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Research Article

Effect of hot water treatments on survival of *E. coli* and *Salmonella* spp. and physical properties in fresh-cut broccoli florets

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Abstract

Hot water treatments were applied to fresh-cut broccoli (*Brassica oleracea* L.) florets to investigate their effects on the survival of food-borne pathogens (*Escherichia coli* and *Salmonella* spp.) and on the physical properties of the broccoli florets during storage. Broccoli florets were treated with hot water at 50, 55 and 60°C for 3 min and immediately cooled with water at 4°C. Non-hot water treated broccoli florets served as controls. The treated broccoli florets were then placed in PVC boxes for 13 days at 4°C. Hot water treatment at 55 and 60°C for 3 min completely controlled *E. coli* and *Salmonella* spp. but these treatments resulted in tissue softening and scald-like symptoms on the broccoli florets. The hot water treatment at 50°C for 3 min resulted in a reduction of both food-borne pathogens for 1.0-1.2 $\log_{10}$CFU.g^{-1} and maintained freshness, firmness and delayed the yellowing of broccoli florets for 4 days. These results suggest that a hot water treatment at 50°C for 3 min has the potential to maintain the physical quality of broccoli florets but this treatment may not be sufficient to control the growth of food-borne pathogens.

Keywords: post harvest, vegetables, heat treatment, food-borne pathogens, quality, Thailand.

Introduction

Broccoli has become a popular vegetable because of its high nutrition, as it contains important levels of vitamins, antioxidants and anti-carcinogenic compounds [1]. The popularity and sales of fresh-cut broccoli has increased due to a rising consumer demand for healthy and convenient food. However, one of the most important problems with fresh-cut produce is the potential microbial contamination by food-borne pathogens which are a cause of human illness. Several disinfectant chemicals or sanitizing treatments have been used to reduce the initial microbial loads on fresh produce destined for fresh-cut processing [2].

Hot water treatment is a commonly used physical treatment to reduce food-borne pathogens and also to delay senescence [3]. At present there is a limited literature dealing with microbial control and maintaining quality of fresh-cut broccoli florets by hot water treatment. Thus, the aim of the present study was to investigate the optimal hot water temperatures required to reduce food-borne pathogens (*E. coli* and *Salmonella* spp.) and to maintain the physical quality of fresh-cut broccoli florets during storage at low temperature.

Materials and Methods

Plant material and hot water treatments

Fresh broccoli (*Brassica oleracea* L.) heads were cut into individual florets and then directly treated with hot water at 50, 55 and 60°C for 3 min. The treated florets were then cooled in water at 4°C for 5 min and any excess water was removed by manual salad spinner. Treated broccoli samples were randomly taken for analysis of microbial growth and physical properties to compare them with the untreated florets as control. A 200 g sample of the broccoli florets was packed in polyvinylchloride (PVC) box and kept at 4°C. Each treatment had 3 replicates (boxes).

Microbial analysis

A 25 g sample of broccoli florets was homogenized in 225 ml of 1% sterile peptone water using a stomacher (IUL Instruments Masticator, Barcelona, Spain) for 2 min. Ten-fold dilution series were made in sterile peptone water as required for plating. The following culture media and conditions were used to enumerate the microbial growth: (1) Plate count agar (HiMedia) incubated at 37°C for 24 h for total bacteria; (2) Eosin Methylene Blue agar, EMB (HiMedia) incubated at 37°C for 24–36 h for *E. coli* counts; and (3) Xylose-Lysine Deoxycholate agar, XLD (HiMedia) at 37°C for 24-48 h for *Salmonella* spp. Microbial counts were expressed as $\log_{10}$ CFU g^{-1} (colony forming units per gram of sample).

Colour measurement

Colour of broccoli floret samples was determined by measuring L*, a*, b* values and hue angle with a Minolta chroma meter (Model CR300, Osaka, Japan) which covered an area of 8 mm^2. Three positions on each of the florets were measured for each treatment and storage time.

Internal gas analysis

CO_2 and O_2 concentrations in PVC boxes of broccoli florets were measured by a Gas Analyzer (OXYBABY® 6.0). A needle sensor was penetrated on the silicone rubber attached on the outer surface (on the top) of the lid for 10 sec. The CO_2 and O_2 concentrations were determined and expressed as a percentage.

Respiration rate

The respiration rate of broccoli florets was determined according to the method of Lemoine *et al*. [4]. Samples of broccoli florets (approximately 150 g) were placed in a 2 L plastic jar, sealed and incubated at 4°C for 3 h. A 1 mL of headspace gas sample was withdrawn with a syringe through a septum fitted in the jar lid and injected into gas chromatograph (Shimadzu-8A, Tokyo, Japan) equipped with Porapak Q column. The data was expressed as mg CO_2 g^{-1} h^{-1}.

Sensory evaluation

The sensory panel consisted of 8–10 untrained members. The sensory attributes were evaluated by visual characteristics such as visual quality, colour and odour/off-odours. A rating score index was given ranging from 1 being completely deteriorated to 9 representing excellent quality and freshness of the analyzed sample. The sample was considered as unacceptable in terms of sensory characteristics when the rating score was lower than 5 (limit of salability).

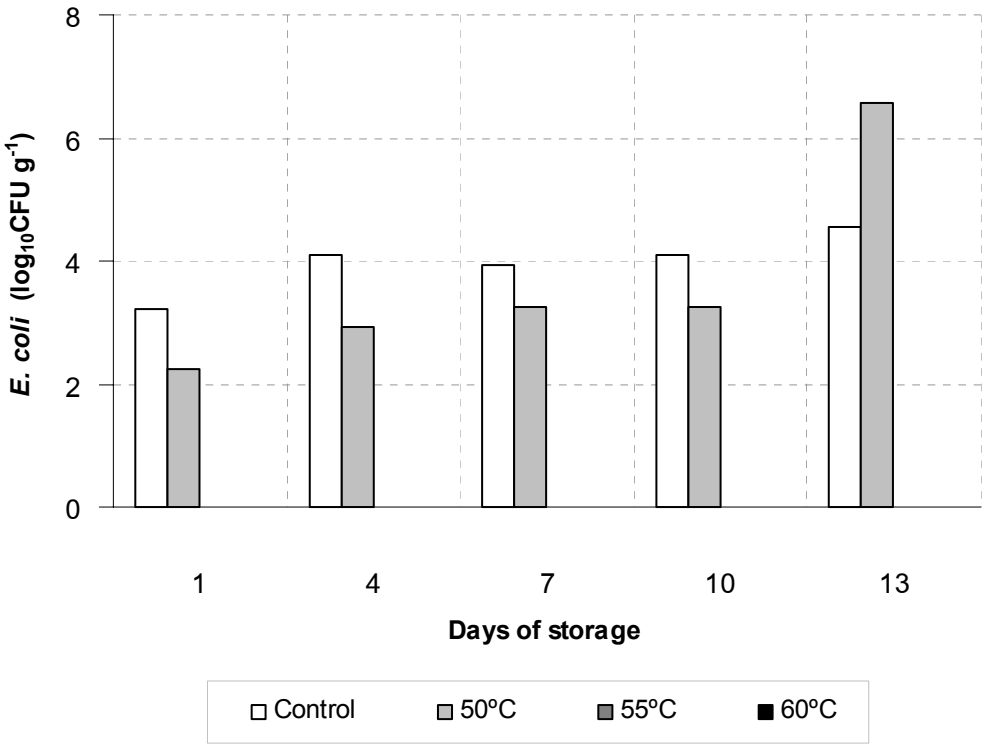
Statistical analysis

Analysis of variance (ANOVA) and Duncan's new multiple range test for comparison of means and least significant differences ($P < 0.05$) were performed on the data using the SPSS version 12.0 software.

Results and Discussion

The effect of hot water (HW) treatments at different temperatures on *E. coli* and *Salmonella* spp. counts in fresh-cut broccoli florets is shown in Figure 1. The initial population of *E. coli* and *Salmonella* spp. in non-hot water (non-HW) treated broccoli florets averaged 3.23 and 3.23 $\log_{10}$CFU g^{-1} respectively. Application of hot water on fresh-cut broccoli florets reduced the numbers of *E. coli* and *Salmonella* spp. compared to that of control. Particularly the HW treatments at 55 and 60°C completely inhibited both food-borne pathogens on day 1 of storage, while the HW treatment at 50°C for 3 min reduced both food-borne pathogens by 1.0-1.2 $\log_{10}$CFU g^{-1} (Fig. 1). A similar result was achieved by Li *et al*. [5] who reported that warm water (50°C), with or without 20 mg/l chlorine, significantly reduced the initial population of mesophilic aerobic microflora in fresh-cut iceberg lettuce by 1.73-1.96 $\log_{10}$CFU g^{-1}. The population counts of both pathogens gradually increased throughout the storage time in all treatments at 4°C. These results suggest that both pathogens can survive and multiply at low temperature, with Li *et al*. [6], Li *et al*. [7] and Moreira *et al*. [8] publishing similar findings. Moreover, the HW treatments were more efficient in controlling the growth of *E. coli* compared with *Salmonella* spp. The differential temperature growth response suggests that *Salmonella* spp. has a greater tolerance to thermal water treatments than *E. coli* (Fig. 1A, 1B).

(A)



(B)

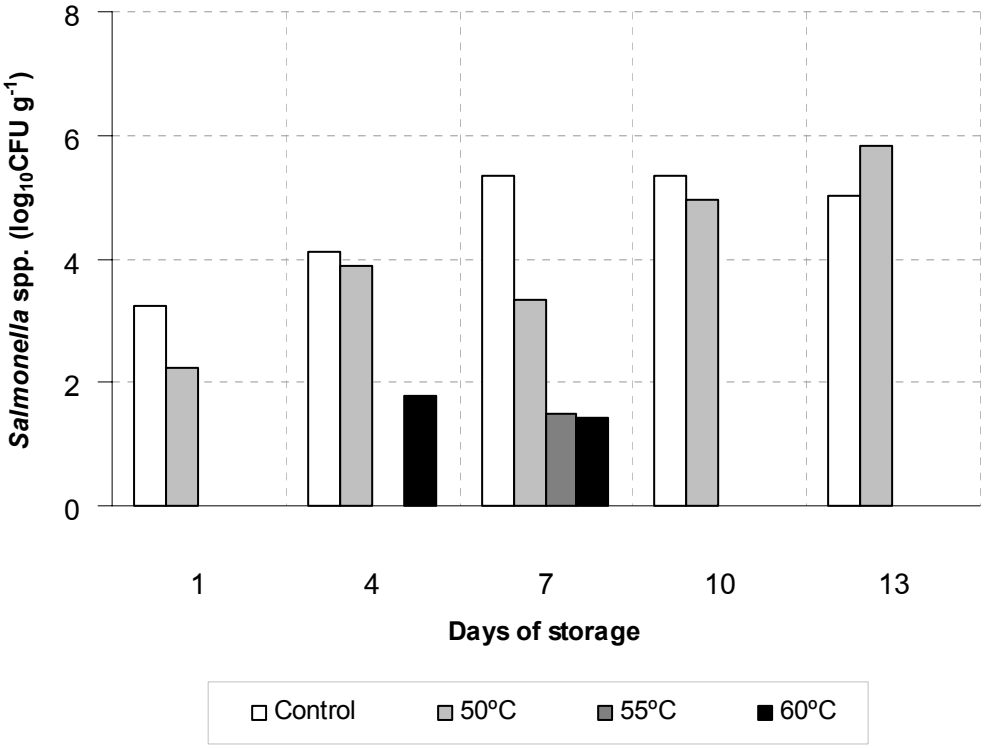


Figure 1. Microbial population of *E. coli* (A) and *Salmonella* spp. (B) in fresh-cut broccoli florets treated with hot water at 50, 55 and 60ºC for 3 min.

Fresh-cut broccoli florets treated with HW at 55 and 60°C showed a lower L* value and hue angle than florets treated with 50°C and than non-treated broccoli throughout the storage time (Fig. 2A, 2D). The L* value and hue angle of florets treated at 50°C was close to that of non-treated broccoli florets. However, HW treatments at 55 and 60°C seemed to decrease the florets quality due to these temperatures caused tissue softening and scald-like symptoms on the broccoli florets. The green colour of broccoli is indicated by a* value. The fresh-cut broccoli treated with HW at 50°C showed a lower a* value than the other treatments (Fig. 2B). This result indicates that HW at 50°C was the best treatment to maintain the green colour of broccoli. Several reports have also shown that heat treatments such as HW or hot air delay the yellowing process and senescence of broccoli florets [3, 9, 10].

A decrease in O_2 and an increase in CO_2 levels in the packaging were observed in all treatments (Fig. 3A, 3B). Gases inside the package were significantly different depending on the degree of water temperature. The lowest levels of O_2 and the highest level of CO_2 were detected in the package of fresh-cut broccoli treated at 50°C for 3 min. Observed gas levels were about 13-16% O_2 and 4.0-5.8% CO_2, whereas the O_2 and CO_2 levels in the package of the other treatments ranged about 16-21% and 0.0-4.4%, respectively.

Toivonen *et al*. [11] suggested that the gas levels of 3.1-4.2% O_2 were acceptable to keep the broccoli heads and when the CO_2 levels reached about 15.0-15.6%, this affected the development of off-odours, which would be found at 4 days of storage at 1°C. Fonseca *et al*. [12] showed that 1-2 kPa O_2 and 15-20 kPa CO_2 in the atmosphere of packaging was effective

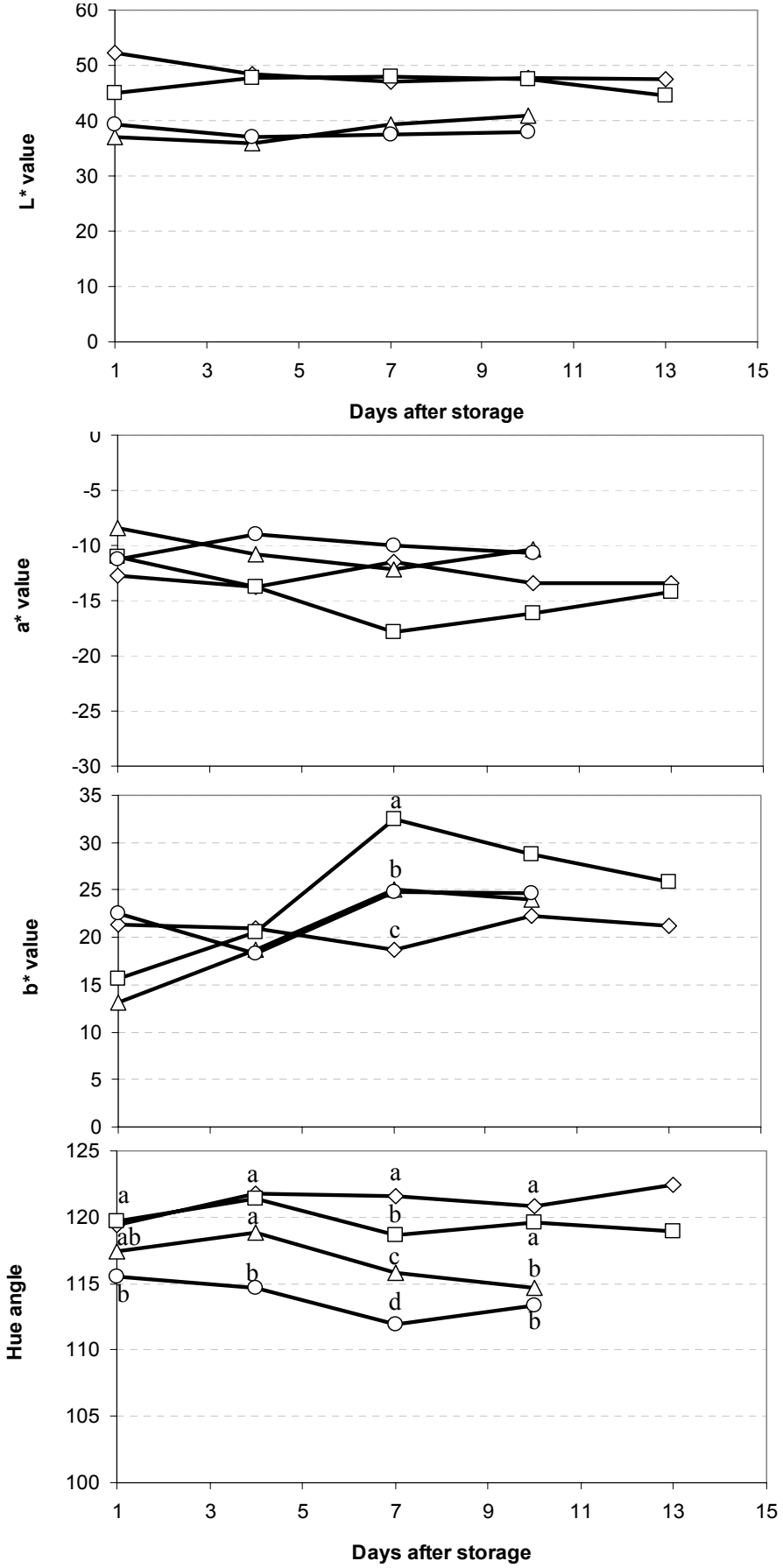


Figure 2. L*, a*, b* value and hue angle of fresh-cut broccoli florets treated with hot water at 50, 55 and 60ºC for 3 min.

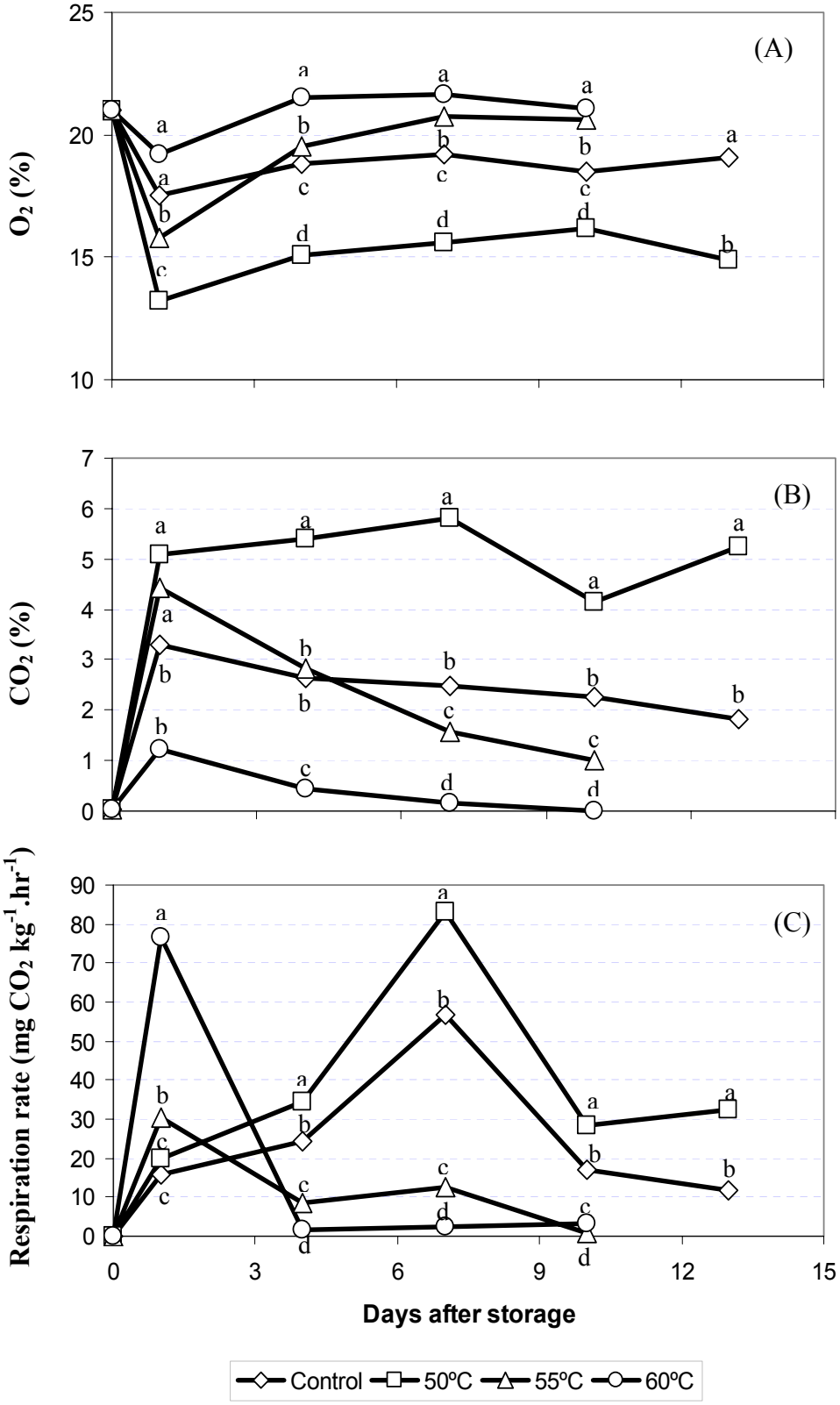


Figure 3. Oxygen (A) and carbon dioxide (B) concentration in package and respiration rate (C) of fresh-cut broccoli florets treated with hot water at 50, 55 and 60ºC for 3 min.

to maintain the quality of shredded Galega kale and extend the storage life for 4-5 days at 20°C. Serrano *et al*. [13] reported that concentration of gas on No-P bags were about 4-12 kPa O_2 and 4.5-6 kPa CO_2, and had extended the storage-life of broccoli heads up to 28 days at 1ºC, with no symptoms of bad aroma and off-flavours could be detected inside the bags after opening. Jia *et al*. [14] reported that MAP with no holes packaging had gas levels around 1-6% O_2 and 8-14% CO_2, which could maintain the quality of broccoli florets for 13 days at 4ºC.

High water temperatures increased the respiration rate of fresh-cut broccoli florets (Fig. 3C). Florets treated with HW at 60°C for 3 min showed sharply increased respiration rates on the first day of storage compared to other treatments and followed by broccoli florets treated with HW at 55°C. The respiration rate of the florets treated with HW at 50°C and of non-treated broccoli florets gradually increased and peaked on day 7 of storage, and florets treated with HW at 50°C had a slightly higher respiration rate than the control (Fig. 3C). Thus, the increase of CO_2 and the decrease of O_2 gas levels in the package were the result of a higher respiration rate of HW treated broccoli florets. The development of the gas levels is mostly based on film permeability, surface area, storage temperature, cultivar, wounding and respiration activity of the produce [13, 15].

The visual quality of HW treated broccoli florets was compared with that of non-HW treated broccoli. The higher temperature HW treatments showed higher scores of visual quality and visual color, and lower scores of odor attributes compared with non-HW treatments (Fig.4A). However, HW treatments at 55-60°C for 3 min are not recommended for fresh-cut broccoli floret because they caused tissue softening and scald-like symptoms.

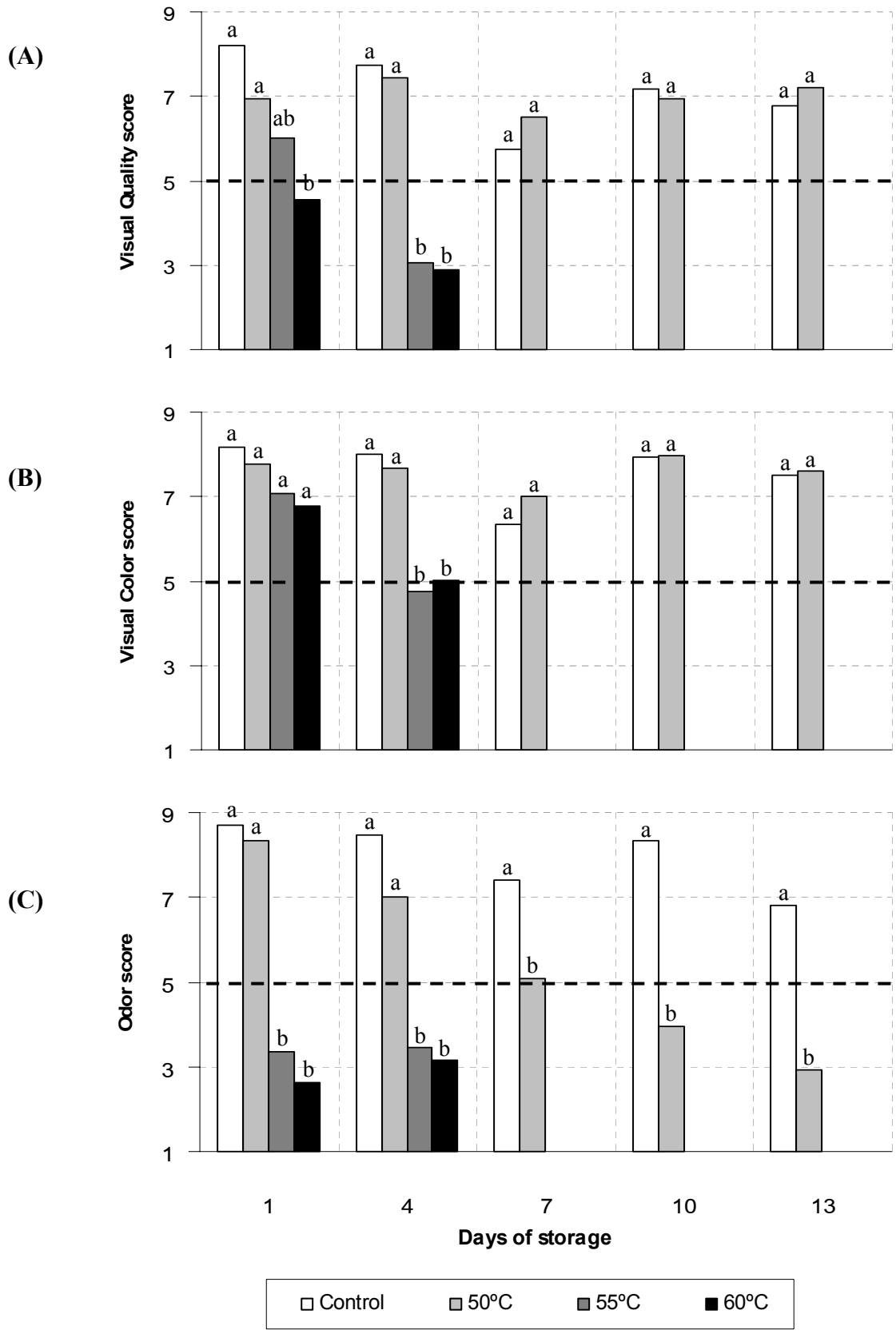


Figure 4 Sensory evaluation using 9-points hedonic scale visual quality (A), visual colour (B) and odour (C) score of fresh-cut broccoli florets treated with hot water at 50, 55 and 60°C for 3 min.

Dash line is representative of the consumer acceptance, if the sensory score is lower than 5 means the consumer will not accept the fresh-cut broccoli florets.

Conclusions

This study confirmed that food-borne pathogens (*E. coli* and *Salmonella* spp.) are found in broccoli florets originating in their general cultivation and handling. HW at 55 and 60°C for 3 min were the most effective treatments to inhibit *E. coli* and *Salmonella* spp. growth in fresh-cut broccoli with 4 days of storage at 4°C, but the tissue of the broccoli florets could not tolerate these temperatures. A HW treatment at 55°C for 3 min successfully reduced pathogenic bacteria contamination and maintained the physical quality of fresh-cut broccoli florets for 7 days. Therefore, these results suggest that a HW treatment at 50°C for 3 min has the potential to maintain the physical quality of broccoli florets but this treatment may not be sufficient to control food-borne pathogen infections.

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