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Moderate vacuum packing and low temperature effects on qualities of harvested mung bean (*Vigna radiata* L.) sprouts

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ABSTRACT

Post-harvest browning, decay, lignification, and other adverse physiological effects limit the shelf-life of mung bean sprouts at ambient temperature. In this study, vacuum packing at pressures of 0, ↓0.02, ↓0.04, and ↓0.06 mPa at 20 °C, and the optimum pressure of ↓0.04 mPa at 4 °C for maintenance of sprouts, was investigated. Chlorogenic acid was identified as the main phenolic compound leading to the browning of mung bean sprouts, along with total flavonoids, total phenols, rutin, and *o*-phthalic acid. Among these, flavonoids were identified as being the main substrate for polyphenol oxidase (PPO) activity. Moderate vacuum packing combined with low temperature storage inhibited PPO, as well as peroxidase (POD) and superoxide dismutase activities, which was associated with a slower rate of browning. In addition, these storage conditions reduced the activity of phenylalanine ammonia lyase (PAL), thereby suppressing the synthesis of phenolic substrates used in the browning reaction. Moderate vacuum packing combined with low temperature storage also maintained the activities of the cell wall-degrading enzymes cellulase and xylanase, and suppressed POD and PAL activities. Together, these effects would be expected to lead to a decrease in cellulose, hemicellulose, and lignin levels, ultimately restricting sprout elongation and maintaining the firmness of the sprouts.

1. Introduction

Mung bean (*Vigna radiata* L. Wilczek), also known as the cyan adzuki bean, is mainly grown in tropical, subtropical, and temperate regions. They are rich in protein, with edible seeds that are enriched in lysine, an essential amino acid in humans (Goyal and Siddiqu, 2014). Germination is an economical and effective method for improving the nutritional value and health-promoting properties of cereals, pseudocereals, cruciferous vegetables, and legumes (Świeca and Gawlik, 2015). The sprouts have a relatively high water content (up to 95%); they are tender, with a fine texture and delicate structure, which makes them susceptible to damage that can affect the sensory properties and nutritional value, and compromise the shelf-life, leading to large economic losses for producers. Low-storage temperatures extend the shelf-life of mung bean sprouts for up to 3 d (Jeon et al., 2010). Additional storage technologies have been evaluated. Gorris et al. (1994) demonstrated that vacuum packing and storage at a temperature below 7 °C could maintain the quality of mung bean sprouts, and inhibit growth of microorganisms. Similarly, DeEll et al. (2000) showed that the morphology of mung bean sprout was maintained under 4 d of storage after vacuum with pre-cooling treatment. However, they also found that storage temperature had a greater effect than cooling temperature on the quality of mung bean sprout quality, with lower temperatures producing white hypocotyls but darker cotyledons. Nishimura et al. (2012) showed that if the mung bean sprouts are soaked in hot water (50 °C) for 60 s and then stored at 8 °C, inhibition of phenylalanine ammonia-lyase (PAL) activity was associated with lower polyphenols concentrations and browning. Goyal and Siddiqui (2014) assessed the effects of ethanol vapor, a hot water dip, ultraviolet irradiation, or a pulsed electric field as respective pre-treatments before storage on the shelf-life of mung bean sprouts. Both water and ethanol vapor treatments extended sprout shelf-life, while ethanol vapor also inhibited the elongation of the sprouts and resulted in weight gain. Moreover, both treatments delayed the onset of sprout decay, and ethanol vapor treatment reduced the occurrence of non-enzymatic browning.

Polyphenol oxidase (PPO), peroxidase (POD), and PAL are the key enzymes related to browning. The enzymes catalyzing the enzymatic browning reaction vary among different products. For example, PPO is

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the leading enzyme responsible for the browning of freshly cut galangal, whereas POD can catalyze the oxidation of phenolic substances into quinone-containing compounds (Lin et al., 2016). PPO, POD, and PAL activities increase during the maturation process of longkong fruit, which result in pericarp browning with phenolic compounds as the substrates of this reaction (Venkatachalam and Meenune, 2012). Numerous studies have examined the different browning substrates in different plants. Zou and Zhang (2012) found that in the main substrate responsible for browning in pear fruit was chlorogenic acid, whereas Wang et al. (2009) showed that gallic acid was the main PPO substrate in lotus root browning and that tannin was the main substrate in pomegranate peel (Zhang and Zhang, 2008).

In the present study, the effects of vacuum packing with storage at a low temperature, alone and in combination, on various quality parameters were examined to optimize the shelf-life and quality of mung bean sprout.

2. Materials and methods

2.1. Plant material

Mung bean seeds were obtained from Dong Sheng Fang Yuan Agricultural Development (DSFYAD) Co., Ltd., Beijing, China. The seeds were sorted, and any flattened or blackened seeds were removed. The seeds were then washed with tap water three times to remove any small particles, and then soaked in distilled water (80 °C, (m/v))for 3 min, followed by soaking in cold distilled water for 5 h. Finally, the seeds were germinated using an automatic bean sprouting machine (DYJ-K, Shandong, China). The water tank temperature was set at 19 °C and the growth chamber temperature was set at 23 °C. Every 4 h, the seeds were drenched in water for 15 s. Sprouts of length of 7–9 cm were used for later experiments.

2.2. Treatments

2.2.1. Vacuum packing

Sprouts of similar thickness and shape were divided into 39 packets of 150 g each and randomly subdivided into four groups with 9 packets per group; the other three packets were considered as the control samples. Sprouts were packed in polyethylene plastic bags (0.03 mm thickness, 24 cm length, and 20 cm width), which were sealed by a vacuum-packaging machine. The sprouts were stored at 20 ± 2 °C, 85 ± 5% relative humidity and vacuum-packed at 0 mPa, −0.02 mPa, −0.04 mPa, and −0.06 mPa. 0 mPa represents the control with an air pressure of 1 bar in the unsealed bags, while −0.02, −0.04, and −0.06 mPa represent the three vacuum-packing treatments in which the air pressure in the bags was 800, 600, and 400 KPa, respectively. Samples collected at days 1, 2, and 3 were used for the determination of shape and quality indicators. The remaining samples were freeze-dried after liquid nitrogen treatment, ground into a powder, filtered through a 60-mesh sieve, stored at ↓40 °C, and then used for subsequent determinations.

2.2.2. Low temperature

Mung bean sprouts were selected and packed as in 2.2.1. Nine packets were stored at 4 ± 2 °C, and the other nine sprouts were stored at 20 ± 2 °C, 85 ± 5% relative humidity was used for both groups. The remaining sprouts served as control samples with all other conditions equalized. Samples taken on days 1, 2 and 3 were used for determinations of shape and quality indicators. The remaining samples were treated as before.

2.2.3. Vacuum packing combined with low temperature

Sprouts were separated into packets of 150 g each. Six packets of sprouts were taken out randomly as the 0 d samples, and the others were divided into two groups. The first group (control) contained 36 samples, which were not vacuum-treated and stored at 20 ± 2 °C, 85 ± 5% relative humidity, whereas the second group was vacuum-packed under the optimal condition of ↓0.04 mPa, as determined in the previous experiment (Section 2.2.1), and stored at 4 ± 2 °C, 85 ± 5% relative humidity. Control sprouts were taken on days 1, 2, and 3, whereas sprouts stored at low temperature were collected on days 1, 3, 5, and 7. Samples of both groups were used to determine shape and quality indicators. Three randomly selected packets of fresh sprouts from the two groups were dried to constant weight at 75 °C in an oven, ground into powder, sieved through 35-mesh, and then used to determine cell wall components. The remaining samples were had the same treatments as above.

2.3. Quality parameters

2.3.1. Weight loss and relative conductivity

The weight loss was determined by weighing the sprouts prior to and during storage using an electronic balance, and calculated using the following formula:

$$\text{Weight loss}\ (\%) = \frac{\text{Wb}-\text{Wa}}{\text{Wb}} \times 100\,\%$$

where Wa is the weight of sprouts before storage, and Wb is the weight of sprouts after storage.

The relative conductivity value was calculated by the simple soaking method described by Wang et al. (2015).

2.3.2. Total microbial counts and soluble sugar content

Microbial loads were determined using the tablet colony counting method (standard plate count) and pour plate method according to the methods of the National Standard of China (GB 4789.2-2010, 2010) and Goyal and Siddiqui (2014). Results are expressed as the aerobic bacterial count in colony-forming units (CFU) per kilogram (LogCFU kg^{-1}) contained in the sample. The total microbial counts in two consecutive dilutions were calculated according to the following formula:

$$\text{N} = \frac{\sum C}{(n_1 + 0.1n_2)} \times \text{d}$$

Where N is the number of colonies in the sample, n_1 is the tablet number in the lower diluent, n_2 is the tablet number in the higher diluent, and d is the first dilution degree.

The soluble sugar content was determined in accordance with the method of Yemm and Willis (1954). The anhydrous glucose standard solution (100–1000 mg L^{-1}) was used to draw the standard curve. The soluble sugar content is expressed as g kg^{-1} on a dry weight basis.

2.3.3. Hypocotyl firmness

The hypocotyl of the samples was measured by a Texture Analyzer (Pro. CT, America) fitted with a Knife Edge 60 mm on the standard probe. The probe velocity was 0.5 mm/s, and load on trigger point 6.8 g. It was measured was 2 cm at the base of the hypocotyl skin. The results were expressed as Newton (N) (Chen et al., 2017).

2.4. Determination of antioxidant activities

2.4.1. PAL, catalase (CAT), POD, and PPO activities

Crude enzymes were extracted according to the methods described by Zhang et al. (2015) and Xu et al. (2016) with slight modifications. In brief, 0.5 g mung bean sprout in 5 mL of precooled phosphate buffer [50 mM, pH 7.8, containing 1% polyvinylpyrrolidone (PVP) and 1 mM Na_2-ethylenediaminetetraacetic acid (EDTA)] were ground to a homogenate in mortar in an ice bath, and then centrifuged at 18,500 × *g* at 4 °C for 20 min. The supernatant was used as the crude enzyme solution.

The supernatant was used to measure the activities of PAL, CAT, POD, and PPO. PAL activity was determined according to the method described by Dong et al. (2015). CAT activity was analyzed according to