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Research Article

Effects of Controlled Freezing-point Storage on Quality of Fresh-cut Broccoli

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Abstract: Broccoli heads (flower buds) senesced rapidly after harvest at ambient temperatures. The study investigated the changes in the weight loss rate, total soluble solids, ascorbic acid, chlorophyll content, malondialdehyde (MDA) content, polyphenoloxidase (PPO) activity, firmness and the rate of ethylene production of the fresh-cut broccoli stored at different temperatures. The freezing point of fresh-cut broccoli was determined and the fresh-cut broccoli was stored under controlled freezing-point condition (-0.5°C) in comparison with room temperature (20±1°C) and cold (4±1°C) storages for up to 12 days. Results showed that peak rate of ethylene production was 73.98 μL/kg·h after storage at -0.5°C at 4 d, which was significantly lower than the other two storage temperatures. Measurements on other parameters such as weight loss rate, ascorbic acid, PPO activity, chlorophyll content, total soluble solids and MDA content also indicated that controlled freezing-point storage at -0.5°C could maintain a better product quality.

Keywords: Freezing-point, fresh-cut broccoli, shelf-life, storage

INTRODUCTION

The current worldwide lifestyle desire for healthier, safer and more convenient fresh food diets correlates these targets by the consumer's desire for high overall quality products. Fresh-Cut (FC) fruits and vegetables meet these consumers' needs and, consequently, its market has enlarged greatly all around the world. The consumer's increasing interest in broccoli is mainly due to its considerable relevance as a health-promoting fresh produce (Seefeldt *et al*., 2012; Lemoine *et al*., 2010). However, broccoli heads, composed by a number of immature floral buds (florets), are harvested totally immature and only have 2-3 days shelf-life at 20°C (Jin *et al*., 2015). Broccoli florets have a shorter shelf-life than intact products due to mechanical damage that can induce high rates of respiration and metabolism, during which many chemical reactions occur. Some of these reactions, if not controlled, can lead to rapid senescence and undesirable quality loss, expressed as surface dehydration, loss of green color and stem firmness, opening of florets, development of undesirable odors and soft rots (Mølmann *et al*., 2015). This is why the development of new technologies to reduce broccoli deterioration and safety problems is much needed. Ultraviolet light C (UV-C) (Martínez-Hernández *et al*., 2013), Controlled Atmosphere (CA) storage (Schouten *et al*., 2009), chitosan coatings (Alvarez *et al*., 2013), ethanol vapour (Fukasawa *et al*., 2010) and 1-MCP (Cefola *et al*., 2010; Gómez-Lobato *et al*., 2012) treatments have been proposed to delay the deterioration. However, the effects of controlled freezing-point storage on the deterioration of fresh-cut broccoli have not been studied extensively.

Controlled Freezing-point Storage (CF Storage) is a new method of preserving foods in minus non-frozen temperature range (Guo *et al*., 2008) to maintain low respiration rate and other metabolic reactions, which has been applied in storing fresh fruits and vegetables, firstly in Japan (Mizuno *et al*., 1990). Research has confirmed that controlled freezing-point storage at non-freezing temperature-zone between the freezing-point of water and that of an individual material can prolong the storage-life of fresh food and also provide good quality retention.

The aim of this study was to investigate the effects of controlled freezing-point storage (-0.5°C) on the physiological changes and nutritional quality changes of fresh-cut broccoli throughout shelf-life.

MATERIALS AND METHODS

Materials: Broccoli heads (*Brassica oleracea* L. var. italica) were directly obained from a local producer of vegetable market in Chengyang, Qingdao. The vegetables were selected for uniform shape, size,

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ripeness, firmness, colour and the absence of visual defects. Broccoli heads were immediately transported to the laboratory within 1 h after the harvesting.

Various treatments: Broccoli heads were separated into florets and stems with a sharp stainless steel knife and sanitized using a 200 μM sodium hypochlorite solution for 2 min, then washed by immersion in tap water for 1 min. The drained broccoli florets were packaged in polypropylene (thickness, 15 μm; size, 25×30 cm) plastic bags which were sealed with rubber band. All the treatments were stored at room temperature (20±1°C), cold (4±1°C) and freezing-point conditions. During storage period, the freezing point, weight loss rate, total soluble solids, PPO activity, ascorbic acid, firmness, MDA content and chlorophyll content were measured. All the experiments were repeated three times and the average values were reported.

Measurement of freezing curves: Freezing curves measurements were performed according to previous report (Rahman *et al*., 2002). A series of experiments were conducted to establish the freezing curves under freezing conditions. Undercooling-point temperatures during freezing were also recorded. Broccoli florets were ground in a mortar and filtered by two layers of gauze. 50 mL the filtrates were placed in a 100 mL beaker. The beaker was sit at a water-ice-salt (3.42 M NaCl) mixtures and the thermometer was placed in the filtrate. When the temperature of the filtrate was 2°C, the temperature was recorded each interval 30s until the filtrate was completely frozen. Then draw the curve of time-temperature. The temperature of broccoli shows good linear downward tend over time before the supercooling point, When lowing than freezing point, the broccoli still doesn't freeze due to the change of phase with the release of latent heat, which means supercooling phenomena. After supercooling phenomena, the temperature comes back to a point and stays stable in a short period of time, that is the freezing point.

Determination of MDA Content: MDA content was determined according to the method reported by Xu *et al*. (2009) with slight modifications. 1 g broccoli florets were ground into mortar with 5.0 mL of 100 mg/mL trichloroacetic acid in an ice bath. The homogenates were centrifuged at 3000×g for 20 min at 4°C. 2 mL of the supernatant was mixed with 2 mL of 0.67% Thiobarbituric Acid (TBA). The reaction mixtures were heat-treated for 25 min at 100°C, immediately cooled in an ice bath and further centrifuged at 3000×g for 20 min. The absorbance of the supernatant was determined at 450, 532 and 600 nm, respectively, with the 754-PC UV/VIS spectrophotometer (Shanghai Spectrum Instruments Co., Ltd., China) and the result was expressed as μmol per gram fresh weight:

$$\text{MDA content} = \frac{[6.452\times(OD_{532}-OD_{600})-0.559\times OD_{450}]}{FW\times V_s}\times V_t \quad (1)$$

where, V_t and V_s were the total volume of the extract solution and the volume of the extract solution contained in the reaction mixture solution, respectively.

Measurement of PPO activity: PPO activity was determined using similar procedures as reported by Gomes *et al*. (2014) with some modifications. 5 g broccoli florets samples were ground into mortar with sodium phosphate buffer (0.1 M, pH 6.8, stored at 4°C), including 2% polyvinyl-polypyrrolidone at a material-to-liquid ratio of 2.0 mL/g in an external ice bath for 3 min. The homogenates were centrifuged at 3000×g for 20 min at 4°C. The reaction mixture that contained 0.1 mL of enzyme sample, 1.0 mL of 50 mM catechol and 4.0 mL 0.1 M pH 6.8 sodium phosphate buffer was used to determine the PPO activity by measuring the absorbance increase at 420 nm. A unit of PPO activity was defined as the change of 0.001 in the absorbance value per minute.

Determination of weight loss rate: Broccoli florets samples were weighed after the treatment and during storage at intervals of 2 days. The value was expressed as a relative percentage and calculated as follows:

$$\text{Weight loss rate}(\%) = \frac{w_i - w_t}{w_i}\times 100 \quad (2)$$

where,

w_i = The initial weight

w_t = The weight measured at the period of storage

Determination of the rate of ethylene production: The ethylene production was determined as the following. Each 500 g sample was placed in a dryer, where ethylene was allowed to accumulate for about 1 h at room temperature. The headspace gas in the dryer was sampled with a 1 mL plastic hypodermic syringe and injected into a gas chromatograph of Shimadzu GC-2010 (Zerbini *et al*., 2015). The temperatures of the column, injector and a flame ionisation detector were 120, 150 and 200°C, respectively. The ethylene production was expressed as μL/kg·h.

Determination of total soluble solids: Total soluble solids were determined according to the method reported by Guo *et al*. (2008) with slight modifications. 5 g broccoli florets were ground into mortar, the homogenate was filtered through a filter paper and the filtrate was centrifuged at 1000×g for 10 min using a desktop centrifuge (TGL-16C, Shanghai Anke Science and Technology Instrument Factory, China). Total soluble solids contents were measured by using a refractometer (WYA-2S, Shenguang Electronics Ltd., Shanghai, China).

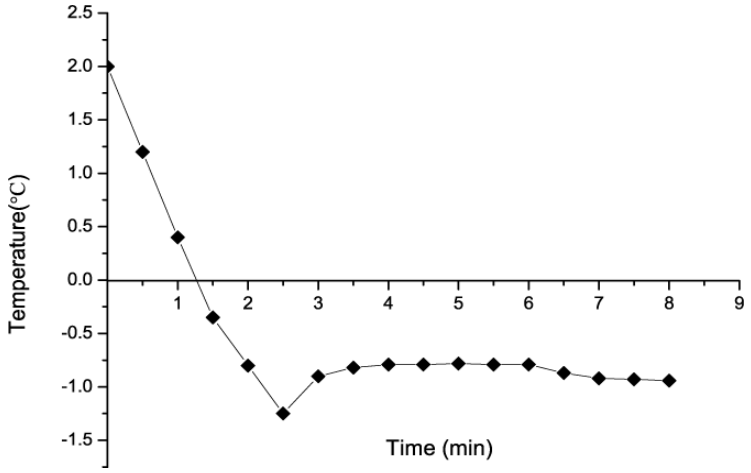

Fig. 1: Temperature-time curve

Determination of ascorbic acid: Ascorbic acid was determined using the 2, 6-dichlorophenol indophenol titrimetric method (Saxena *et al*., 2013). Briefly, 5 g of broccoli florets samples were extracted with metaphosphoric acid and volume was made up to 50 mL. 10 mL of the filtrate was titrated with dye until the distinct rose pink color persisted for 15-20 s. Results were expressed as average value of three replicate samples.

Determination of the chlorophyll content: The Chlorophyll content of each fresh-cut sample was measured using the method described by Zhang *et al*. (2015) with some modifications. Broccoli florets (2 g) of each sample was ground in 80% acetone/water (v/v) and extracted overnight at 4°C in the dark. The extract was filtered and the final volume was adjusted to 20 mL with the same acetone/water solution. The filtered solution was used for the spectrophotometric determination of chlorophyll a and chlorophyll b at wavelengths of 663 and 645 nm, respectively:

$$\text{Chlorophyll content} = \frac{(20.29 \times OD_{645} + 8.05 \times OD_{663})}{2000} \times V \quad (3)$$

where, V was the total volume of the extract solution. The result was expressed as mg per 100 g of fresh weight (mg/100 g).

Determination of firmness: The firmness of each fresh-cut broccoli florets sample was measured using the method described by Oms-Oliu *et al*. (2008) with some modifications. The puncture test was performed on the flat side of each slice using a CT3-4500 from BROOKFIELD company (UK). Cylindrical probe was pressed vertically against the surface of the samples. The operating condition was as follows: 3 mm of probe diameter, 2.00 mm/s of puncture protest speed, 0.5 mm/s of puncture test speed, 0.5 mm/s of puncture posttest speed, 4.00 mm of puncture distance and 6.8 g of trigger force. The firmness was reported as the maximum force (N) from the computer.

Statistical analysis: All samples were analyzed at least in triple and all results are presented as means±SD. Collected data were analysed using Analysis of Variance (ANOVA), Statistical analysis was performed using the SPSS software package version 17.0 (SPSS Inc), Differences of $p<0.05$ were considered to be significant.

RESULTS AND DISCUSSION

Determination of freezing point: Figure 1 showed the representative freezing curve for broccoli. The freezing point was readily determined in such a system: the broccoli pulps first supercools to a temperature below its freezing point, then, as ice crystals form, the temperature rises and temporarily forms a distinct plateau that is the broccoli's freezing point. With the time passing, the temperature of broccoli pulps gradually decreased. When achieving the freezing point, the temperature would not continue to fall because of exothermic phenomena in the freezing process. The freezing point and undercooling point was -0.79°C and -1.25°C from the freezing curve for broccoli, respectively. The temperature of storage was set just above the freezing point to keep broccoli alive with minimal quality deterioration rate and avoid freezing damage. Taking account of temperature fluctuation and practical application, the temperature of -0.5°C was chosen for storage in each experiments.

Changes in MDA content: The thiobarbituric acid-reactive substance MDA is the product of membrane peroxidation and has been used as a direct indicator of membrane injury. Postharvest senescence of broccoli is correlated with lipid peroxidation, leading to cell-membrane disintegration. The effect of storage temperature on MDA content of fresh-cut broccoli

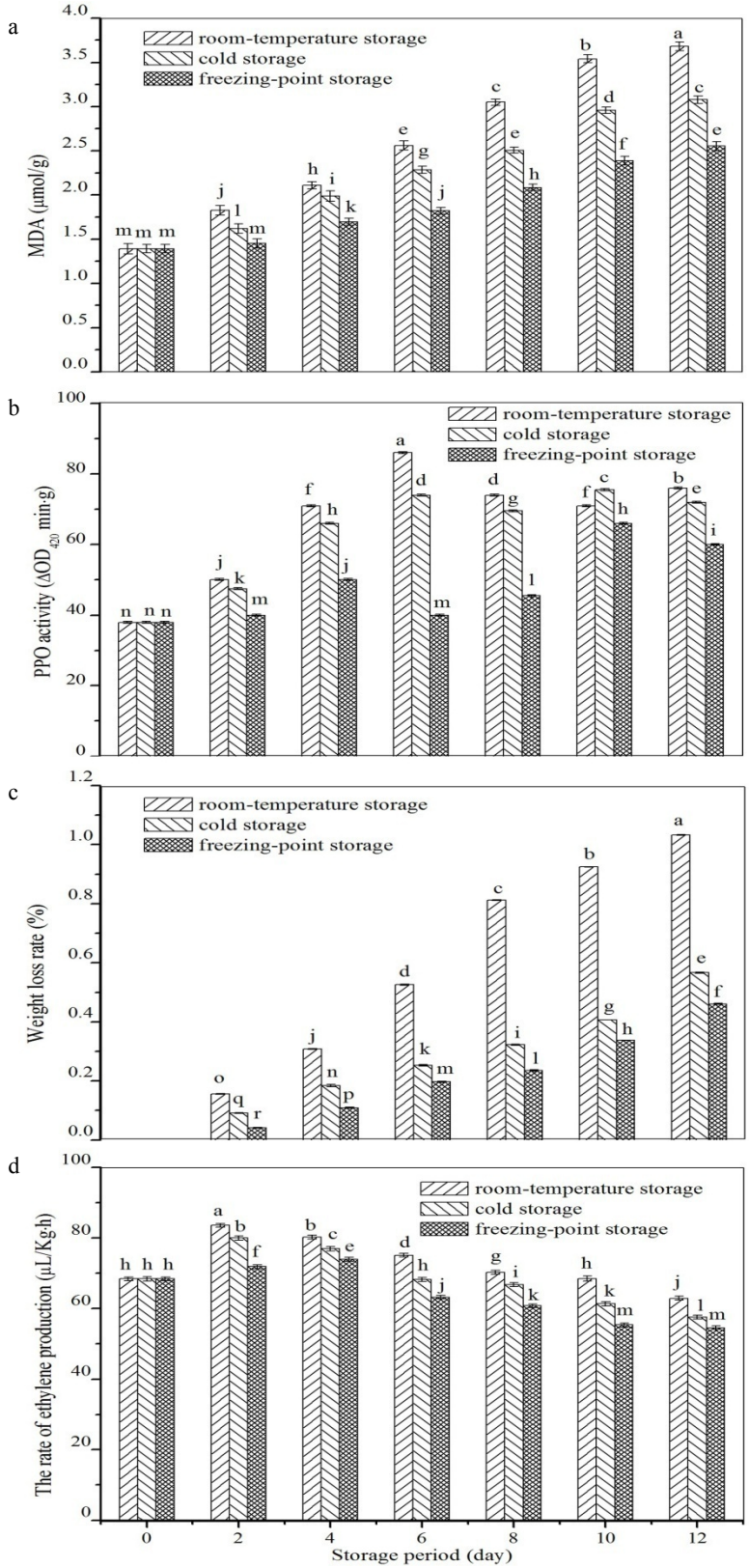

Fig. 2: (a): Changes of weight loss rate; (b): MDA content; (c): PPO activity; (d): The ethylene production rate of fresh-cut broccoli during storage. Bars represent standard errors of the mean. n = 3; Different letters above bars indicate significant differences *p<0.05 according to Least Significant Difference test (LSD)

florets was shown in Fig. 2. The initial content of MDA was 1.39 μmol/g and insignificant difference in MDA content was observed among different storage temperatures at the first day of storage. Subsequently, a marked rise in MDA content was found in florets stored at cold and room temperature than the florets stored at freezing point condition. The MDA content of samples stored at -0.5°C was lower than the other two samples during the whole storage period. The mean of MDA content after 12 days' storage at room temperature, 4°C and -0.5°C were 3.68, 3.08 and 2.55 μmol/g, respectively. The results indicated that the freezing-point storage could significantly inhibit the rise of MDA content ($p<0.05$).

Changes in PPO activity: The generally higher and more rapid accumulation of enzyme activities in fresh-cut tissue might represent a general or global response to wounding. Enzymatic browning of the cut surfaces, leading to serious quality deterioration, has became a matter of concern for the food industry searching for efficient ways to inhibit this reaction (Luo *et al*., 2011). Enzymatic reactions, leading to the appearance of pink, grey or brown colour, can appear within minutes if the tissues are not treated with inhibitors (Cacace *et al*., 2002). Enzymatic browning is a consequence of the PPO catalyzing oxidation of phenolic substrates into the quinones, which undergo further reactions to dark pigments called melanins. These phenolic compounds have been widely reported to have beneficial effects on the maintenance of health and the prevention of cancer and cardiovascular diseases. Biological properties of phenolic compounds result from their ability to act as free radical scavengers and terminate the radical chain reactions that occur during the oxidation of unsaturated fats. The increase of the PPO activity might be due to the activation of soluble tyrosinase forms existing in a latent state, which can be activated during storage period by several factors. As showed in Fig. 2, the PPO activity in the freezing-point condition had two peaks and arrived the first peak at 4 d, decreasing after the 4 d and afterward ascended gradually. The PPO activity of samples stored at -0.5°C was lower than the other two samples during the whole storage period. The means of PPO activity after 12 days' storage at room temperature, 4°C and -0.5°C were 76.01, 72.10 and 60.00 ΔOD_{420}/min·g, respectively. The results indicated that the freezing-point storage could significantly inhibit the rise of PPO activity ($p<0.05$).

Changes in weight loss rate: Generally speaking, respiration or transpiration is one factor that affects the quality of fresh-cut products (Luo *et al*., 2012). Figure 2 showed the changes of weight loss rate of fresh-cut broccoli during storage. The means of rate after storage at room temperature, 4°C and -0.5°C were 1.033, 0.567 and 0.467%, respectively. Results revealed that the controlled freezing-point storage could significantly inhibit the moisture loss of fresh-cut broccoli ($p<0.05$).

Changes in the rate of ethylene production: The proteins and other substances in fruits during post-harvest storage and transportation process can be decomposed into ethylene and carbon dioxide due to the metabolism, resulting in fruit ripening and senescence (Guo *et al*., 2013). Ethylene production is induced by various kinds of environmental stress, such as wounding, physical load, disease, exposure to low temperature and chemicals and water stress in plants, which promotes fruit ripening and flower and vegetable senescence (Hyodo *et al*., 1991). The senescence of broccoli florets is closely associated with endogenous ethylene. It was clearly seen from Fig. 2 that ethylene production rates of the samples stored at room temperature and 4°C increased rapidly, reaching a maximum at 2 d and decreased thereafter. However, the ethylene production peak of the samples stored at freezing point appeared at 4 d. The average value of the ethylene production rates after 12 days' storage at room temperature, 4°C and -0.5°C were respectively 62.89, 57.56 and 54.56 μL/kg·h. The results indicated that the freezing-point storage significantly reduced the rate of ethylene production during storage period compared with the other samples ($p<0.05$).

Changes in total soluble solids: Maintaining the original total soluble solids content is important in storage of fresh-cut broccoli to preserve the sensory qualities. Figure 3 indicated that the mean of total soluble solids after 4 days' storage at room temperature, 4°C and -0.5°C were 7.1, 7.5 and 7.6 Briº respectively which revealed that the controlled freezing-point storage and cold storage could significantly maintain the total soluble solids content ($p<0.05$). The total soluble solids content is descended in the early storage due to the mechanical damage leading to the increase of the respiration rate and the loss of nutrient in the early storage. A obvious change of each treatments at 4, 6 and 8 d could also be seen in Fig. 3. The total soluble solids content raised gradually and the rising rate of samples stored at freezing point was obviously slower than the other two treatments ($p<0.05$). Results revealed that the freezing point could significantly control the decomposition of macromolecule substances and the caducity of tissue. The decomposition of macromolecule substances at the end of storage caused the increase of total soluble solids.

Changes in ascorbic acid: Fresh fruits and vegetables are significant sources of dietary vitamin C and offer more than 90% of the vitamin C required for humans (Büchert *et al*., 2010). The main biologically active form of vitamin C is L-ascorbic acid, although its oxidation product, L-dehydroascorbic acid, is also active (Wills *et al*., 1984). The cutting process imparts physiological stress in fresh-cut commodities, which results in a significant reduction of ascorbic acid content. It seems that low temperatures are one of the most important factors in reducing vitamin C

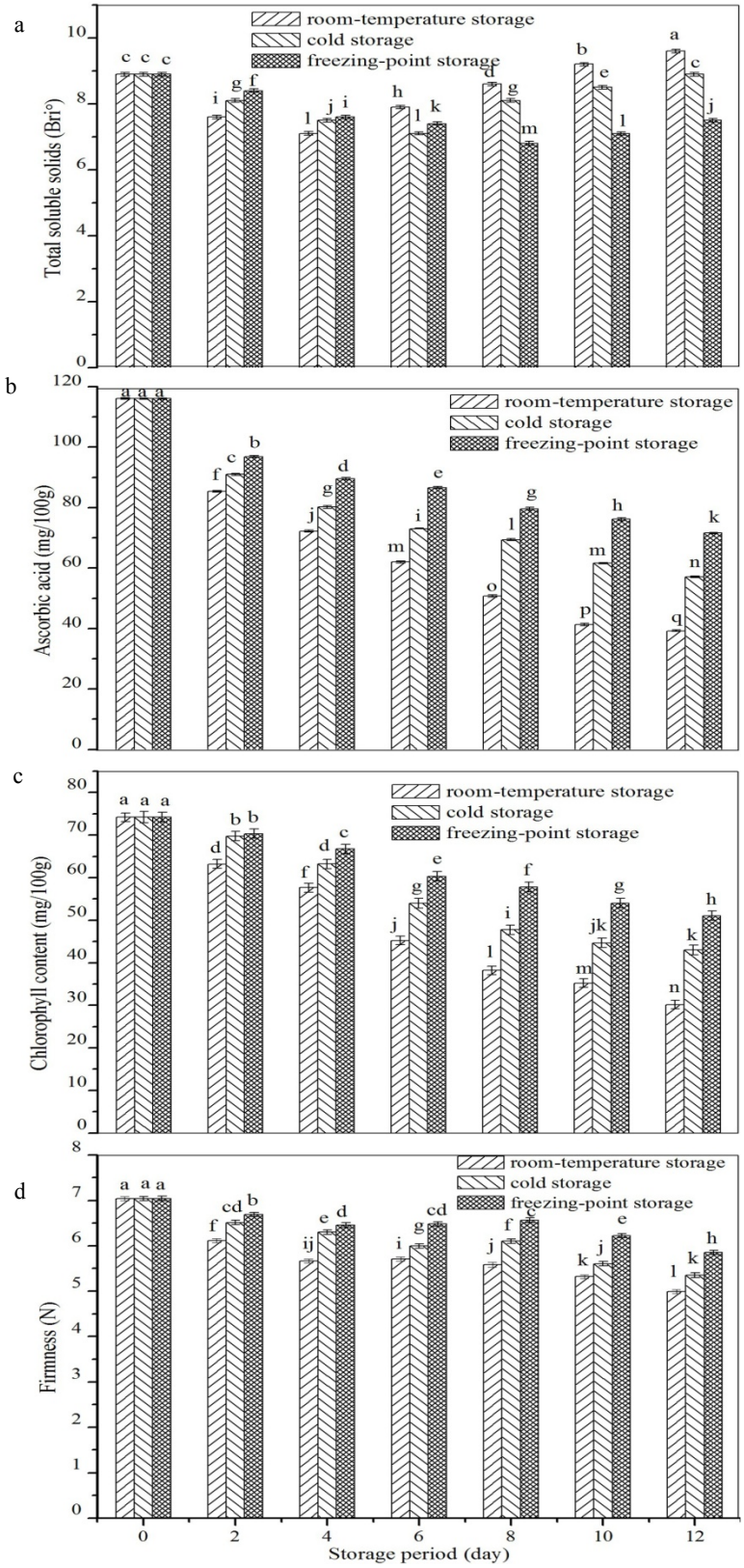

Fig. 3: (a): Changes of total soluble solid content; (b): Ascorbic acid content; (c): Chlorophyll content; (d): Firmness of fresh-cut broccoli during storage. Bars represent standard errors of the mean. n = 3; Different letters above bars indicate significant differences *p<0.05 according to Least Significant Difference test (LSD)

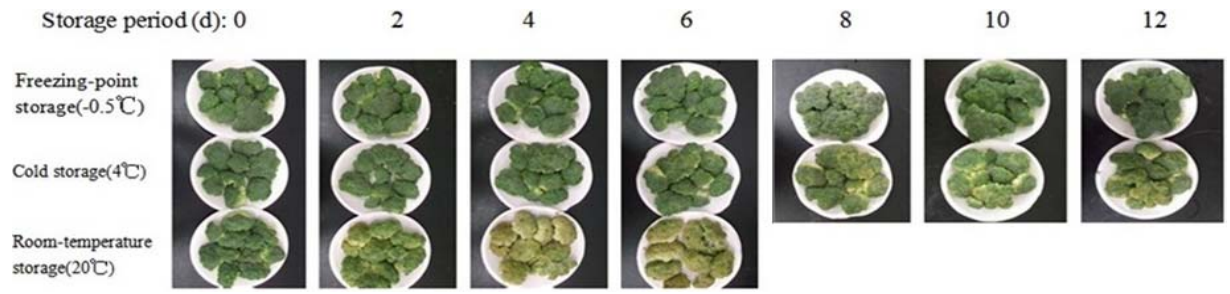

Fig. 4: Effect of controlled different temperatures storage on sensory of fresh-cut broccoli

degradation of fresh-cut produce (Odriozola-Serrano *et al*., 2008). In this study, the degradation trend of the three curves were similar. The lost of samples stored at -0.5°C was less than the other two storage environments. The ascorbic acid in samples stored at room temperature markedly decreased over time and resulted in 66.21% deterioration at the last day of storage ($p<0.05$). Overall, the freezing-point condition preserved higher levels of ascorbic acid reducing 38.31% throughout storage. Hence, commodity value of the fresh-cut broccoli at freezing-point storage is the best. The maintenance of total vitamin C in fresh-cut broccoli stored at -0.5°C may be related to the inhibition of PPO activity because Zhang *et al*. (2005) found that PPO could reduce the level of vitamin C.

Changes in chlorophyll content: Chlorophyll content showed significant degradation over time regardless of what treatments. The main symptom of broccoli senescence is yellowing due to chlorophyll catabolism. The activity of chlorophyll-degrading enzymes in coleslaw was enhanced by the mechanical damage (Aiamla-or *et al*., 2012). In the present study, fresh-cut broccoli was observed a degradation phenomenon over time, which was supported by chlorophyll content evaluation. As showed in Fig. 3, the mean of chlorophyll content after 12 days' storage at room temperature, 4°C and -0.5°C were 30.11, 42.99 and 51.03 mg/100 g, respectively. This result evaluated by chlorophyll content assay illustrated that the chlorophyll degradation speed of the samples stored at -0.5°C was significantly slower than the other two control treatments ($p<0.05$, Fig. 4). Freezing-point condition might be beneficial for fresh broccoli green tissue to inhibit the activity of photosynthetic enzymes during the storage period. So the storage at freezing point markedly alleviated the deterioration of chlorophyll content.

Changes in firmness: Dehydration causes important structural and physicochemical changes in foods affecting to their final quality. Hernando *et al*. (2008) examined dried mushrooms by using scanning electron microscopy, observing that dehydration makes tissue more flattened and collapsed than flesh mushrooms. Texture is one of the quality attributes in the fresh-cut broccoli. Softening of the texture is related to the weight loss and the cell-wall degradation by degrading enzymes such as polygalacturonase and pectinesterase. Figure 3 showed that the decreasing tendency of various samples' firmness was similar. Values of 4.99, 5.35 and 5.85 N were severally the average value of firmness after 12 day's storage at room temperature, 4°C and -0.5°C. The results indicated that the freezing-point storage significantly reduced the degree of softening during storage period compared with the other samples ($p<0.05$). The loss of firmness is mainly due to the freezing point, which reduces the rate of water diffusion and delays the time of dehydration. At the same time, in the tissue cells destroyed by cutting, decomposition of macromolecule substances accelerates caducity and softening of the fresh-cut broccoli. The rapid emollescence and deterioration of fresh-cut broccoli likely refer to catabolism of the cytomembrane and cytoderm sensitized to physical wounding.

Experiments were conducted to store fresh-cut broccoli for up to 12 days under three conditions, i.e., controlled freezing-point storage at -0.5°C, cold storage at 4°C and room-temperature storage at 20°C. Compared with the other two storage conditions, controlled freezing-point storage at -0.5°C showed preferable higher physiological (ascorbic acid, total soluble solids, chlorophyll content, MDA content, PPO activity and the rate of ethylene production) and commercial (weight loss rate and firmness) qualities. Therefore, the fresh-cut broccoli stored at -0.5°C should be recommended for prolonging the shelf-life of the product. Research on the application of the controlled freezing-point storage technique should be extended to cover more fruit and vegetable varieties.

CONCLUSION

Experiments were conducted to store fresh-cut broccoli for up to 12 days under three conditions, i.e., controlled freezing-point storage at -0.5°C, cold storage at 4°C and room-temperature storage at 20°C. Compared with the other two storage conditions, controlled freezing-point storage at -0.5°C showed preferable higher physiological (ascorbic acid, total soluble solids, chlorophyll content, MDA content, PPO

activity and the rate of ethylene production) and commercial (weight loss rate and firmness) qualities. Therefore, the fresh-cut broccoli stored at -0.5°C should be recommended for prolonging the shelf-life of the product. Research on the application of the controlled freezing-point storage technique should be extended to cover more fruit and vegetable varieties.

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