



## Preparation of resveratrol-enriched and poor allergic protein peanut sprout from ultrasound treated peanut seeds



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### ABSTRACT

Peanut sprout is a kind of high quality natural food which has important effect on health-care. It contains abundant bioactive substances such as resveratrol and lower fat. Naturally, resveratrol occurs in stilbene phytoalexin phenolic compound produced in response to a variety of biotic and abiotic stresses. In this study, the influence of ultrasonic stimulation on the resveratrol accumulate in germinant peanut prepared from three varieties (FH12, FH18, and BS1016) in the dry state before steeping were investigated. All experiments were performed using an ultrasonic cleaner bath operating at three frequencies (28, 45 and 100 kHz) for 20 min at constant temperature 30 °C. The resulted amounts of resveratrol in peanut sprout were increasing by 2.25, 3.34, and 1.71 times compared with the control group of peanut germinated from FH12, FH18, and BS1016, respectively, after 3d with decreasing the amounts of allergic protein. After ultrasound, the germination rate and total sugar content increased slightly while the crude fat decreased and protein remained unchanged. Overall, the study results indicated that ultrasound treatment combined with germination can be an effective method for producing enriched-resveratrol and poor allergic protein peanut sprout as a functional vegetable.

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## 1. Introduction

Peanuts are important dietary food source of resveratrol (3,4',5-trihydroxystilbene) with potent antioxidant properties implicated in reducing risk of cancer, cardiovascular and Alzheimer's disease, and delaying aging [1,2]. Over the last 10 years, peanut has been attracted extensive interests of the public due to its benefits to human health. In recent years, resveratrol has been detected in edible peanut and commercial peanut commodities [3–8]. Resveratrol contents in peanut and peanut-related products are varied as affected by cultivar and growth stage of peanuts. During normal cultivation, the increase of resveratrol in peanut was observed after 9 days germination [8]. Thus, the author proposed that it is essential to prepare peanut sprout as a functional vegetables. Resveratrol is a naturally occurring stilbene phytoalexin phenolic compound produced in response to biotic stresses, such as microbial invasion [9] and abiotic stress, such as injury, UV

irradiation and ultrasound [2]. The amounts of resveratrol in peanut kernels were shown to increase in response to the treatment of UV irradiation and ultrasound [6,10]. The increased amount of resveratrol in peanut kernels can be directly linked to the production of resveratrol-enriched peanut products.

Peanut allergy is one of the most severe food allergies due to its life-threatening nature and persistency [11]. Many methods have been applied to control the peanut allergy. However, the major lupine allergens possess high thermal resistance, which was only slightly affected by microwave, boiling, and extrusion-cooking [12]. Furthermore, it was reported that roasted peanuts are more allergenic than raw peanuts [13]. Besides, more recent study showed that autoclaving could decreased the IgE-binding capacity of peanut allergens [14]. Therefore, it is of great significance to find new approaches, instead of thermal processing in order to decrease the allergy in peanut or peanut and its products.

Ultrasound is an emerging technology in the food synthesis of bioactive compounds. Previously, ultrasound could increase the amount of resveratrol in peanuts, especially in sliced peanut [15]. In grapes juice prepared from ultrasound treated grape cultivars, for example it was increased 1.53, 1.15 and 1.24 times in grape

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Campbell Early, MBA and Kyoho, respectively [16]. In addition, the ultrasound treatment increased the amount of taxol by 3 times in *Taxus baccata* cell culture [17], shikonin by 60–70% in *Lithospermum erythrorhizon* cell culture [18] and ginsenoside saponins by 75% in ginseng cell [19]. Further, it was observed that ultrasound have profound effects on the extraction efficiency of bioactive compounds. According to Chukwumah et al. [20], the highest amounts of resveratrol and biochanin were obtained in peanut after 150-min ultrasound-assisted extraction. Regarding to its functionality, ultrasounds-assisted has been proven to be three times faster than conventional methods for the isolation of ginsenosides (tripentene saponins) from various types of ginseng roots Wu et al. [21]. Ultrasound at low intensity has been shown to have sublethal effects due to cavitation, formation of gas, or vapor bubbles after ultrasound, which increases the biosynthesis of secondary metabolites, membrane permeability, and changes cell morphology.

Therefore, in this study, we investigated the effect of ultrasound on the amounts of resveratrol and allergic protein in peanut sprout prepared from three different peanut cultivars. To the best of our knowledge, this study is the first to report increased amounts of resveratrol and decrease amount of allergic protein in peanut sprout following ultrasound of peanut seeds.

## 2. Materials and methods

### 2.1. Peanuts and chemicals

Three varieties of peanut (*Arachis hypogaea L.*) cultivars Fuhua12 (Tang8252 × Luhua9, FH12), Fuhua 18 (Jihua4 × Tang8252, FH18), and Baisha1016 (Shitouqi × Fuhuasheng, BS1016) were selected from Liaoning Province of China and used for this study. All the selected peanuts cultivars were supplied by Liaoning Academy of Agricultural Sciences in the middle of October, 2013. Standard trans-resveratrol was obtained from Solarbio (Solarbio Science & Technology Co., Ltd, Beijing, China), and stock trans-resveratrol solution was kept at  $-20^{\circ}\text{C}$ . The chemicals and solvents used for analysis in this study were of analytical or high performance liquid chromatography (HPLC)-grade and purchased from Sigma–Aldrich (St. Louis, MO, USA) or Solarbio (Solarbio Science & Technology Co., Ltd, Beijing, China).

### 2.2. Ultrasound treatment

The raw peanuts used were stored in the refrigerator at  $4^{\circ}\text{C}$  for 3 months. All processing implements and surfaces were washed and sanitized with 1% sodium hypochlorite prior to use. About 100 seeds were washed twice with 500 mL de-ionized water, drained, surface sanitized in 500 mL of 1% sodium hypochlorite solution for 15 min, and rinsed with sterilized deionized water. Similar-sized seeds were selected for treatment with or without ultrasound treatment. The selected peanuts were treated by ultrasound at 28, 45 and 100 kHz using an ultrasonic cleaner bath (KQ-300VDV, overall dimensions:  $410 \times 350 \times 420$  mm, tank dimensions:  $300 \times 240 \times 180$  mm, weight: 12 kg, Kunshan Ultrasonic Instrument Co., Ltd, China). Then, 100 peanut seeds per batch were put into a 500 mL beaker which carried 400 mL sterilized deionized water for disinfection. A total of ten groups' peanuts were subjected to ultrasound at three frequencies 28, 45 and 100 kHz, for 15, 20 and 30 min. After ultrasound treatment, peanuts were steeping for 6 h at  $25^{\circ}\text{C}$  in the dark place. Same procedures were followed for the control samples, excluding the ultrasound treatment. Application of ultrasound treatment and all subsequent analyses were conducted in dark place to avoid iso-

merization of trans-resveratrol to cis-resveratrol as described by Trela et al. [22].

### 2.3. Peanut sprout preparation

Each batch of treated peanut kernels (in Section 2.3), after ultrasound or control were placed on a Ceramic tray and germinated under dark in a growth chamber (RDN-300G, NBDN Co., Ningbo, China) at  $28^{\circ}\text{C}$  and 90% relative humidity for 5 days. After 5 days of germination, the number of sprouts for each batch was counted, as calculating the germination rate.

### 2.4. Sample pretreatment

For each batch, 100 visibly sound kernels were subjected to germination as that described above. After 0, 1, 2, 3, 4, and 5 days of germination, each peanut cultivar (placed in a tray) was harvested and divided evenly into three sublots. From each subplot, twelve kernels or sprouts were randomly sampled, weighed, and followed by lyophilization (LGJ-25, Beijing sihuan scientific instrument factory co., LTD, Beijing, China). After lyophilization and weight determination, the dried materials were pulverized with a cyclone mill to prepare whole sprout powders. The powders were sealed in polyethylene plastic bags and stored under  $-40^{\circ}\text{C}$  for resveratrol, allergic protein, and compositional analyses.

### 2.5. Analysis of resveratrol

The extraction of resveratrol in peanut sprout was conducted according to the method described by Chen [23], with slight modification on ratio of material to solvent. 0.1 g of dehydrated peanut sprout was deposited into a 10 mL centrifuge tube. The powder was homogenized with 5 mL of 80% methanol and shocked blending with vortex mixer. The tubes were screw-capped, heated in a water bath at  $80^{\circ}\text{C}$ , and shaken occasionally for 45 min. After centrifugation (8000 r/min, for 15 min, at  $20^{\circ}\text{C}$ ), the supernatants were filtered through a  $0.45\ \mu\text{m}$  syringe filter for HPLC analysis.

The resveratrol were determined as previously described by Hasan [12] with minor modification. HPLC was performed using an Agilent 1100 Series HPLC system (Agilent Technologies, Palo Alto, CA) equipped with a UV detector (Young-Lin, YL9100, Seoul, Korea) and a C18 reversed column ( $4.6\ \text{mm} \times 150\ \text{mm}$ ,  $5\ \mu\text{m}$ ). The wavelength and column temperature were set at 320 nm and  $40^{\circ}\text{C}$ , respectively. The mobile phase was a mixture of methanol: 20 mM phosphoric acid (20:80) and methanol: 20 mM phosphoric acid (80:20) in the ratio of 70:30 (v/v). The injection volume was  $20\ \mu\text{L}$  with the flow rate of 1 mL/min. The amounts of resveratrol in the peanut sprout samples were calculated using the regression curve, which was calculated from the peak areas of resveratrol generated by analysis of various concentrations of the standard.

### 2.6. Analysis of allergic protein

The content of allergic protein was measured according to the enzyme-linked immunosorbent assay (ELISA) method [24]. 1 g of peanut sprout flour was mixed with 20 mL phosphate buffer (10 mmol/L) preheated to  $60^{\circ}\text{C}$ . The mixture was extracted at heat  $60^{\circ}\text{C}$  for 10 min, by stirring. After centrifugation (2500 r/min, for 20 min, at  $4^{\circ}\text{C}$ ), the supernatants were diluted with phosphate buffer (10 mmol/L) at a 1:4 v/v ratio and recovery to room temperature. After extraction, peanut allergen was measured by using a commercially available test kit targeting a specific peanut allergen (Tepnel BioSystems, UK). The ELISA test kits were used according

to the manufacturer's instructions. The range of detection of the assay was from 2.5 to 25  $\mu\text{g}/\text{mL}$  (g). All the tests were performed in triplicate.

### 2.7. Analysis of total sugars, crude fat, and crude protein

The total soluble sugar content was determined using vitriolic acid-phenol method [25], with slightly modification. About 15 mg of sprout powder was placed in an anaerobic jar with 15 mL of deionized water. It was then hydrolyzed using vitriolic acid at the room temperature for 3 h and heated in a vacuum oven at 105 °C for 4 h. Finally, it was determined by using UV-3010 spectrophotometer.

The crude fat content was determined using Soxhlet extraction as described by Wilkin with minor modifications [26]. Approximately 1 g of sprout powder was placed in a thimble and refluxed for 2 h using petroleum ether (60–90 °C) as the solvent. After 2 h, the solvent was recovered and the residue was weighed.

The crude protein content was analyzed according to Kjeldahl method with minor modifications [26]. Approximately 25 mg of sprout powder was enclosed in foil and subject to pyrolysis. The protein conversion factor from nitrogen to protein is 5.46, which was used to calculate the protein content of the samples.

### 2.8. Statistical analysis

The statistical significance was assessed by one-way ANOVA followed by Tukey test at a probability ( $p$ ) of 0.05 using the Microcal Origin software (V8.0). The treatments were performed in triplicate. All data are presented as mean  $\pm$  standard deviation.

## 3. Results and discussion

Ultrasound treatment was applied to peanut seeds prior to preparation of resveratrol-enriched peanut sprout. The resveratrol in the peanut sprout was identified based on comparison to the retention time of standard resveratrol by HPLC analysis (Fig. 1).

### 3.1. Effect of ultrasound treatment on germination rate in peanut sprout

As shown in Table 1, the germination rate varied greatly depending on the peanut cultivars (FH12, FH18, and BS1016) and ultrasound frequencies (28, 45, and 100 kHz). It shows that germination rate was most high for 100 kHz ultrasonic waves treated peanut kernels, especially for cultivar peanut FH18. After 5 days of germination of the peanut kernels for sprout preparation, germination rate of the controls and 100 kHz ultrasonic waves treated peanut were 89.7% and 94.06% for FH12, 89.35% and 90.47% for BS1016, and 87.97% and 94.01% for FH18, respectively. Since seed viability is essential for the kernels as destined for peanut sprout preparation, the use of ultrasound treatment is valuable to enhance seed viability. This result was in accordance with previous study that ultrasound treatment increased the growth of cultured *Panax ginseng* cells through mechanical stress and microstreaming induced by acoustic cavitation, which disrupts the cell wall [14].

### 3.2. Optimized conditions for ultrasound and germinal treatment

Through the single-factor conditions test of FH12, the optimum condition in which the soaking time and ultrasound time were found (Table 2 and Fig. 2). We investigated the effect of ultrasound time (0, 10, 20, 30, 40 min) and soaking time (2, 4, 6, 8, and 10 h) respectively for accumulating resveratrol. The resveratrol content

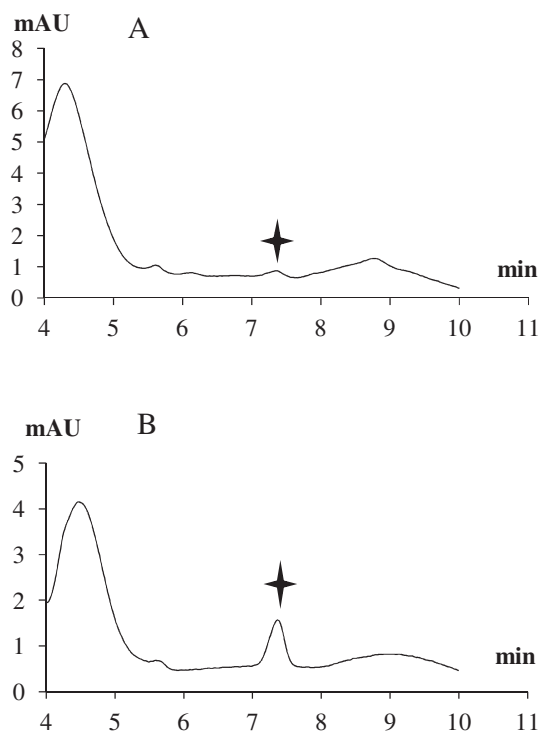


Fig. 1. HPLC chromatogram of resveratrol in peanut prepared from control (A) and ultra-sonication-treated FH12 peanut for 1d germination (B).

Table 1

The effect of ultrasound treatment on germination rate in peanut.

Peanut variety	Treatments	Germination rate (%)
FH12	CK	89.71 $\pm$ 1.34 <sup>a</sup>
	US-28	91.98 $\pm$ 0.56 <sup>a</sup>
	US-45	90.90 $\pm$ 0.47 <sup>ab</sup>
	US-100	94.06 $\pm$ 0.86 <sup>b</sup>
FH18	CK	87.97 $\pm$ 1.51 <sup>a</sup>
	US-28	91.30 $\pm$ 0.88 <sup>ab</sup>
	US-45	92.43 $\pm$ 1.41 <sup>ab</sup>
	US-100	94.01 $\pm$ 2.66 <sup>b</sup>
BS1016	CK	89.35 $\pm$ 0.78 <sup>a</sup>
	US-28	89.99 $\pm$ 0.20 <sup>a</sup>
	US-45	88.75 $\pm$ 1.22 <sup>a</sup>
	US-100	90.75 $\pm$ 2.50 <sup>a</sup>

<sup>a</sup>Data are expressed as the mean  $\pm$  SE of triplicate samples. Different superscript letters within a column indicate significant differences ( $p < 0.05$ ).

of whole sprouts changed significantly as the ultrasound time and soaking time increasing. Consequently, we selected the optimum ultrasound treatment 20 min and soaking 6 h condition in the following experiment.

### 3.3. Effect of ultrasound treatment on resveratrol content in peanut sprout

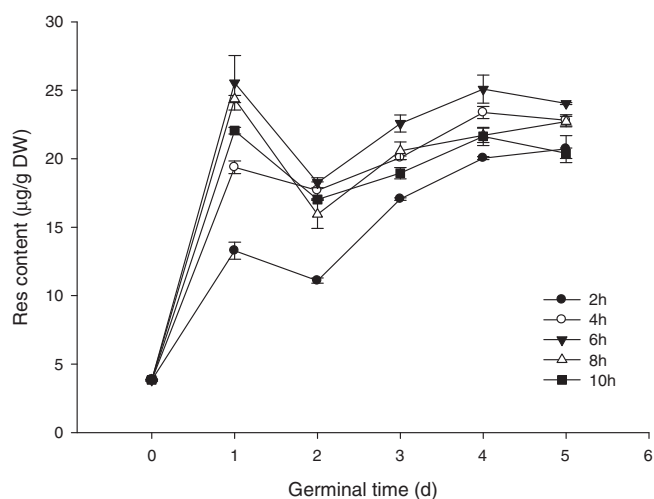
Significantly higher amount of resveratrol was observed in peanut sprout prepared from peanut treated by ultrasound for all peanut varieties (Fig. 3). The resveratrol content of whole sprouts increased significantly ( $p < 0.05$ ) with an increase of ultrasound frequency and germination time. Obviously, the resveratrol content increased fastest in the sprouts prepared from the 100 kHz ultrasonic waves treated peanut. After 3 days of germination, the resveratrol content reached to the highest value in sprouts for all varieties. The resveratrol contents of peanut sprout prepared from

**Table 2**

Changes of resveratrol content in peanut sprout prepared from different ultrasound treatments during germination.

Ultrasound frequencies (kHz)	Ultrasound time (min)	Resveratrol content ( $\mu\text{g/g DW}$ )				
		1d	2d	3d	4d	5d
28	10	$9.72 \pm 0.47^b$	$7.62 \pm 0.76^a$	$16.10 \pm 0.31^c$	$22.05 \pm 1.04^d$	$22.92 \pm 0.41^d$
	20	$25.55 \pm 1.41^c$	$18.25 \pm 0.25^a$	$22.57 \pm 0.63^b$	$25.08 \pm 0.72^{bc}$	$24.03 \pm 0.05^{bc}$
	30	$14.19 \pm 0.81^a$	$13.88 \pm 1.83^a$	$19.00 \pm 1.06^b$	$17.54 \pm 1.32^{ab}$	$19.41 \pm 0.59^b$
45	10	$9.59 \pm 0.25^a$	$10.80 \pm 0.16^b$	$19.07 \pm 0.31^c$	$22.61 \pm 0.49^d$	$24.36 \pm 0.31^e$
	20	$23.46 \pm 0.44^b$	$13.21 \pm 1.88^a$	$31.38 \pm 1.34^c$	$23.96 \pm 0.39^b$	$24.70 \pm 0.31^b$
	30	$19.97 \pm 0.12^{bc}$	$13.03 \pm 0.43^a$	$19.00 \pm 1.06^b$	$21.01 \pm 0.02^c$	$23.29 \pm 0.30^d$
100	10	$8.56 \pm 0.29^a$	$10.11 \pm 0.25^b$	$20.96 \pm 0.37^c$	$21.05 \pm 0.60^c$	$9.30 \pm 0.25^d$
	20	$16.17 \pm 0.23^a$	$15.06 \pm 0.37^a$	$27.83 \pm 0.77^d$	$22.74 \pm 0.27^b$	$25.45 \pm 0.64^c$
	30	$10.36 \pm 0.10^a$	$11.70 \pm 0.36^b$	$16.72 \pm 0.28^c$	$19.29 \pm 0.27^d$	$20.49 \pm 0.54^d$

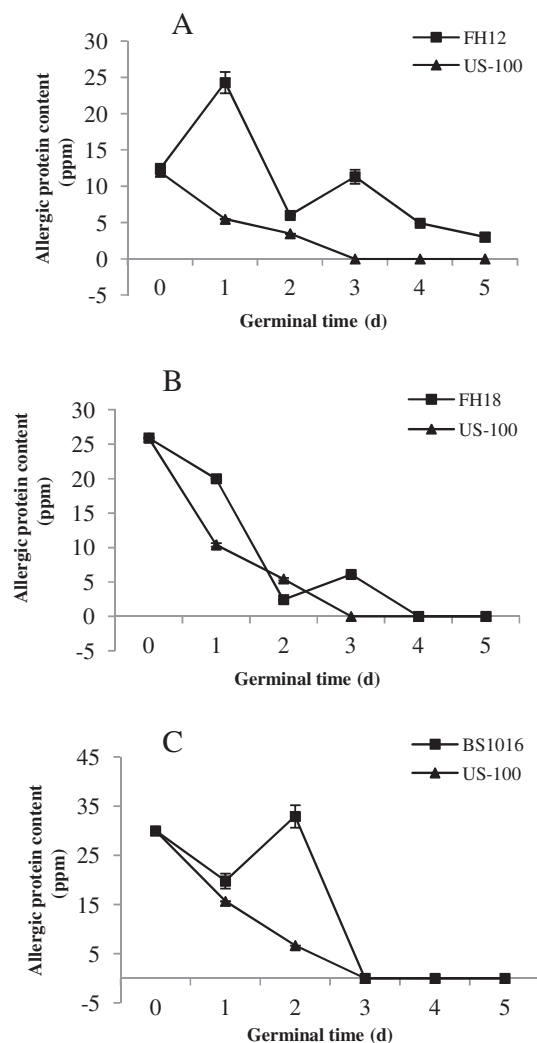
Data are expressed as the mean  $\pm$  SE of triplicate samples. Different superscript letters within a column indicate significant differences ( $p < 0.05$ ).



**Fig. 2.** Changes of resveratrol content during germination in peanut sprout prepared from different soaking time.

ultrasound-treated peanut were increase from 3.89 to 31.38, 2.32 to 25.06, and 1.66 to 13.28  $\mu\text{g/g}$ , which were 3.32, 3.34, and 1.71 times higher than that in controls for the corresponding peanut cultivars FH12, FH18, and BS1016. The highest resveratrol content was detected in FH12 peanut sprout and lowest in BS1016 peanut sprout. It was revealed that resveratrol contents deviated considerably as affected by cultivar and individual sprout. This finding corresponds with previous studies that resveratrol contents varied greatly depending on peanut cultivar or variety and growth stages [8,27] (Table 3).

To our knowledge, no studies have investigated the amount of resveratrol in peanut sprout prepared following ultrasound-assisted cleaning of peanut kernels. We previously found an increase in the amount of resveratrol in the peanut kernels treated with ultrasound for 4 min followed by 24 h incubation in dark at 25 °C [11]. Most of recent studies found a higher resveratrol concentration achieved when peanuts treated with ultrasound using different parameters [10,28]. In addition, ultrasound has been used to increase resveratrol content in fruits, such as grape skin and grape juice [12,29]. Therefore, in this study we applied the ultrasound treatment to prepare resveratrol-enriched peanut sprout using different peanut varieties. There was a dispute about explaining the increase of resveratrol in peanuts after exposure to ultrasound. In one respect, it was concluded that the ultrasound caused rapid increase in the concentrations of phenylammonia



**Fig. 3.** Changes of allergen Ara h 1 content in peanut sprout prepared from ultrasound treated peanut of cultivars FH12 (A), FH18 (B), BS1016 (C) during germination.

lyase, which could be responsible for increased concentrations of resveratrol in ultrasound-treated peanuts [2]. While in another study, it was pointed out that the increase of trans-resveratrol in peanuts after exposure to ultrasound can be attributed to induced plant cell defense responses, as demonstrated by the production of

**Table 3**  
Changes of resveratrol content in peanut sprout prepared from ultrasound treated peanut of cultivars FH12, FH18, BS1016 during germination.

Peanut variety	Treatments	Resveratrol content ( $\mu\text{g/g DW}$ )					
		0d	1d	2d	3d	4d	5d
FH12	CK	3.84 $\pm$ 0.04 <sup>a</sup>	4.02 $\pm$ 0.11 <sup>a</sup>	7.17 $\pm$ 0.64 <sup>b</sup>	9.74 $\pm$ 0.31 <sup>c</sup>	21.47 $\pm$ 1.04 <sup>d</sup>	23.43 $\pm$ 0.93 <sup>d</sup>
	US-28		23.46 $\pm$ 0.44 <sup>c</sup>	13.21 $\pm$ 1.88 <sup>b</sup>	31.38 $\pm$ 1.34 <sup>d</sup>	23.96 $\pm$ 0.39 <sup>c</sup>	24.70 $\pm$ 0.31 <sup>c</sup>
	US-45		16.17 $\pm$ 0.23 <sup>b</sup>	15.06 $\pm$ 0.37 <sup>b</sup>	27.83 $\pm$ 0.77 <sup>c</sup>	22.74 $\pm$ 0.27 <sup>c</sup>	25.45 $\pm$ 0.64 <sup>d</sup>
	US-100		25.55 $\pm$ 1.41 <sup>d</sup>	18.25 $\pm$ 0.25 <sup>b</sup>	22.57 $\pm$ 0.44 <sup>c</sup>	25.08 $\pm$ 0.72 <sup>d</sup>	24.03 $\pm$ 0.05 <sup>c</sup>
FH18	CK	2.32 $\pm$ 0.07 <sup>a</sup>	2.73 $\pm$ 0.05 <sup>ab</sup>	4.01 $\pm$ 0.14 <sup>b</sup>	7.23 $\pm$ 0.28 <sup>c</sup>	11.05 $\pm$ 0.60 <sup>d</sup>	20.75 $\pm$ 0.70 <sup>e</sup>
	US-28		12.80 $\pm$ 0.21 <sup>b</sup>	8.90 $\pm$ 0.43 <sup>c</sup>	18.85 $\pm$ 0.73 <sup>c</sup>	14.56 $\pm$ 0.70 <sup>d</sup>	19.58 $\pm$ 0.76 <sup>d</sup>
	US-45		13.23 $\pm$ 0.20 <sup>b</sup>	16.82 $\pm$ 0.53 <sup>d</sup>	15.31 $\pm$ 0.08 <sup>c</sup>	16.31 $\pm$ 0.32 <sup>cd</sup>	19.66 $\pm$ 0.33 <sup>e</sup>
	US-100		16.80 $\pm$ 0.21 <sup>b</sup>	17.25 $\pm$ 0.26 <sup>b</sup>	25.06 $\pm$ 0.63 <sup>c</sup>	22.02 $\pm$ 0.96 <sup>d</sup>	21.35 $\pm$ 1.23 <sup>d</sup>
BS1016	CK	1.66 $\pm$ 0.03 <sup>a</sup>	2.55 $\pm$ 0.05 <sup>ab</sup>	4.41 $\pm$ 0.24 <sup>bc</sup>	6.43 $\pm$ 1.36 <sup>cd</sup>	7.83 $\pm$ 0.34 <sup>de</sup>	9.30 $\pm$ 0.14 <sup>e</sup>
	US-28		5.92 $\pm$ 0.34 <sup>b</sup>	6.87 $\pm$ 0.18 <sup>c</sup>	12.22 $\pm$ 0.36 <sup>f</sup>	9.05 $\pm$ 0.07 <sup>d</sup>	10.03 $\pm$ 0.15 <sup>e</sup>
	US-45		8.55 $\pm$ 0.32 <sup>d</sup>	5.64 $\pm$ 0.08 <sup>b</sup>	6.90 $\pm$ 0.23 <sup>c</sup>	9.52 $\pm$ 0.33 <sup>e</sup>	9.615 $\pm$ 0.38 <sup>e</sup>
	US-100		8.15 $\pm$ 0.18 <sup>b</sup>	10.30 $\pm$ 0.57 <sup>c</sup>	13.28 $\pm$ 0.29 <sup>d</sup>	10.98 $\pm$ 0.55 <sup>c</sup>	13.06 $\pm$ 0.18 <sup>d</sup>

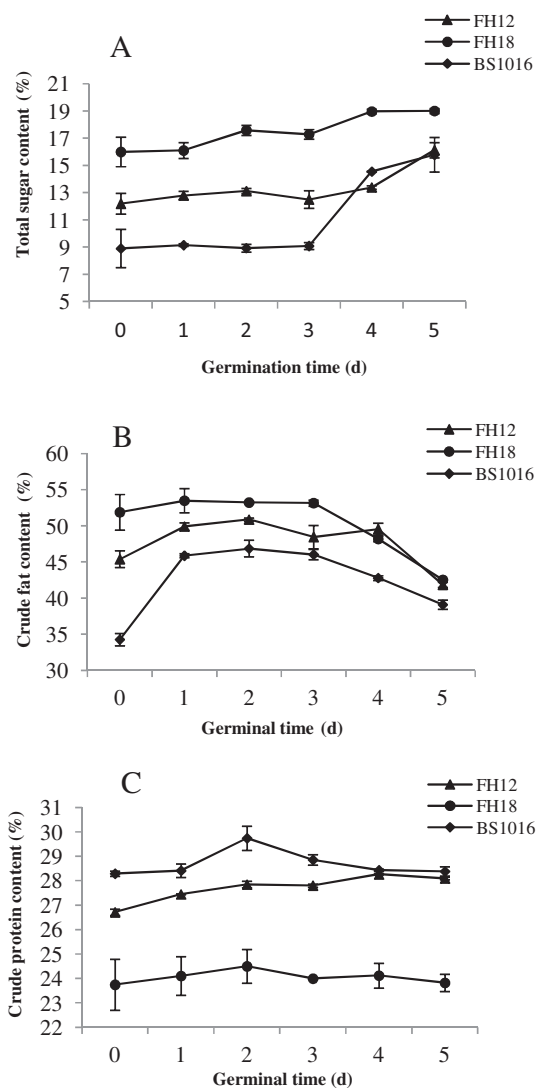
Data are expressed as the mean  $\pm$  SE of triplicate samples. Different superscript letters within a column indicate significant differences ( $p < 0.05$ ).

$\text{H}_2\text{O}_2$  [30]. The states above could make an explanation for the increasing of resveratrol in peanut sprout prepared from ultrasound-treated peanuts.

#### 3.4. Effect of ultrasound treatment on allergic protein content in peanut sprout

Peanut allergy is the food allergen most capable of causing severe, life-threatening, and even fatal allergic reactions, which is an enormous clinical problem [24]. Food allergy is difficult to control; avoidance is the only available treatment for food allergy at this time. In this study, the change of allergic protein content in controls and 100 kHz ultrasonic waves treated peanut during germination time was studied. It was observed that allergic protein content in the ultrasound-treated samples was reduced faster than that in controls for all peanut varieties (Fig. 3A–C). Specially, after 3 days of germination, the allergic protein content in controls decreased from 11.96 to 10.65, 25.93 to 6.11, and 30.02 to 8.62  $\mu\text{g/g}$  for peanut cultivars FH12, FH18, and BS1016, correspondingly. The allergic protein content in the 100 kHz ultrasonic waves treated peanut were degraded completely for all varieties and studied the effect of allergic protein content under 28 and 45 kHz frequencies, but the effects are not so significant compared with 100 kHz. Therefore the author can conclude that ultrasound is a useful method to reduce the allergen allergic protein content in peanut.

To the best of our knowledge, few have been reported on the reduction of allergic protein in the peanut by ultrasound. Previous studies suggested that autoclaving produces an important decrease in allergenicity of roasted peanut due to changes in the structure of the proteins [21]. It was demonstrated that most of the  $\alpha$ -helical structure was lost after autoclave treatments. This is because many of the IgE binding epitopes in the major allergens of peanuts (i.e. Ara h 1, Ara h 2 and Ara h 3) are located on the  $\alpha$ -helical regions of these proteins [21,31]. Whereas, it has been recognized that thermal processing (roasting) enhanced the allergenicity of peanut by protein modification, which contributed to Maillard reaction [13]. However, in this study, the treatment on peanut was different from previous studies, which maintained the viability of peanut seeds. After ultrasound treatment, the allergic protein content was not change instantly. The decrease of allergic protein content was observed in 100 kHz ultrasonic waves treated peanut during germination time, and decreased faster than that in controls. It is known that ultrasound increased the levels of enzymes, phenylammonia lyase, polyphenol oxidase, and peroxidase in ginseng cell



**Fig. 4.** Changes of total sugar (A), crude fat (B), and crude protein (C) contents in ultrasound treated peanut during germination.

cultures [18]. Therefore, we were supposed not to do with the modification of protein, instead promoted the synthesis of allergenicity hydrolysis protease.



### 3.5. Effect of ultrasound treatment on total sugar, crude fat, and crude protein contents in peanut sprout

Protein, fat and sugar are important factors for maintenance of the quality of peanut. In this study, changes of crude protein, crude fat, and total sugar content were detected in 100 kHz ultrasonic waves treated peanut during germination. We also investigated the protein, fat and sugar content under 28 and 45 kHz frequencies, but there were no significances vs. 100 kHz. The total sugar contents in of peanut cultivars differs from various peanut cultivars and germination time (Fig. 4). The total sugar content of peanut kernels increased significantly with an increase of germination time. As shown in Fig. 4, total sugar content remained largely unchanged in the early 3 days, while it increased steadily thereafter. After 5 days of germination, total sugar content contents increased from 12.18% to 16.12%, 16.00% to 19.01%, and 8.99% to 15.79% for peanut cultivars of FH12, FH18, and BS1016, correspondingly. This result resembles the observation of Wang [8], who reported that the sucrose and glucose contents of peanut kernels after 9 days germination time increased remarkably.

Change of crude fat in the peanut sprout prepared from 100 kHz ultrasonic waves treated peanut during germination as affected by germination were very significant (Fig. 4). Before germination, the highest crude fat content was observed in the kernels of FH18, followed by kernels of FH12 and BS1016. In general, the crude fat contents of the peanut kernels increased significant in the 3 days of germination and decreased afterward. Specially, after 5 days of germination, the crude fat was decrease from 45.38% to 41.84%, 51.88% to 34.26%, and 34.26% to 33.10% (w/w) corresponding to peanut cultivars FH12, FH18, and BS1016.

As shown in Fig. 4, the germination didn't have significant effect on the crude protein content. The crude protein varied as affected by the peanut cultivars. This is contrary to the observation of Wang [8] who reported that the protein decreased during germination time. This difference may be caused by different treatments applied on peanut.

## 4. Conclusion

In this study, it was shown that ultrasound had profound effects on resveratrol and peanut allergy in peanut sprout at 100 kHz for 20 min. An increase of resveratrol was occurred when peanut sprout was exposed to postharvest stress by ultrasound and germination, with the decreasing of peanut allergy. Additionally, the germination rate of the peanut kernels was increased after ultrasound treatment. The total sugar in ultrasound treatment peanut increased slightly while the crude fat decreased and protein remained unchanged during germination. Therefore, the use of ultrasound treatment has the potential for application in the commercial production of resveratrol-enriched and poor allergic protein peanut sprout. Further development is needed to investigate the mechanism for the increase of resveratrol and loss of allergy in peanut.

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