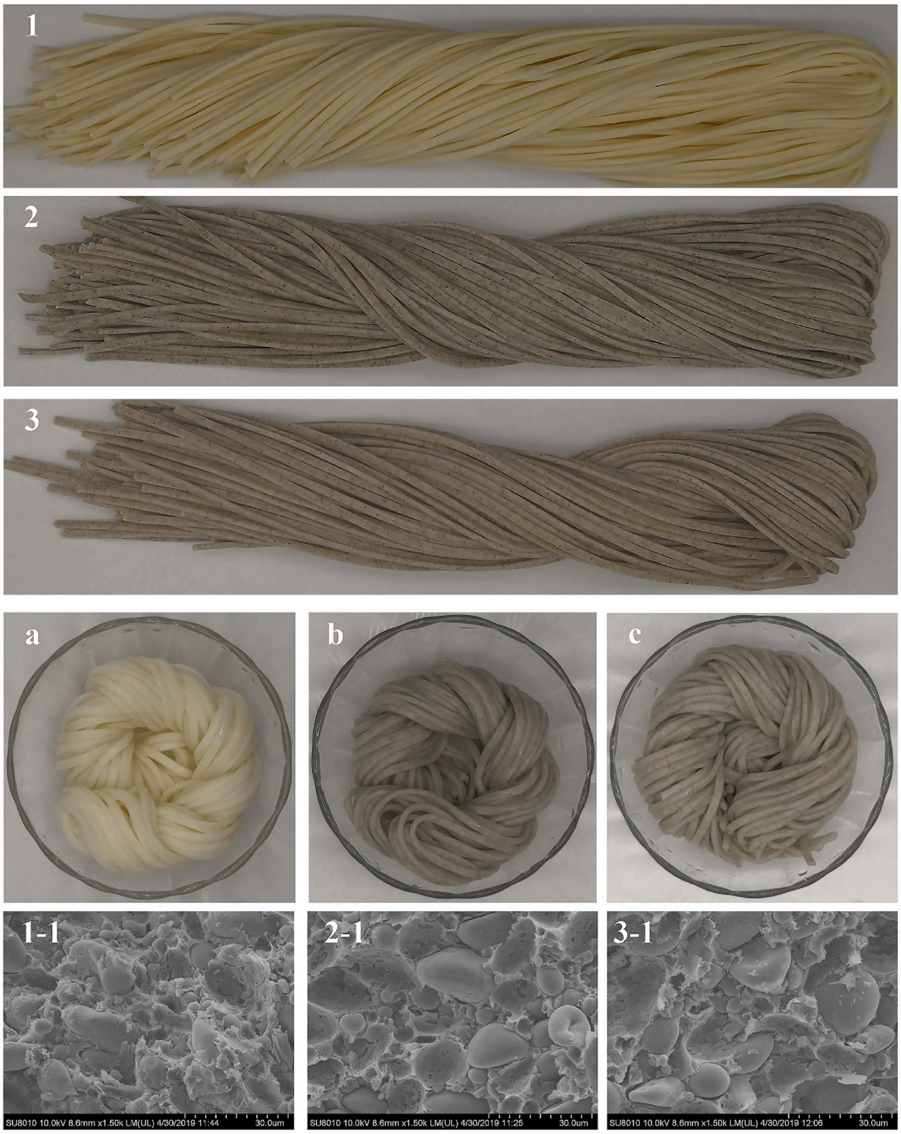

Fig. 4. Morphology and microstructure of noodles.

been considered as an important factor affecting the firmness of cooked noodles (Kaur, Shevkani, Singh, Sharma, & Kaur, 2015; Wang, Warkentin, Vandenberg, & Bing, 2014). Therefore, the results might be due to the differences of protein and amylose between wheat and MB. However, compared with mixed MB-wheat noodles, the TPA parameters of mixed HRH-MB-wheat noodles including hardness, gumminess and chewiness were significantly decreased ($P < 0.05$). The results indicated that the HRH treatment weakened the adverse effects of MB on the texture of noodles.

3.7. GABA content and distribution of flour and noodles

The content of GABA in wheat flour, mixed MB-wheat flour and mixed HRH-MB-wheat flour were 1.42, 1.46 and 21.60 mg/100 g DW, respectively (Fig. 5) A similar result was reported by Youn et al. (2011), which showed the GABA content of wheat was 0.80 mg/100 g. Besides, as shown in Fig. 2, HRH treatment increased the GABA content of MB from 1.59 to 102.32 mg/100 g DW. Thus the GABA content of mixed HRH-MB-wheat flour was much higher than mixed MB-wheat flour. Interestingly, compared with wheat flour and mixed MB-wheat flour, the total GABA content of cooked noodles of wheat and mixed MB-wheat had a significant increase ($P < 0.05$) respectively, especially for cooked MB-wheat noodles (from 1.46 to 9.88 mg/100 g DW). However, the GABA content of cooked noodles of mixed HRH-MB-wheat had a significantly decrease compared with its flour (from 21.66 to 16.56 mg/100 g DW, $P < 0.05$).

For cooked wheat and mixed MB-wheat noodles, the increases of GABA should be attributed to the activation of enzymes related to GABA synthesis during noodles making and/or cooking (Komatsuzaki et al., 2007). Besides, different GABA content between cooked wheat noodles and mixed MB-wheat noodles might due to their differences of GABA accumulation capacity (Fukumori et al., 2013). In terms of cooked mixed HRH-MB-wheat noodles, the decrease of GABA should be attributed to the degradation of GABA by MRs (Lamberts et al., 2008). In addition, the results indicated that 44.32%, 52.83% and 55.37% of GABA of wheat noodles, MB-wheat noodles and HRH-MB-wheat noodles were distributed in the soup after cooking, respectively. As we all know, GABA was highly soluble in water, thus, the distribution of GABA in soup should be due to the water solubility of GABA, and the differences of GABA distribution should be related to the structure of noodles and their cooking loss. Nevertheless, the GABA content of cooked HRH-MB-wheat noodles was the highest. Therefore, HRH treatment was not only an effective method for GABA accumulation in MB seeds, but also an efficient approach for the development of GABA-enriched MB

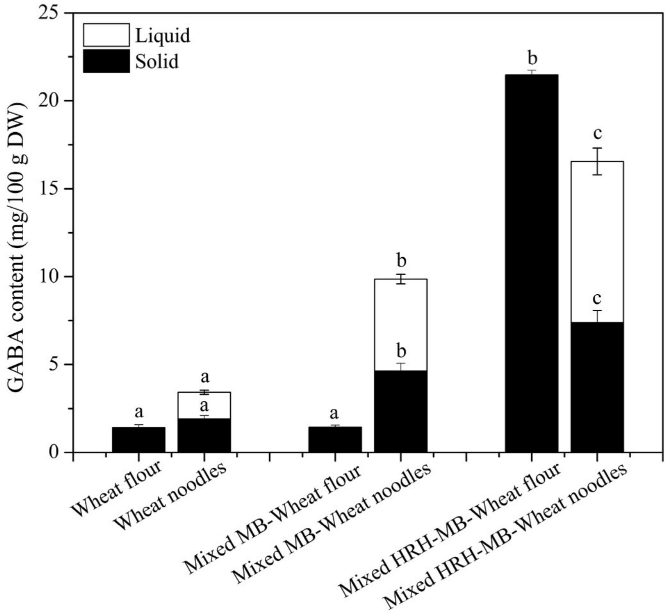

Fig. 5. GABA content and distribution of noodles.

noodles.

4. Conclusion

The present study demonstrated that HRH treatment was an effective approach to enhance GABA of MB, especially for black MB varieties. Even though different cooking methods, including steaming, H-steaming, boiling, H-boiling, roast and microwave, slightly changed the GABA content of MB and HRH-MB, the GABA content of cooked HRH-MB was still much higher than that of cooked MB. Soaking increased the GABA content of MB, but most of GABA of HRH-MB were distributed in the soaking solution. After boiling and H-boiling, about 50.50%–66.35% GABA of MB and HRH-MB were distributed in the soup. The GABA loss of HRH-MB was about 19.37% after accelerated storage for 5 months. In addition, compared to mixed MB-wheat flour and noodles, the pasting properties (PV, TV, BDV, FV and SBV) of flour, color (L* and a*) and texture properties (hardness, adhesiveness, gummines and chewiness) of noodles with the addition of HRH-MB were closer to wheat flour and noodles respectively. Compared to cooked wheat noodles and mixed MB-wheat noodles, the cooked mixed HRH-MB-wheat noodles had the highest GABA content (16.56 mg/100 g). Therefore, HRH-MB could be used as a good ingredient to increase the GABA content of MB noodles and improve the quality of wheat flour noodle.

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Statement of interest conflict

The authors declare no competing financial interest.

Ethical guidelines

Ethics approval was not required for this research.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Yuling Ma: Conceptualization, Writing – original draft, Writing – review & editing, Data curation. **Aixia Wang:** Methodology, Software. **Mei Yang:** Writing – review & editing. **Shanshan Wang:** Visualization. **Lili Wang:** Formal analysis, Investigation. **Sumei Zhou:** Conceptualization, Supervision, Validation, Funding acquisition, Project administration, Resources. **Christophe Blecker:** Supervision, Validation, Project administration, Resources.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2021.112783.

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