

Original article

Effect of heat and relative humidity treatment on γ -aminobutyric acid accumulation, other micronutrients contents, antioxidant activities and physicochemical properties of mung bean (*Vigna radiata* L.)

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Summary A novel approach to heat and relative humidity (HRH, 65–75 °C, 98% RH) treatment was used to enhance γ -aminobutyric acid (GABA) of mung bean with a low moisture (12–20%) within several hours (4–6 h). Meanwhile, the changes in micronutrients, antioxidant activities and physicochemical properties were also evaluated. Results showed that the GABA content was significantly accumulated, increasing from 1.73 to 109.78 mg 100 g⁻¹ dry weight (DW) under the conditions of 16.72% moisture, 68.97 °C, 98% RH for 5.34 h. Meanwhile, the amino acids, including aromatic (tyrosine and phenylalanine), sulphur-containing (methionine and cystine) and branched-chain amino acids (leucine, isoleucine and valine), were significantly increased ($P < 0.05$). Although HRH treatment decreased the total anthocyanin content of mung bean, the total phenolic, vitexin and isovitexin contents, and antioxidant activities were significantly increased ($P < 0.05$). Furthermore, HRH treatment caused the wrinkled structure of mung bean coat, increased the paste viscosity of mung bean flour and decreased the time of complete gelatinisation of starch in mung bean by one third during cooking. These results suggested that HRH treatment could effectively elevate the above functional components, especially GABA, improve the antioxidant activities, pasting and cooking properties of mung bean.

Keywords Functional properties, Heat and relative humidity treatment, mung bean, physicochemical characteristics, γ -aminobutyric acid.

Introduction

γ -Aminobutyric acid (GABA), a non-proteinogenic amino acid, has various health benefits, such as anti-hypertensive, anti-diabetic and neuroprotective effects (Diez-Gutiérrez *et al.*, 2020). According to the previous studies, GABA is present in small amounts in grains and legumes, which are consumed in the human daily diet (Chua *et al.*, 2019). Multitudinous methods have been used to increase the GABA contents in grains and legumes, such as soaking, germination and fermentation. However, these methods commonly consumed a lot of water and time (generally more than 24 h), and the shape and flavour were also changed

compared with the non-processed materials (Komatsuzaki *et al.*, 2007; Liao *et al.*, 2013). Heat and relative humidity (HRH, 60–80 °C, 80–98% RH) treatment may be a potential method to address these challenges (Fukumori *et al.*, 2013). Studies have shown that the GABA content of rice with insufficient moisture (16 ~ 18.5%) was increased significantly, from 1.5 to 17.8 mg 100 g⁻¹ DW under HRH treatment (50 °C and 90% RH or more) within several hours (Fukumori *et al.*, 2013). This method can not only reduce the cost of hydration and dehydration during processing, such as soaking and germination, but also avoid the changes of rice taste, which make it can be consumed like the native rice.

Mung bean (*Vigna radiata* L.) has been recommended as an excellent nutritional and functional

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legume to prevent many chronic degenerative diseases due to its health benefits (Ganesan & Xu, 2018). It is also popular with vegetarian and celiac disease populations because of high protein content and gluten-free (Zhu *et al.*, 2018). At present, soaking, germination and fermentation under different conditions have been shown to increase the GABA content of mung bean, as well as other nutritional components (Nikmaram *et al.*, 2017). Moreover, these GABA-enriched mung beans have been applied in the production of functional products (Wu *et al.*, 2015; Liu *et al.*, 2018). However, the studies about the effect of different HRH treatment conditions on GABA and other chemical components contents of mung bean were deficient, thus restricting its application. Furthermore, physico-chemical properties of mung bean and flour, as crucial characteristics of processing and utilisation, such as colour, thermal and pasting properties, may also be modified under HRH treatment. Liu *et al.* (2018) reported that although mung bean sprout had high nutritional and functional properties, the brightness and pasting viscosity decreased with the increase of germination time, which eventually affected the quality of mung bean noodles. In addition, the thermal properties of mung bean starch were increased significantly under heat-moisture treatment (Li *et al.*, 2011a), while its pasting properties had a significant decrease under repeated and continuous dry heat treatments (Liang *et al.*, 2020). It has been illustrated that viscosity, gelatinisation and retrogradation of starch played a vital role in the qualities of noodles and 'rigid' structure of gluten-free pasta (Li *et al.*, 2012; Marti & Pagani, 2013). However, mung bean, as a gluten-free and starch-enriched food, its thermal and pasting properties under HRH treatment have not been explored. Notably, the hard coat of mung bean made it difficult to cook, which limited its consumption, especially for people with the accelerating pace of life. HRH treatment might address this problem in consideration of the changes in the structure of mung bean coat during hydration and dehydration. However, no reports focussed on cooking properties of mung bean.

Therefore, the purposes of this study are 1) to explore the effect of different HRH treatment conditions on GABA content of mung bean, and optimise HRH treatment conditions for obtaining a highest GABA content; 2) to study the changes of other micronutrients, antioxidant activities and physico-chemical properties of mung bean after HRH treatment; 3) to explore the starch gelatinisation degree of native and HRH-treated mung bean with different cooking time. Our study focussed on the comprehensive understanding about the effects of HRH treatment on mung bean, eventually promoting the development of GABA-enriched mung bean products.

Materials and methods

Materials and reagents

Mung bean (Jilv No.9) was obtained from Sandou Food Co., Ltd, Hebei province, China. The GABA, gallic acid (GA), Folin-Ciocalteu reagent, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS (2,2'-Azino-bis 3-ethylbenzothiazoline-6-sulphonic acid) were all purchased from Sigma-Aldrich Chemical Co. Ltd (St. Louis, MO, USA). Mixed free amino acid standards were provided by Wako Pure Chemical Industries (Osaka, Japan). Dabsyl chloride was purchased from Med Chem Express (MCE, America). All the chemicals were of analytical and/or HPLC grade.

HRH treatment

According to the method of Fukumori *et al.* (2013), Supplement Fig. 1 shows the HRH-treated mung bean preparation flow sheet. Briefly, in the tempering stage, 50 g of mung bean was washed and packed in a sealed vessel to absorb water under different tempering conditions (tempering moisture, temperature and time) by manually shaking. In the HRH treatment stage, the tempered mung beans were treated under different HRH conditions (HRH treatment temperature, relative humidity and time) in a temperature and humidity test chamber (KW-TH-49T, Dongguan KOWIN Testing Equipment Co., Ltd, Guangdong, China). After that, the samples were freeze-dried and were pulverised to flour by a laboratory mill (CT 410 Cyclotec TM, FOSS Scino Co. Ltd, Suzhou, China). Then, these samples were sealed in plastic bags with some desiccant inside and stored at 4 °C for further analysis.

Optimisation of HRH treatment conditions for GABA accumulation

The effects of HRH treatment conditions on GABA accumulation were determined by single-factor tests (Supplement Fig. 2). Then, the GABA content was used as the response value for response surface methodology (RSM). A Box-Behnken design with three independent factors (A, tempering moisture; B, HRH treatment temperature; C, HRH treatment time) at three variation levels was performed. According to the ranges of the value of factors A, B and C, 17 experiments were employed, which include 12 factorial points and 5 replicates of the central point, which were used to optimise the GABA content (Table 1). The GABA content (Y) was the response value. Design-Expert software (Trial Version 11.1.0.1, Stat-Ease Inc., Minneapolis, Minnesota, USA) was used to analyse

Table 1 Response surface design for GABA optimisation in mung bean under HRH treatment and the observed response

Run	Independent variables			Response GABA (mg/100 g, DW)
	A (%)	B (°C)	C (h)	
1	12	65	5	74.58 ± 1.84
2	20	65	5	95.75 ± 2.46
3	12	75	5	69.98 ± 2.1
4	20	75	5	74.56 ± 2.18
5	12	70	4	89.51 ± 1.88
6	20	70	4	93.52 ± 1.84
7	12	70	6	95.37 ± 2.62
8	20	70	6	95.60 ± 2.6
9	16	65	4	86.58 ± 1.19
10	16	75	4	79.59 ± 1.48
11	16	65	6	100.15 ± 2.17
12	16	75	6	80.64 ± 0.95
13	16	70	5	109.46 ± 2.43
14	16	70	5	112.28 ± 2.98
15	16	70	5	105.97 ± 2.36
16	16	70	5	111.14 ± 2.28
17	16	70	5	109.89 ± 2.42

the experimental data. Then, the data were fitted to a second-order polynomial model, and the regression coefficients were obtained. The fitted quadratic mode for GABA (Y) of HRH-treated mung bean was shown in Eq. (1):

$$\begin{aligned}
 Y(\text{GABA, g/100g, DW}) = & -4317.05125 + 40.40519A \\
 & + 111.90415B + 86.79750C - 0.207375AB \\
 & - 0.236250AC - 0.526000BC - 0.742828A^2 \\
 & + 0.765810B^2 + 4.36275C^2
 \end{aligned} \quad (1)$$

Where Y is the response value of GABA; A, B and C are the independent variable of tempering moisture, HRH treatment temperature and time respectively. Based on the optimised conditions, HRH-treated mung beans were dried, milled and stored as above methods, then were used for the determination of other micronutrients, antioxidant activities and physicochemical properties.

GABA content determination

The GABA content was analysed by HPLC as described previously (Ma *et al.*, 2021). In detail, mung bean flour (1.0 g) was mixed with 70% ethanol (10 mL) at room temperature for 30 min. Then, the mixture was centrifuged at 11000 g, 25 °C for 10 min. Samples were extracted in triplicate. Then, all of the supernatant layers was mixed. Subsequently, 1.0 mL of the mixed supernatant, 0.2 mL of NaHCO₃ (0.4 g,

in 10 mL water) and 0.4 mL of dabsyl chloride (20 mg, in 10 mL acetonitrile) were mixed and incubated at 70 °C for 20 min. Then, the GABA content was determined using Agilent 1260 HPLC (Agilent Technologies Inc., USA) with a ZORBAX SB-C18 column (5 µm), 4.6 × 250 mm i.d. Mobile Phase A and B was sodium acetate buffer solution (50 mM, 69%) and acetonitrile (31%), respectively. HPLC was run at 1.0 mL min⁻¹ flow rate and 436 nm wavelength.

Free amino acids determination

Free amino acids (FAAs), including methionine (Met), isoleucine (Ile), leucine (Leu), valine (Val), phenylalanine (Phe), threonine (Thr), lysine (Lys), tyrosine (Tyr), histidine (His), cystine (Cys), arginine (Arg), taurine (Tau), ornithine (Orn), aspartic acid (Asp), glycine (Gly), serine (Ser), hydroxyproline (Hypro), alanine (Ala) and glutamic acid (Glu), were measured according to the method as described previously (Zhao *et al.*, 2017). Mung bean flour (0.5 g) and 0.1 M HCl (5.0 mL) were mixed. Then, the mixture was sonicated for 10 min at room temperature and was centrifuged at 4193 g for 15 min at 25 °C. Afterwards, the obtained supernatant was adjusted to 5.0 mL. Then, 1.0 mL of supernatant was mixed with 1.0 mL of 8% sulphosalicylic acid to purify the FAAs. After centrifugation at 16770 g for 15 min at 25 °C, 1.0 mL of supernatant was dried by using flushing nitrogen. Then, 1.0 mL of 0.02 M HCl was used for reconstitution solution, and the reconstituted solution was filtered by using a 0.45 µm filter membrane. Subsequently, the FAAs were analysed using an amino acid analyzer (L-8800, Hitachi, Japan).

Phytochemical compounds determination

The extraction of phytochemical compounds of mung bean was carried out based on the previous method with some modifications (Yao *et al.*, 2011). 2.0 g of mung bean flour was extracted with 20 mL of 80% methanol–water and acidified methanol–water (v/v), respectively. The mixture was centrifuged at 4500 g for 10 min after shaking for 2 h. The extraction was conducted in triplicate. Then, all the supernatant were collected and evaporated at 45 °C to less than 5.0 mL. Finally, the concentrated extracting solution was diluted to 10 mL with extraction solvent. The extracts were stored at –20°C in the dark until analysis.

Total phenolic content (TPC) was measured using Folin–Ciocalteu method according to Yao *et al.* (2010). Briefly, 1.0 mL of 1 N Folin–Ciocalteu was thoroughly mixed with 1.0 mL of methanol–water extracts or GA solution, and then 3.0 mL of Na₂CO₃ (10 g, in 100 mL distilled water) was added into the

above mixture. Then, the mixture was placed at room temperature in the dark for 60 min before the absorbance was measured at 765 nm. The result was expressed as mg GA equivalents (GAE) per gram dry sample.

Total anthocyanins content (TAC) determination was performed according to Hosseinian *et al.* (2008) with some modifications. The absorbance of acidified methanol–water extract was measured at 520 nm and at 700 nm in buffers at pH 1.0 and 4.5, respectively. TAC of extracts (mg L^{-1}) = $\{[(A_{510} - A_{700})_{\text{pH } 1.0} - (A_{510} - A_{700})_{\text{pH } 4.5}] \times \text{MW} \times \text{DF} \times 10^3\} / (\epsilon \times 1)$, where MW is the molecular weight (g mol^{-1}) = 449.2 g mol^{-1} for cyanidin-3-glucoside (Cy-3-glc), DF is the dilution, and ϵ is the extinction coefficient ($\text{L} \times \text{cm}^{-1} \times \text{mol}^{-1}$) = 26,900 for Cy-3-glc. The result was expressed as Cy-3-glc equivalents per gram dry sample.

Vitexin and isovitexin were measured according to Yao *et al.* (2011).

Antioxidant activities determination

The DPPH and ABTS radical scavenging activity of methanol–water extract were estimated according to the method of Yao *et al.* (2010). For DPPH radical scavenging activity, 1.0 mL of DPPH solution (8.0 mg DPPH powder was dissolved with 100 mL 80% methanol) was added to 1.0 mL of extract or Trolox solution. The mixture was placed at room temperature in dark for 30 min before the absorbance was measured at 517 nm. ABTS⁺ working solution was prepared by reacting ABTS solution (7 μM ABTS, in redistilled water) with 2.45 mM $\text{K}_2\text{S}_2\text{O}_8$ and kept at room temperature in the dark for 16 h, and then diluted with distilled water to an absorbance of 0.70 (± 0.02) at 734 nm. For ABTS radical scavenging activity, 50.0 μL extracts or Trolox solution and 3.0 mL ABTS⁺ working solution were mixed and put at room temperature in dark for 20 min before the absorbance was measured at 734 nm. The results were expressed as Trolox equivalents (TE) per gram dry sample.

The ferric reducing antioxidant power (FRAP) assay was conducted according to Chen *et al.* (2014). The FRAP working solution was prepared by mixing 300 mM pH3.6 acetate buffer (3.1 g $\text{C}_2\text{H}_3\text{NaO}_2 \cdot 3\text{H}_2\text{O}$ and 16 mL $\text{C}_2\text{H}_4\text{O}_2$), 10 mM IPTZ (2,4,6-tripyridyl-s-triazine, 40mM HCl) and 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution in a ratio of 10:1:1. Then, 6.0 mL of FRAP working solution, 0.2 mL of extracts or FeSO_4 solution and 0.6 mL of distilled water were mixed and kept at 37 °C for 10 min before the absorbance was measured at 593 nm. The result was expressed as FeSO_4 equivalents.

The colour determination

The colour (L^* , a^* and b^* value) was characterised by Digi Eye digital electronic eye (Shanghai Eutin International Trading Co., Ltd.). A standard white and colour board were used to standardise the colorimeter, respectively.

The micro-structures determination

The internal morphology was investigated by scanning electron microscopy (SEM Hitachi S-570, Hitachi, Co., Ltd., Tokyo, Japan) at an acceleration voltage of 10 kV. The samples were frozen in liquid nitrogen and then sublimated rapidly to ensure that no water was presented in the samples. The samples were coated with sputtered gold before being photographed. The mung bean seeds and flour samples were observed at magnifications of $\times 500$ and $\times 1,000$, respectively.

Thermal properties determination

Thermal properties of samples were analysed using a differential scanning calorimeter (DSC-Q20, CT, USA). 3.5 mg of mung bean flour was mixed with 7.0 mg of deionised water in DSC-pans. It was then sealed and placed overnight at 4 °C for equilibration. The samples were analysed from 20 to 120 °C with the change in temperature of 10 °C / min^{-1} .

Pasting properties determination

The pasting properties of mung bean flour were investigated using Rapid Visco Analyzer (TechMaster, Perten, Sweden). Viscosity profiles of samples were recorded using flours suspensions (4.5 g flour:20.0 g water, w/w). The temperature–time conditions included a heating step from 50 to 95 °C at 6 °C / min^{-1} 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^{-1} and a holding phase at 50 °C for 2 min.

Degree of starch gelatinisation determination during different cooking time

Cooking: native and HRH-treated mung bean were boiled in water for different times (seeds:water = 50 g:500 g w/w; cooking time 15 min, 30 min and 45 min, respectively). Then, the samples were freeze-dried, milled and sealed in plastic bags with some desiccant inside and stored at 4 °C for further analysis.

The degree of starch gelatinisation of cooked samples was measured by enzymatic determination (Liu & Liu, 2020). Double 200 mg of flour was weighed and put in two 25 mL tubes separately. Then, 15 mL of buffer solution (sodium acetate, pH 4.5) was added to

each tube. One tube was put in boiled water for 1 h to gelatinise totally, while another tube was put at room temperature to measure the degree of starch gelatinisation. Notably, the tube without flour was set as the blank. After that, 1.0 mL of enzyme solution (180 U amyloglucosidase / mL) was added and was incubated 1 h at 40 °C (shaking every 15 min). Then, 2.0 mL of 10% $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and 1.0 mL of 5 N NaOH were mixed in the tube and made the volume reach to 25 mL with water. Then, 0.1 mL of above solution was mixed with 2 mL of copper reagents (40 g Na_2CO_3 and 7.5 g tartaric acid were mixed in 400 mL distilled water, and 4.5 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ was added to get 1000 mL with distilled water) in 25 mL tube. Then, the tube with sample solution was put in boiling water for 6 min, and added 2.0 mL of phosphomolybdate reagents, and continued to boil for 2 min (phosphomolybdate reagents: 70 g molybdic acid and 10g sodium tungstate were mixed with 400 mL 10% NaOH and 400 mL distilled water, after boiling for 20-40 min, the NH_3 was expelled. After that, 700 mL distilled and 250 mL 85% H_3PO_4 were added. Then the volume of mixed solution was adjusted to 1000 mL using distilled). Finally, the tube was cooled and diluted to 25 mL. The absorbance was determined at 420 nm. Degree of starch gelatinisation (%) = $(A_{1-1} - A_0) \times 100\% / (A_{1-2} - A_0)$, where A_{1-1} means the parboiling native or HRH-treated mung bean flour; A_{1-2} means the completely gelatinised native or HRH-treated mung bean flour; A_0 means the blank.

Statistical analysis

The data of the experiments were replicated three times. The statistical analysis was depended on the SPSS version 11.5. An analysis of the variance (ANOVA) test followed by Duncan Multiple Range Test was used for significant analysis. Data obtained from native and HRH-mung bean were analysed using independent samples t test. All significant differences were detected at $P < 0.05$.

Results and disscussing

Optimisation for GABA accumulation in mung bean under HRH treatment using RSM.

The results of single-factor tests are presented in Supplement Fig. 2. The results showed that tempering moisture, HRH treatment temperature, RH and time had a significant influence ($P < 0.05$) on GABA accumulation according to one-way ANOVA analysis. Specifically, there was a gradual increase in GABA content with the increases of tempering moisture, but a sharp decrease occurred when tempering moisture exceeded 16% (Supplement Fig. 2 A). The GABA content increased following the increases in processing temperature from 60 to 70 °C, while the GABA

content reduction was observed when the temperature was above 70 °C (Supplement Fig. 2 D). As shown in Supplement Fig. 2 E, the GABA content continuously increased with the increase of RH. Considering the importance of moisture on GABA accumulation (Supplement Fig. 2 A), the highest relative humidity (98% RH) was chosen, that's because the moisture must be lost with a low relative humidity. The GABA content continuously increased and reached the maximum level of 109.46 mg 100 g⁻¹ DW at 5 h (Supplement Fig. 2 F). Therefore, tempering moisture (A, 12%, 16% and 20%), HRH treatment temperature (B, 65 °C, 70 °C and 75 °C) and time (C, 4 h, 5 h and 6 h) were selected as the key factors of Box-Behnken design (Table 1), and the analysis of variance (ANOVA) of the result of Box-Behnken was presented in Supplement Table. 1. The fitted quadratic mode for GABA (Y) of HRH-treated mung bean was estimated by RSM and was shown in Eq. (1).

The p -value of model (0.0001) and p -value of lack of fit (0.1255) proved that the model fits with the data and can be used to optimise the variables (Supplement Table. 1). Besides, the value of coefficient of determination (R^2) was 0.9728, which indicated that 97.28% of the variation could be explained excellently (Supplement Table. 1). Based on RSM, HRH treatment temperature (B) and tempering moisture (A) significantly affected the GABA content of HRH-treated mung bean, followed by HRH treatment time (C). The quadratic term (A^2 , B^2 and C^2) was significant ($P < 0.05$), and AB terms were also significant ($P < 0.05$). The other terms were insignificant.

The visual interaction between independent and dependent variables was clarified by three-dimensional response surface plots. The accumulation of GABA ranged from 69.98 to 112.28 mg 100 g⁻¹ DW, which depended on the tempering moisture, HRH treatment temperature and time, and their interaction (Fig. 1 a-c).

Figure 1 (a) presented that the content of GABA was increased with the increase of tempering moisture and HRH treatment temperature within a certain range. Both of them exerted a significant effect on GABA accumulation (P -value = 0.0181 and 0.0013 respectively). The linear factor of their interaction also showed a significant influence on GABA accumulation (P -value = 0.0474). Figure 1 (b) showed that the linear factor of HRH treatment time did not exert a significant influence on GABA content (P -value = 0.0734), and its interaction with tempering moisture also had no significant effect on GABA enhancement of mung bean, with a P -value of 0.6012. From Fig. 1 (c), we can see that the GABA content increased with HRH treatment temperature to a certain value thereafter decreased. But the interaction of HRH treatment temperature and time showed no significant effect on GABA accumulation (P -value = 0.1716).

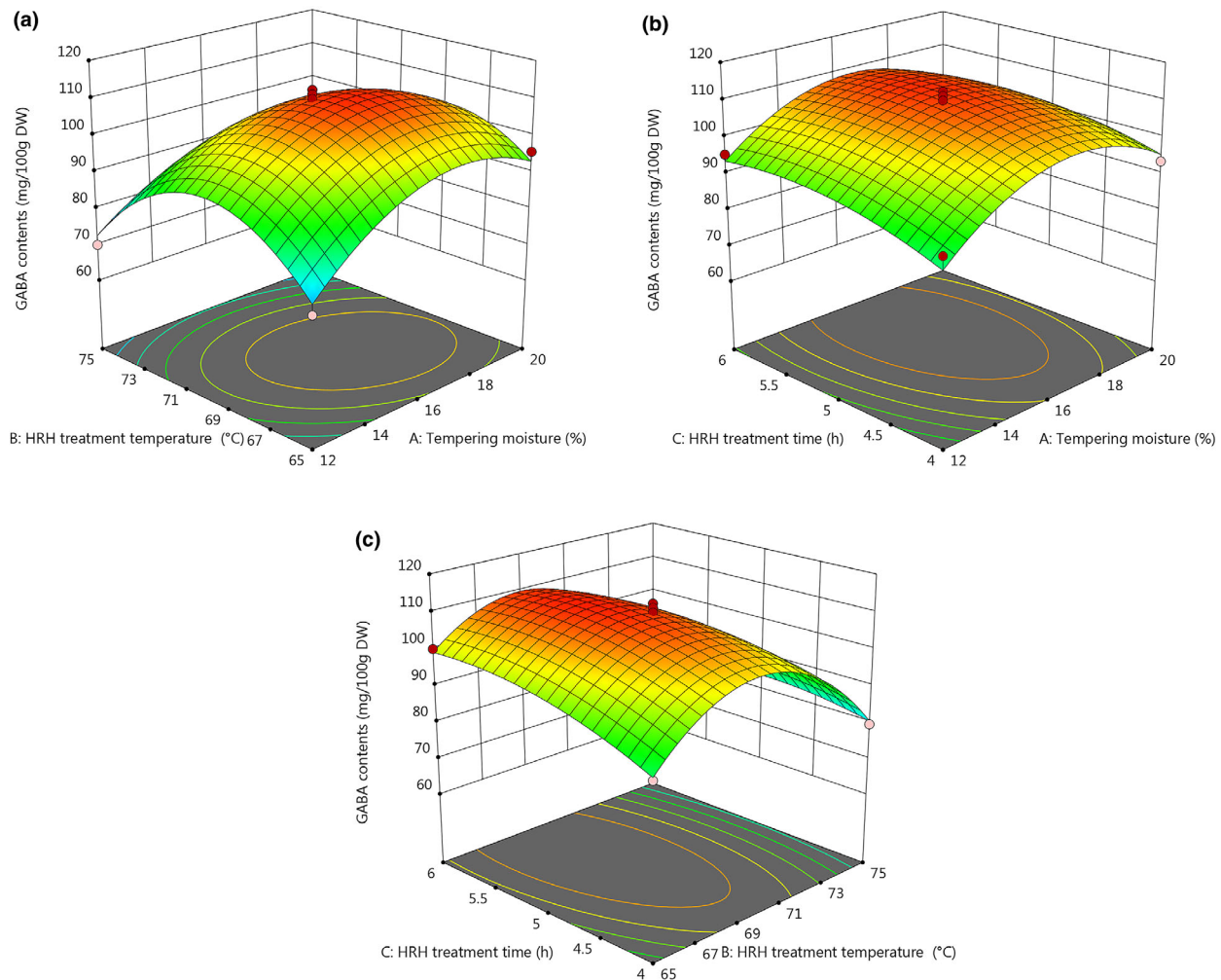


Figure 1 (a) Response surface for GABA accumulation in mung bean under HRH treatment. (b) Response surface for GABA accumulation in mung bean under HRH treatment (c) Response surface for GABA accumulation in mung bean under HRH treatment.

The GABA accumulation of mung bean under HRH treatment might be due to the residual rate-limiting enzymes GAD of GABA shunt and DAO of polyamine degradation pathway that got activated as result of HRH treatment (Yang *et al.*, 2013). In previous studies, 45 °C was an optimal temperature for GABA accumulation of adzuki bean during soaking and germination (Liao *et al.*, 2013), while 40 °C appeared optimal for GABA synthesis in rice bran with 30% moisture contents (Kim *et al.*, 2015). It suggested that the optimum generation temperature and moisture of GABA were different among various plants or treatment methods. In our study, the results suggested that for mung bean, GABA could be accumulated in an extremely low moisture content. Thus, HRH treatment was a promising approach to develop mung bean-based GABA-enriched functional foods.

Verification of predictive models

By prediction with computing program, the optimum conditions to produce the highest GABA content ($111.17 \text{ g } 100\text{g}^{-1} \text{ DW}$) were obtained as follows: tempering moisture of 16.72%, HRH treatment temperature of 68.97 °C and HRH treatment time of 5.34 h. Then, an experiment was carried out in triplicate under the optimal conditions. The result showed that the GABA content was $109.78 \pm 2.95 \text{ g } 100 \text{ g}^{-1} \text{ DW}$, which was in good agreement with the predicted data.

FAs profiling

As presented in Fig. 2, the Glu content ($95.26 \text{ mg } 100 \text{ g}^{-1} \text{ DW}$) in native mung bean was the highest, followed by Arg ($68.04 \text{ mg } 100 \text{ g}^{-1} \text{ DW}$) and

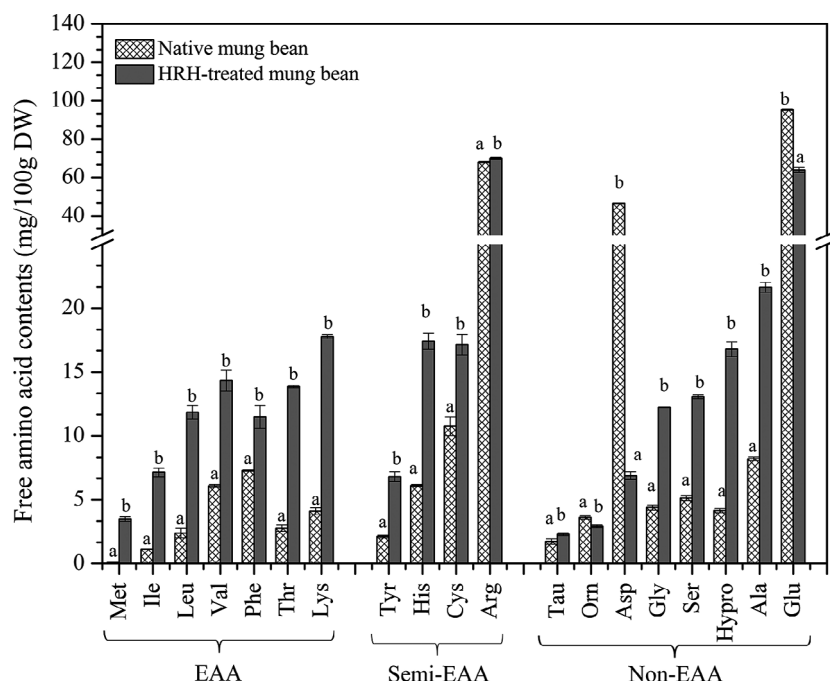


Figure 2 Free amino acid contents in native and HRH-treated mung bean.

Asp ($46.50 \text{ mg } 100 \text{ g}^{-1} \text{ DW}$), which were in agreement with the previous study (Ali *et al.*, 2016). However, HRH treatment not only changed the profiles of FAAs in mung bean, but also significantly increased the total amount of FAAs (from 279.86 to $331.18 \text{ mg } 100 \text{ g}^{-1} \text{ DW}$). Overall, except for Non-EAAs, including Glu, Asp and Orn, showed a significant decrease ($P < 0.05$), and all other FAAs had a significant increase ($P < 0.05$). Among them, the amounts of Met, Ile and Thr increased more than 5 times, and those of Leu, Lys, Hypo and Tyr increased 3-5 times. Especially, the contents of sulphur-containing amino acids, including Met and Cys, increased from 0.10 to 3.49 and 10.76 to $17.15 \text{ mg } 100 \text{ g}^{-1} \text{ DW}$, respectively. The aromatic amino acids, including Tyr and Phe, increased from 2.11 to 6.81 and 7.28 to $11.49 \text{ mg } 100 \text{ g}^{-1} \text{ DW}$, respectively, and the branched-chain amino acids, including Leu, Ile and Val, also augmented from 1.13 to 7.15 , 2.38 to 11.85 and 6.09 to $14.34 \text{ mg } 100 \text{ g}^{-1} \text{ DW}$, respectively. A similar tendency to the changes of FAAs was also found in germinated brown rice under soaking and gaseous treatment (Komatsuzaki *et al.*, 2007).

Normally, the seeds are dormant, but they can be activated in suitable conditions, such as germination. Under HRH treatment, the mung bean absorbed water and situated in a low oxygen and high-temperature environment, which also made it possible to induce various biological reaction by activating various residual enzymes. Kim *et al.* (2015) also found similar changes in FAAs in rice bran treated by an anaerobic

storage. It has been reported that Glu and Asp were direct or indirect precursor of GABA and other FAAs, and Glu-to- α -ketoglutarate conversion is used to provide an amino group in various transamination reactions which allow conversion of Glu and Asp to Met, Thr, Ile and Lys by the plastidial aspartate-family pathway (Fait *et al.*, 2008; Podlešáková *et al.*, 2019). Therefore, we speculated that the increase of other FAAs was related to the consumption of Asp and Glu. However, the transformation among FAAs of mung bean under HRH treatment is still not entirely clear. In view of the antioxidant properties of aromatic and sulphur-containing amino acids, and the important role of branched-chain amino acid on skeletal muscle and liver (Plate II *et al.*, 2000; Garzón & Drago, 2018), the approach was valuable in manipulating the FAAs profiles and improving the functional amino acid compounds of mung bean.

Phytochemical compounds and antioxidant activities

As shown in Table 2, the contents of TPC, TAC, vitexin and isovitexin of mung bean were 2.06 , 1.74 , 2.11 and $1.20 \text{ mg } \text{g}^{-1} \text{ DW}$, respectively, which were similar to the report of Yao *et al.* (2011). After HRH treatment, the contents of TPC, vitexin and isovitexin in mung bean increased significantly ($P < 0.05$), while the content of TAC decreased significantly ($P < 0.05$). However, the values of FRAP, DPPH and ABTS free radicals scavenging activity of mung bean showed a significant increase ($P < 0.05$). The decreases in the

Table 2 Phytochemical components and antioxidant activities of native and HRH-treated mung bean

	Native mung bean	HRH-treated mung bean
Phytochemical components		
TPC mg/g DW	2.06 ± 0.12b	2.49 ± 0.19a
TAC mg/g DW	1.74 ± 0.06a	0.93 ± 0.11b
Vitexin mg/g DW	2.11 ± 0.10b	2.46 ± 0.16a
Isovitexin mg/g DW	1.20 ± 0.05b	1.40 ± 0.04a
Antioxidant activities		
DPPH µM/g DW	28.88 ± 0.57b	32.72 ± 0.53a
ABTS µM/g DW	16.15 ± 0.40b	17.44 ± 0.63a
FRAP µM/g DW	14.76 ± 0.82b	18.49 ± 0.73a

TPC, (total phenolic content), TAC (total anthocyanins content), DPPH (1,1-diphenyl-2-picrylhydrazyl), ABTS (2,2'-azino-bis 3 ethylbenzothiazoline-6-sulphonic acid) FRAP (ferric reducing antioxidant power). Results are the mean of three samples ± standard deviation. Different letters in the same row represent significant differences between native mung bean and HRH-treated mung bean ($p < 0.05$). Same as follows.

TAC content of mung bean under HRH treatment should be attributed to its thermolability. The increases in TPC content and antioxidant activities of mung bean under HRH treatment are possibly due to the activation of related enzymatic involved in the biosynthesis of phenolic compounds, such as esterase and phenylalanine-ammonialyase (Perales-Sánchez *et al.*, 2014; Gómez-Favela *et al.*, 2017). Similarly, results were also found in soaked barley (Al-Ansi *et al.*, 2020). Besides, Garzón & Drago (2018) reported that some FAAs had a positive correlation with antioxidant activities, especially aromatic and sulphur-containing amino acids. In this study, the contents of aromatic and sulphur-containing amino acids of HRH-treated mung bean were higher than native mung bean (Fig. 2).

Particle morphology, micro-structure and colour

Result showed that the particle morphology of HRH-treated mung bean seeds is consistent with native mung bean seeds, while the glossiness was a little weak (Supplement Fig. 3 a and b), which may be due to the damage of waxy structure of mung bean seeds surface under HRH treatment. Besides, the surface of the mung bean seeds had a little shrinkage because of water absorption and drying processing. From the perspective of appearance (Supplement Fig. 3 c and d) and micro-structure (Supplement Fig. 3 g and h) of mung bean flour, HRH treatment almost had no influence on them. However, the micro-structure of mung bean seeds was changed greatly under HRH treatment

(Supplement Fig. 3 e and f). The starch granules in native mung bean seeds cells were clear and uniform, but were almost invisible after HRH treatment, which reflected that partial starch was gelled and wrapped other starch after treatment. Huang *et al.* (2016) also found the partial gelatinisation of the sweet potato starch during heat-moisture treatment and the degree of gelatinisation increased with the processing time.

Supplement Fig. 4 showed that HRH-treated mung bean seeds had higher L^* and a^* than native for the seeds. It might be due to the degradation of anthocyanins (Castañeda-Ovando *et al.*, 2009) or the loss of water-soluble pigments. For HRH-treated mung bean flour, it showed a lower value of L^* , a^* and b^* than native mung bean flour. It might be attributed to the transformation of pigments from the seed coat to the endosperm layer or maillard reaction (Lamberts *et al.*, 2008).

Thermal and pasting properties

In order to better understand the processing properties of HRH-treated mung bean flour, the thermal and pasting properties were summarised in Table 3 and Supplement Fig. 5. In terms of thermal properties, HRH treatment significantly increased ($P < 0.05$) the value of T_0 of mung bean flour, which indicated that partial starch in mung bean flour was gelatinised during the processing of HRH treatment. The results were in accordance with the results of SEM (Supplement Fig. 3 e and f). The reason for the increase of T_0 should be attributed to the preferential melting of less stable crystallites of starch in mung bean flour, leaving behind more stable residual crystallites with a higher melting temperature (Wang *et al.*, 2016).

From the perspective of pasting properties, except for PT, HRH treatment resulted in a significant increase ($P < 0.05$) in all other parameters of mung bean flour including PV, TV, FV, SBV, BDV and Pt. Similar results were observed in the high hydrostatic pressure-treated mung bean starches (Li *et al.*, 2011b). These alterations in viscosity and Pt following the HRH treatment might result from the changes of granular structures during the transformation and reorganisation of crystalline structure (Katopo *et al.*, 2002; Li *et al.*, 2011a). The significant increase ($P < 0.05$) of viscosity of HRH-treated mung bean flour was demonstrated to produce a more viscous paste than native mung bean flour. The higher SBV was observed in the HRH-treated mung bean flour, suggesting their higher tendency towards retrogradation. Li *et al.* (2012) reported that better quality of noodles required high viscosity of flour. The gelatinisation and retrogradation of starchy flour played a vital role on the 'rigid' structure of gluten-free pasta (Marti & Pagani, 2013). Therefore, the positive effect of HRH treatment on

Table 3 Thermal and pasting properties of native and HRH-treated mung bean flour

	Native mung bean	HRH-treated mung bean
Thermal properties		
T ₀ (°C)	62.57 ± 0.26b	65.04 ± 0.44a
T _p (°C)	72.52 ± 0.42	72.09 ± 0.01
ΔH (J/g)	2.95 ± 0.27	3.44 ± 0.24
Pasting properties		
PV (cp)	2844 ± 99b	4115 ± 25a
TV (cp)	1592 ± 23b	2536 ± 25a
FV (cp)	2907 ± 47b	4836 ± 37a
BDV (cp)	1251 ± 77b	1579 ± 20a
SBV (cp)	1315 ± 24b	2301 ± 39a
Pt (min)	7.75 ± 0.04b	8.58 ± 0.08a
PT (°C)	72.62 ± 0.23	72.73 ± 0.03

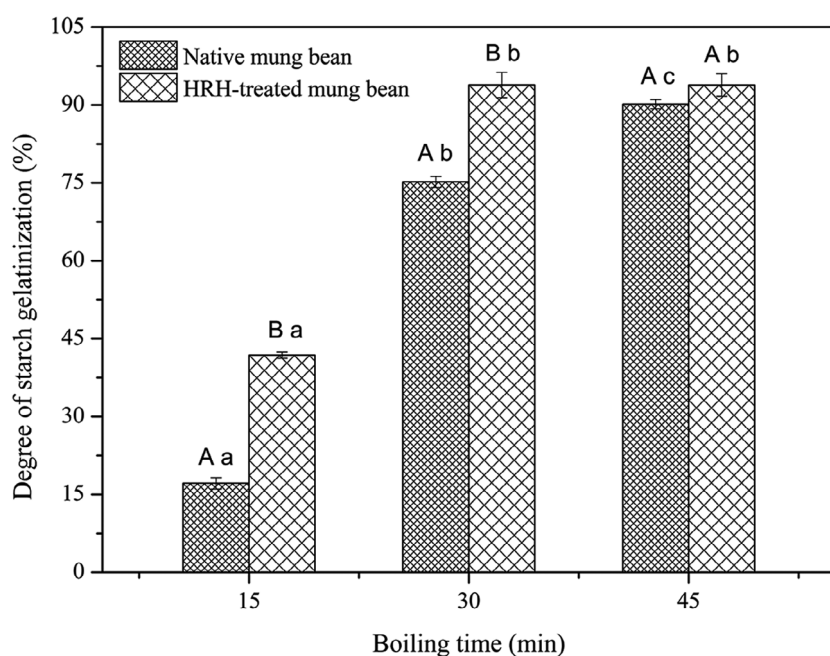
T₀ (Onset temperature), T_p (endothermic peak temperature), ΔH, (gelatinisation enthalpy), PV, (peak viscosity), TV (trough viscosity), (FV) final viscosity, BDV (breakdown viscosity), SBV (set-back viscosity), Pt (peak time), PT (pasting temperature).

pasting properties should be concerned. However, the quality characteristics of HRH-treated mung bean-correlated products need further research.

The degree of starch gelatinisation of mung bean under different cooking time

It is well known that mung bean needs longer time to cook thoroughly in the boiling water than cereals, which limited their consumption and application to some extent. Figure 3 has shown that the starch gelatinisation degrees of native and HRH-treated mung bean seeds were increased with the increasing cooking

time. Besides, the starch gelatinisation degree of HRH-treated mung bean was significantly higher ($P < 0.05$) than native mung bean within 30 min. However, there were no significant differences in the starch gelatinisation degrees of HRH-treated mung bean with 30 min and 45 min boiling time. Briefly, the HRH-treated mung bean is easier to cook thoroughly than native mung bean, and the cooking time would be shortened by 15 min. On the one hand, as we described above (3.5), mung bean starch has been partially gelatinised in advance under HRH treatment. Therefore, less starch in HRH-treated mung bean needed to be gelatinised during cooking. On the other hand, the dense

**Figure 3** Starch gelatinization degree of native and HRH-treated mung bean under different boiling time.

structure of mung bean coat was destroyed under HRH treatment Supplement Fig. 3 b), which made it easier to absorb water and provided sufficient moisture for starch gelatinisation quickly.

Conclusion

The GABA content of mung bean was more than 60 times higher than that of native mung bean (from 1.73 to 109.78 mg 100 g⁻¹) under HRH treatment conditions (tempering: 16.72% moisture, 25 °C, 5 h; HRH treatment: 98% RH, 68.97 °C for 5.34 h). Meanwhile, the contents of some FAAs in mung bean, such as aromatic (Tyr and Phe), sulphur-containing (Cys) and branched-chain amino acids (Leu and Leu), were significantly increased ($P < 0.05$) under HRH treatment, while Non-EAA (Glu, Arg and Asp) was significantly decreased ($P < 0.05$). Besides, although the content of TAC in mung bean had a significant decrease ($P < 0.05$) after HRH treatment, the TPC, vitexin and isovitexin contents and antioxidant activities had a significant increase ($P < 0.05$). In terms of physicochemical properties, HRH treatment did not change the particle morphology of mung bean, but caused the wrinkled structure of mung bean coat, significantly changed its internal micro-structure and increased pasting viscosity, which eventually caused the cooking time of HRH-treated mung bean was shortened by 15 min compared with native mung bean. Our results provide a comprehensive understanding of HRH-treated mung bean and will be useful in promoting its consumption, especially for chronic patients and consumers with a fast-paced life.

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Author Contribution

Yuling Ma: Investigation (lead); Software (lead); Writing-original draft (lead); Writing-review & editing (lead). **Li-Tao Tong:** Data curation (lead). **Shanshan Wang:** Formal analysis (lead). **Tingyu Liu:** Methodology (lead). **Lili Wang:** Visualization (lead). **Liya Liu:** Writing-review & editing (equal). **Xianrong Zhou:** Project administration (equal). **Sumei Zhou:** Conceptualization (lead); Funding acquisition (lead); Project administration (lead); Resources (lead); Supervision (lead); Writing-review & editing (equal). **Christophe Blecker:** Project administration (equal); Supervision (equal); Validation (equal); Writing-review & editing (equal).

Ethical Guidelines.

Ethics approval was not required for this research.

Conflict of interest.

The authors declare no conflict of interest.

Peer review

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Data availability.

Research data are not shared.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Supplementary Material