Ultrasonics Sonochemistry 28 (2016) 334–340

Contents lists available at ScienceDirect

Ultrasonics Sonochemistry

journal homepage: www.elsevier.com/locate/ultson

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

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ARTICLE INFO

Article history:
Received 6 January 2015
Received in revised form 30 April 2015
Accepted 12 August 2015
Available online 12 August 2015

Keywords:
Peanut sprout
Ultrasound
Resveratrol
Allergic protein

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.

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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http://dx.doi.org/10.1016/j.ultsonch.2015.08.008
1350-4177/

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 °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 °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 × 350 × 420 mm, tank dimensions: 300 × 240 × 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 °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 isomerization 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 °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 sublot, 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 °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 °C, and shaken occasionally for 45 min. After centrifugation (8000 r/min, for 15 min, at 20 °C), the supernatants were filtered through a 0.45 μm 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 mm × 150 mm, 5 μm). The wavelength and column temperature were set at 320 nm and 40 °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 μL 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 °C. The mixture was extracted at heat 60 °C for 10 min, by stirring. After centrifugation (2500 r/min, for 20 min, at 4 °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