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Quality of low temperature heat-shocked green asparagus spears during short-term storage

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The present study evaluated the effects of heat-shock treatment (washing in 48°C distilled water for 5 min) on quality of green asparagus spears stored at 25 or 4°C for 7 days. The results showed that the quality characteristics including toughness, lignin and cellulose contents, and phenylalanine ammonia-lyase, cinnamyl alcohol dehydrogenase and peroxidase activities were increasing in both heat-shocked and control spears stored at 25°C; however, the heat-shocked spears exhibited lower degrees of increases than control spears. Similar increasing trends but with even smaller magnitudes in these characteristics were obtained from the heat-shocked spears stored at 4°C. Heat-shocked spears stored at 25 or 4°C also demonstrated lesser proliferations of total mesophilic aerobes, *Escherichia coli* and mold than their respective controls. Thus, a combination of 48°C heat-shock treatment and 4°C cold storage can be used to conserve the quality of stored green asparagus spears.

Key words: Green asparagus, heat-shock, lignification, microbial load, storage.

INTRODUCTION

Green asparagus (*Asparagus officinalis* L.) spear is a healthy but highly perishable vegetable, which has a very short shelf life (generally three to five days) with normal post-harvest handling at ambient temperature. Many physical and chemical changes such as the loss of water and the increase of toughness that reduce the quality of green asparagus spears occur during harvesting, handling and storage (Rodriguez-Arcos et al., 2002; Liu and Jiang, 2006). Among these changes, the increase of toughness is an important factor in de-grading the quality of green asparagus spears and consequently decreasing their market value. The toughness of green asparagus spears is reported to be related to the degree of lignification in their tissues, which is regulated by enzymes Phenylalanine Ammonia-Lyase (PAL), cinnamyl alcohol dehydrogenase (CAD) and peroxidase (POD) (Boudet, 2000; Chen et al., 2002; Cai et al., 2006; Li and Zhang, 2006). Additionally, many pathogenic micro-organisms can contaminate the produced green asparagus spears during cultivation, harvest and post-harvest handling. The physical and chemical changes of green asparagus spears that occurred during storage would facilitate the proliferation of loaded microorganisms if the harvested green asparagus spears were not properly sterilized. As a result, the increased microbial load would further reduce the quality of green asparagus spears and shorten their shelf life (Sothornvit and Kiatchanapaibul, 2009). Many methods have been developed to ensure microbial safety and extending shelf life of green asparagus spears.

Washing asparagus with chlorine was reported to reduce the microbial load and maintained its quality (Sothornvit and Kiatchanapaibul, 2009). Aqueous ozone treatment plus a subsequent modified atmosphere

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packaging also proved effective in decreasing the toughness and extending the shelf life of treated asparagus (An et al., 2007). A combination of edible coating of chitosan and 2°C cold storage also exhibited a reduction of decay of green asparagus and extended its shelf life (Qiu et al., 2013). Nevertheless, heat treatment is still extensively used to eradicate pathogens and preserve the quality of various fresh and fresh-cut produces because of the increased concern about the safety of chemicals used in post-harvest treatments (Sivakumar and Fallik, 2013). The applied heating parameters (for example, time and temperature regime) can vary to accomplish almost any degree of microbial inactivation, ranging from limited reduction of microbial load to complete sterilization (Rajkovic et al., 2010). Heat treatment has also been used to preserve the quality of harvested asparagus spears (Lau et al., 2000). Heat-shock is a method which usually implies a washing step at a temperature ranging from 43 to 70°C for a few minutes (usually less than 5 min) (Fallik, 2004; Rico et al., 2007). This method is proved useful to preserve the quality of produces such as green onion (Cantwell et al., 2001), lettuce (Murata et al., 2004) and kiwi fruit (Beírãao-da-Costa et al., 2008). But it has not been tested for controlling the proliferation of microorganisms and extending the shelf life of green asparagus spear.

In the present study, attempts were made to characterize the physical and chemical changes in green asparagus spears that have been subjected to 48°C heat- shock, and then stored at 4 or 25°C conditions for seven days. The activities of PAL, CAD and POD in the stored asparagus spears were determined and compared. The effectiveness of heat-shock treatment on initial microbial de-contamination and proliferation during storage was also examined. Knowledge of these variations should help to identify the potential value of low temperature heat-shock treatment on safety control and quality preservation of green asparagus spears.

MATERIALS AND METHODS

Plant materials and chemicals

Spears of fresh green asparagus (*Asparagus officinalis* L. cultivar Tainan 12) were obtained from Tainan District Improvement Station (Tainan county, Taiwan). The spears were cut at ground level between 8:00 and 9:30 AM, then placed in crushed ice and transported to the laboratory within three h on the day of harvest. The spears that were 15 to 20 mm in diameter and 15 cm (from the tip) in length with closed bracts were used in the study. The selected and cut spears (15 cm in length) were washed with distilled water, then drained and cleaned with paper towels to remove the water. A preliminary heat shock trial by using several temperature regimes (ranged from 45 to 57°C) had shown that a heat shock at 48°C water for 5 min was capable of reducing the increases of toughness to some extent in the treated asparagus spears stored at ambient temperature for five days. Therefore, in this study, the 48°C heat-shock treatment was used for asparagus spear quality control. While a portion of selected asparagus spears was not heat-shocked (the control group), another portion of the selected asparagus spears (5 kg) underwent the heat-shock treatment in 48°C distilled water for 5 min. The heated-shocked spears were then immersed in ice-cold water for 10 min. Following cooling, the spears were drained and dried with paper towels and then packed into an airtight plastic container (20 × 10 × 5 cm), and then stored in airtight chambers (with about 85% relative humidity) at 4 or 25°C for seven days. Spears from the control samples were also immersed in ice-cold water for 10 min and then drained and stored in the same way as the heat-shocked spears. The stored spears were sampled on day 0, 1, 3, 5 and 7, and the top portions of spears (first 12 cm from the tip) were used for physical and chemical analyses.

The polyvinylpyrrolidone, 2-mercaptoethanol, ethylenediaminetetraacetic acid (EDTA) sodium salt, and the chemicals including tris (hydroxymethyl) aminomethane-HCl, xylenol, L-phenylalanine, borate buffer, coniferyl alcohol, Nicotinamide adenine dinucleotide phosphate, guaiacol used for enzyme activity assays were purchased from Sigma-Aldrich (St. Louis, MO, USA). The growth media for microbial analyses were purchased from Chisso Corporation (Tokyo, Japan). All other chemicals used in this study were of analytical grade.

Fresh weight loss and texture toughness determinations

The fresh-weight loss of the spears (harvested asparagus spears 15 cm in length) was determined with a precision balance during the entire storage period. The tissue toughness measurements were performed in a Tahdi Texture Analyzer (Stable Micro-Systems Ltd, Godalming, UK) with a 500 N load cell and a 5 mm diameter probe. A single puncture measurement was made on each spear sample (10 mm depth of penetration) at a speed of 1.0 mm s^{-1}. The recorded breaking force (maximum peak force, N) was used as an indicator of texture toughness parameter.

Lignin, cellulose and H_2O_2 contents determinations

For lignin determination, five randomly selected spears (harvested asparagus spears 15 cm in length) were diced and frozen in liquid nitrogen and then stored at -20°C prior to analyses. Lignin was extracted and measured by using the procedures detailed by Bruce and West (1989). Different samples from the same frozen bulked and diced spears that were used for lignin analyses were also used for the cellulose assay following the procedures detailed by Oomena et al. (2004). The content of H_2O_2 content was determined by using the procedures detailed by Lin et al. (1988).

Enzymes activities determinations

For phenylalanine ammonia-lyase (PAL) activity assay, 5 g of diced and bulked frozen spears prepared from the harvested asparagus spears (15 cm in length from the tip) were homogenized for 2 min in 15 ml 0.1 M borate buffer (pH 8.8) containing 6 g polyvinylpyrrolidone, 5 mM 2-mercaptoethanol and 2 mM EDTA. The homogenate was centrifuged for 15 min at 14,000 g and the supernatant was collected for enzyme activity determination. The PAL activity was measured according to the method of Cheng and Breen (1991). For cinnamyl alcohol dehydrogenase (CAD) activity assay, 5 g of diced and bulked frozen samples were extracted by using 10 ml Tris-HCl buffer (0.2 M, pH 7.5). The homogenized mixture was centrifuged at 14,000 g for 15 min and the supernatant was used as enzyme extract. The CAD activity was assayed by using the procedures detailed by Goffner et al. (1992). For peroxidase (POD) activity determination, the enzyme was extracted from 5 g of diced and bulked frozen samples in 0.05 M phosphate buffer (pH 7.0). The homogenate was centrifuged at 14,000 g for 15

Table 1. The fresh weight loss (%) and toughness (N force cm^{-2}) measured in the control and heat-shocked green asparagus spears stored at 25 or 4°C for seven days.

Treatment	Storage temperature (°C)	Storage period(days)				
		0	1	3	5	7
			Fresh weight loss (%)			
Control	25	-	1.12±0.19^{bc}	1.62±0.37^{d}	3.94±0.18^{g}	10.01±1.61^{f}
Heat-shocked		-	0.71±0.09^{a}	1.05±0.13^{c}	1.84±0.28^{e}	7.41±0.63^{d}
Control	4	-	0.68±0.08^{a}	0.81±0.03^{b}	0.89±0.09^{b}	0.94±0.07^{b}
Heat-shocked		-	0.61±0.06^{a}	0.67±0.04^{a}	0.73±0.05^{a}	0.81±0.04^{a}
			Toughness (N force cm^{-2})			
Control	25	2.37±0.06	2.83±0.41^{a}	3.51±0.11^{c}	3.87±0.10^{c}	4.22±0.14^{c}
Heat-shocked			2.78±0.19^{a}	3.15±0.19^{b}	3.56±0.14^{b}	3.83±0.11^{b}
Control	4		2.61±0.34^{a}	2.73±0.10^{a}	3.07±0.32^{a}	3.74±0.12^{b}
Heat-shocked			2.53±0.13^{a}	2.67±0.23^{a}	2.94±0.26^{a}	3.28±0.30^{a}

Values for fresh-weight loss and toughness with different superscript letters within columns were significantly different at $P < 0.05$, respectively.

min at 4°C, and the supernatant was used to determine the POD activity by using the method described by Sheu and Chen (1991).

Microbiological analyses

The diced green asparagus spears samples (25 g) were added to 225 ml of 0.85% (w/v) NaCl solution, and then homogenized and diluted (6-fold dilution) with distilled water. Following dilution, 1 ml of diluted sample was surface-plated on media prepared following the manufacturer's instructions (Chisso Corporation, Tokyo, Japan) for the counting of total mesophilic aerobes, *Escherichia coli* and mold. All the plates were incubated at 35°C for 48 h, 35°C for 24 h or 25°C for 5 days for total mesophilic aerobes, *E. coli* or mold counting, respectively. After incubation, the colonies of microbes were enumerated and expressed as log colony forming units per g (log cfu g^{-1}).

Statistical analysis

The experiments were conducted in a completely randomized design with four replicates. Analyses of variance (ANOVA) were performed and means were compared using Duncan's multiple range tests at $P < 0.05$. Correlation analyses were also used to characterize the relationships between the contents of lignin, cellulose and H_2O_2, and toughness, as well as the related enzymes.

RESULTS AND DISCUSSION

Fresh weight loss and toughness at 25°C storage condition

The fresh-weight loss is a crucial parameter for stored green asparagus spears because the loss in fresh weight represents an economic loss. In the present study, the fresh-weight loss in control and heat-shocked green asparagus spears stored at 25°C increased progressively over the seven days of storage (Table 1). For non-treated control, the fresh-weight loss detected over seven days of storage was 10.01% of the initial weight. The fresh-weight loss of stored spears is mainly due to the loss of water (Albanese et al., 2007). The heat-shock treatment was effective in slowing down the fresh-weight loss of green asparagus spears (Table 1). The fresh-weight loss was 7.41% (26% less than control) in the heat-shocked spears stored at 25°C for seven days. The toughness of stored green asparagus spears is an important factor that determines the acceptance or rejection by consumers. As shown in Table 1, a steady increase of breaking force (an indicator of tissue toughness) was detected in the spears stored at 25°C for seven days. After seven days of storage, the measured toughness for non-treated control spears gradually increased to 4.22 N force cm^{-2}.

Increases in toughness were also obtained from the heat-shocked spears over seven days of storage. However, the extent of increases was much lower in the heat-shocked spears; the measured toughness only reached to 3.83 N force cm^{-2} at the end of seven days storage. These results suggest that the heat-shock treatment is effective in retarding the increased tissue toughness during 25°C storage.

Lignin, cellulose and H_2O_2 contents at 25°C storage condition

Lignin is a complex polymer of phenylpropanoid residues occurring in large quantity in the secondary cell walls of fibers, xylem vessels and tracheids. These vascular tissues of green asparagus spear are reported to continually produce lignin after harvest (Hennion et al., 1992; Waldron et al., 2003). Boudet (2000) reported that the changes in toughness of control and heat-shocked asparagus spears during 25°C storage were linked to the

Table 2. The contents of cellulose (% of fresh weight), lignin (% of fresh weight) and H_2O_2 (μmole g^{-1} fresh weight weight) measured in the control and heat-shocked green asparagus spears stored at 25 or 4°C for seven days.

Treatment	Storage temperature (°C)	Storage period(days)				
		0	1	3	5	7
		Lignin content (% fresh weight)				
Control	25	0.50±0.09	0.65±0.02^{b}	0.76±0.05^{b}	0.90±0.06^{b}	1.21±0.11^{d}
Heat-shocked			0.59±0.04^{ab}	0.71±0.03^{b}	0.84±0.07^{b}	0.94±0.09^{c}
Control	4		0.53±0.10^{a}	0.57±0.05^{a}	0.63±0.04^{a}	0.76±0.04^{b}
Heat-shocked			0.52±0.11^{a}	0.55±0.04^{a}	0.60±0.04^{a}	0.67±0.03^{a}
		Cellulose content (% fresh weight)				
Control	25	0.55±0.12	0.89±0.05^{b}	1.48±0.05^{d}	1.79±0.05^{c}	2.24±0.12^{d}
Heat-shocked			0.61±0.07^{a}	1.32±0.10^{c}	1.49±0.05^{b}	1.89±0.08^{c}
Control	4		0.60±0.09^{a}	0.88±0.01^{b}	1.36±0.08^{b}	1.62±0.06^{b}
Heat-shocked			0.58±0.16^{a}	0.71±0.03^{a}	1.14±0.06^{a}	1.46±0.03^{a}
		H_2O_2 content (μmol g^{-1} Fresh weight)				
Control	25	25.43±1.95	36.31±0.72^{d}	42.55±2.15^{c}	45.27±2.25^{c}	56.13±3.09^{d}
Heat-shocked			31.16±0.80^{b}	36.42±3.08^{b}	40.21±2.20^{b}	49.24±2.59^{c}
Control	4		28.82±0.61^{a}	31.74±1.46^{a}	31.73±1.53^{a}	37.01±2.01^{b}
Heat-shocked			27.21±0.66^{a}	28.25±1.48^{a}	30.73±2.24^{a}	33.26±1.47^{a}

Values for lignin, cellulose and H_2O_2 with different superscript letters within columns were significantly different at $P < 0.05$, respectively.

concomitant changes in their lignin contents. Therefore, in the present study, the contents of lignin, cellulose and H_2O_2 in the tested asparagus spears were determined and compared. As shown in Table 2, the lignin content in the control spears was increased from 0.53 to 1.21% over the seven days of 25°C-storage (Table 2). The lignin content in heat-shocked asparagus spears was also increasing, but the extent of lignin increases was lower than non-treated control spears (Table 2). These results indicate that the 48°C heat-shock treatment prior to storage could retard the lignin increases in green asparagus spears during seven days of storage at 25°C. The content of cellulose also plays a key role in the texture attributes of asparagus cell wall (An et al., 2007).

In the present study, the changes in cellulose content were also observed in control spears (increased from 0.55 to 2.04%) stored at 25°C for seven days (Table 2). The cellulose content in the heat-shocked spears was also increasing, but with the extent of cellulose increases lower than those in the controls (Table 2). H_2O_2 is an ubiquitous and dynamic constitute of plant cells. It serves as a substrate in several cell-wall formation reactions such as the apoplastic cross-linking of cinnamyl alcohols during lignin biosynthesis (Ros Barceló, 1998). Our results showed that the changing trend of H_2O_2 contents in the control and the heat-shocked spears stored at 25°C were basically the same, which increased gradually throughout the entire storage period (Table 2). However, the heat-shock treatment did reduce H_2O_2 accumulations in the treated spears (increased to 49.24 μmol g^{-1} fresh weight) as compared to those in the control spears (increased to 56.13 μmol g^{-1} fresh weight) during seven days of 25°C-storage (Table 2). The reduced H_2O_2 contents indirectly supported the findings of decreased lignin formation in green asparagus spears heat-shocked at 48°C. Boudet (2000) reported that the increases in toughness of stored asparagus spears were coupled with increases in lignin and cellulose contents. The calculated positive correlations ($P < 0.01$) between toughness and lignin, cellulose and H_2O_2 contents (across control and heat-shocked spears) supported his findings (Table 3).

Phenylalanine ammonia-liase (PAL), cinnamyl alcohol dehydrogenase (CAD) and peroxidases (POD) activities at 25°C storage condition

The degree of lignification of plant tissues is reported to be enhanced by enzymes such as PAL, CAD and POD (Chen et al., 2002; Cai et al., 2006; Li and Zhang, 2006). PAL catalyzes the first reaction in the biosynthesis of plant phenylpropanoid products (Cai et al., 2006). CAD plays a down-regulating role in the last step of monolignol pathway, while POD catalyzes the polymerization of monolignol like p-coumaroyl, coniferyl, 5-hydroxyconiferyl and sinapyl alcohols to complete the process of

Table 3. The correlation coefficients between the tested physical and chemical characteristics of green asparagus spears (across control and heat-shocked spears) stored at 25 or 4°C.

Parameter	Lignin	Cellulose	H_2O_2
25°C			
Toughness	0.903**	0.913**	0.865**
PAL activity	0.571**	0.626**	0.593**
CAD activity	0.193	0.355*	0.201
POD activity	0.539**	0.529**	0.563**
4°C			
Toughness	0.923**	0.837**	0.892**
PAL activity	0.907**	0.815**	0.829**
CAD activity	0.445**	0.552**	0.510**
POD activity	0.735**	0.656**	0.639**

*, ** Values are significant at $P < 0.05$ and 0.01, respectively.

lignification (Holm et al., 2003). As shown in Table 4, an increase in PAL activity was observed in the control spears stored at 25°C for three days (increased from 16.81 to 54.47 units g^{-1} fresh weight), and then followed by a rapid decrease (reached to 26.01 units g^{-1} fresh weight) afterwards. Heat-shocked spears showed retardation and delay in the changing trend of PAL activity, with the PAL activity increasing from 16.81 (day 0) to 48.15 units g^{-1} fresh weight (day 5) and then declining (Table 3) afterwards. Both CAD and POD activities in the control and heat-shocked spears stored at 25°C followed the changing trends of PAL activity, but each peaked at different sampling time (Table 4). The reduced PAL, CAD and POD activities may explain in part why heat-shock treatment retards the lignifications of green asparagus spears and preserves their quality to some extent as compared to non-treated control spears. These notions were supported by the positive correlations (across control and heat-shocked spears) between the contents of lignin, cellulose and H_2O_2 and the activities of enzyme activities (except lignin or H_2O_2 vs. CAD) shown in Table 3.

Liu and Jiang (2006) pointed out that, in green asparagus, the plant hormone ethylene played a key role in the accumulation of lignification that resulted from the enhanced PAL, CAD and POD activities. Ethylene is produced in response to various kinds of environmental stress, including wounding, and the wound-induced ethylene is involved in plant lignification (Luo et al., 2007). The fresh-cut asparagus spear is considered as a wounded tissue; therefore, the observed lignification in fresh-cut spears can be a wound-induced ethylene effect. It appears that the wound-induced ethylene evolution of heat-shocked spears might be lower than that of non-treated control spears. Accordingly, the activities of PAL, CAD and POD are reduced to some extent, and result in a less lignin accumulation in heat-shocked asparagus spears during 25°C storage (Falik, 2004).

Microbial loads at 25°C storage condition

Table 5 showed the populations of foodborne pathogens proliferated on the green asparagus spears during seven days of 25°C-storage. The initial levels of total mesophilic aerobes, *E. coli*, and mold measured in the green asparagus spears were 2.36, 2.07 and 1.06 log cfu g^{-1} fresh weight, respectively. After seven days of 25°C-storage, the populations of total mesophilic aerobes, *E. coli*, and mold were increased to 4.87, 3.36 and 2.37 log cfu g^{-1} fresh weight, respectively. The populations of total mesophilic aerobes, *E. coli*, and mold in heat-shocked asparagus spears were increased to 3.58, 3.19 and 2.17 log cfu g^{-1} fresh weight, respectively. The values were 24, 5 and 8% lower than the total mesophilic aerobes, *E. coli* and mold counts recorded on non-treated controls, respectively. These results are expected because the application of optimal pre-storage heat treatment to fresh-cut produces is shown to be beneficial in decreasing the proliferation of various microorganisms during storage (Fallik, 2004). Thus, it appears that the heat-shock treatment (washing in 48°C distilled water for 5 min) can be used to reduce the microbial load in fresh-cut green asparagus spears stored at 25°C.

Cold (4°C) storage effects on the tested physical and chemical characteristics

Temperature management is an important factor affecting the quality of fresh produce, and cold storage is generally recommended for highly perishable vegetables such as green asparagus (Albanese et al., 2007). In the present study, cold (4°C) storage period of seven days significantly reduced the fresh-weight losses in green

Table 4. The activities (unit g^{-1} fresh weight) of phenylalanine ammonia-lyase (PAL), cinnamyl alcohol dehydrogenase (CAD) and peroxidase (POD) measured in the control and heat-shocked green asparagus spears stored at 25 or 4°C for seven days.

Treatment	Storage temperature (°C)	Storage period(days)				
		0	1	3	5	7
		Phenylalanine ammonia-lyase activity (unit g^{-1} fresh weight)				
Control	25	16.81±0.93	28.61±1.26^{c}	54.47±3.19^{d}	38.13±2.61^{c}	26.01±1.82^{a}
Heat-shocked			25.60±1.43^{b}	40.41±1.89^{b}	48.15±2.16^{d}	38.90±2.11^{c}
Control	4		18.22±0.92^{a}	25.30±2.90^{a}	30.21±1.56^{b}	34.14±1.34^{b}
Heat-shocked			16.73±1.41^{a}	21.67±2.39^{a}	24.11±2.14^{a}	27.22±1.45^{a}
		Cinnamyl alcohol dehydrogenase activity (unit g^{-1} fresh weight)				
Control	25	4.20±0.24	5.14±0.11^{b}	9.37±0.50^{e}	5.71±0.24^{b}	3.45±0.44^{a}
Heat-shocked			4.70±0.16^{a}	7.57±0.72^{c}	6.77±0.42^{c}	4.92±0.99^{b}
Control	4		4.66±0.14^{a}	6.27±0.47^{b}	5.73±0.23^{b}	5.21±0.21^{b}
Heat-shocked			4.41±0.17^{a}	4.84±0.22^{a}	5.04±0.32^{a}	5.24±0.43^{b}
		Peroxidase activity (unit g^{-1} fresh weight)				
Control	25	17.42±0.83	24.42±0.72^{b}	38.62±2.91^{cd}	36.22±2.11^{b}	24.24±1.80^{b}
Heat-shocked			20.13±0.64^{a}	36.63±0.93^{c}	36.70±0.63^{b}	23.62±1.81^{a}
Control	4		18.57±0.73^{a}	25.91±2.23^{b}	23.74±1.05^{a}	21.48±1.24^{a}
Heat-shocked			18.10±1.63^{a}	21.82±1.46^{a}	20.58±0.67^{a}	22.19±1.26^{a}

Values PAL, CAD and POD with different superscript letters within columns were significantly different at $P < 0.05$, respectively.

asparagus spears (Table 1). Both control and heat-shocked green asparagus spears stored at 4°C showed considerably less fresh-weight losses than the spears stored at 25°C. The relatively less fresh-weight loss obtained from the spears stored at 4°C might be explained by the reduced water loss at low temperature. Cold (4°C) storage also significantly reduced the increasing trend of toughness in the stored asparagus spears (Table 1). Both control and heat-shocked spears stored at 4°C exhibited less toughness than the spears stored at 25°C at respective sampling time (Table 1).

The differences in toughness between control and heat-shocked spears were also significant, with the toughness values obtained from the heat-shocked spears lower than the values obtained from control spears. These results confirm that a combination of heat shocking and 4°C cold storage is effective in preserving the quality of green asparagus spears. As shown in Table 2, the contents of lignin, cellulose and H_2O_2 in control and heat-shocked green asparagus spears were also increasing during 4°C storage. But the increased lignin, cellulose and H_2O_2 contents within seven days of storage at 4°C were significantly lower than those of the spears stored at 25°C. Additionally, the heat-shocked spears contained less lignin, cellulose and H_2O_2 than their respective controls during seven days of 4°C-storage (Table 2).

As a result, positive correlation coefficients (across control and heat-shocked spears) between toughness and lignin, cellulose or H_2O_2 contents were also obtained from the green asparagus spears stored at 4°C (Table 3).

Cold (4°C) storage effects on PAL, CAD and POD activities

The changes in PAL activities obtained from the spears stored at 4°C for seven days differed from the PAL activities obtained from the samples stored at 25°C (Table 4). Both the control and the heat-shocked spears stored at 4°C showed a steady increase in PAL activities throughout the cold storage period. The maximum PAL activities obtained from the spears stored at 4°C were lower than the maximum activities obtained from the spears stored at 25°C (Table 4). Additionally, the PAL activities obtained from the heat-shock spears were lower than the PAL activities obtained from the non-treated controls. The activities of CAD and POD obtained from the spears stored at 4°C were also lower than the activities of CAD and POD obtained from the spears stored at 25°C (Table 4). Minor differences in CAD and POD activities were detected between the control and the heat-shocked spears, with the heat-shocked spears generally having slightly lower CAD and POD activities developed lignification of asparagus spears during 4°C storage are probably due to their lower PAL, CAD and POD activities (Villanueva et al., 2005).

As shown in Tables 1, 2 and 4, it is noticeable that the green asparagus spears stored at 4°C maintain better physical and chemical qualities than that of the spars

Table 5. The counts of total mesophilic aerobes, *E. coli* and mold (log cfu g^{-1} fresh weight) measured on the bud segment of green asparagus subjected to various treatments and stored at 25 or 4°C for 7 days.

Treatment	Storage temperature (°C)	Storage period(days)				
		0	1	3	5	7
		Total mesophilic aerobes (log cfu g^{-1} fresh weight)				
Control	25	2.36±0.31	2.94±0.20^{a}	3.38±0.20^{a}	4.12±0.27^{a}	4.87±0.38^{a}
Heat-shocked			2.60±0.12^{b}	2.68±0.14^{c}	2.98±0.16^{c}	3.58±0.21^{b}
Control	4		2.87±0.10^{a}	3.02±0.11^{b}	3.25±0.11^{b}	3.56±0.18^{b}
Heat-shocked			2.58±0.09^{b}	2.78±0.16^{c}	2.99±0.10^{c}	3.04±0.17^{c}
		E. coli (log cfu g^{-1} fresh weight)				
Control	25	2.07±0.23	2.50±0.19^{a}	2.57±0.11^{a}	2.92±0.11^{a}	3.36±0.18^{a}
Heat-shocked			2.18±0.10^{b}	2.49±0.21^{a}	2.82±0.21^{a}	3.19±0.14^{ab}
Control	4		2.32±0.10^{ab}	2.58±0.18^{a}	2.79±0.24^{a}	2.91±0.19^{b}
Heat-shocked			1.86±0.21^{c}	2.01±0.17^{b}	2.22±0.26^{b}	2.47±0.21^{c}
		Mould (log cfu g^{-1} fresh weight)				
Control	25	1.06±0.02	1.16±0.12^{a}	1.56±0.11^{a}	1.93±0.07^{a}	2.37±0.13^{a}
Heat-shocked			1.08±0.23^{a}	1.38±0.09^{b}	1.71±0.13^{b}	2.17±0.11^{a}
Control	4		1.18±0.10^{a}	1.46±0.11^{ab}	1.72±0.08^{b}	2.11±0.18^{ab}
Heat-shocked			1.05±0.19^{b}	1.32±0.06^{b}	1.68±0.14^{b}	1.92±0.15^{b}

Values total bacterial, *E. coli* and mold counts with different superscripts were significantly different at the level of $P < 0.05$.

stored at 25°C. The improved physical quality of 4°C-stored green asparagus spears is possibly due to the relatively low ethylene production rate during 4°C storage (Zamorano et al., 1994).

Cold (4°C) storage effects on microbial loads

As shown in Table 5, both control and heat-shocked spears stored at 4°C showed steady increases in the populations of total mesophilic aerobes, *E. coli* and mold throughout the seven days of cold storage period. Nevertheless, the increased populations of total mesophilic aerobes, *E. coli* and mold in the spears stored at 4°C were significantly lower than those of the spears stored at 25°C. Additionally, the populations of total mesophilic aerobes, *E. coli*, and mold obtained from the heat-shocked spears were consistently lower than the populations of total mesophilic aerobes, *E. coli* and mold obtained from the non-treated controls. The asparagus spears subjected to heat shock treatment showed 1.83, 0.89 and 0.45 log cfu g^{-1} fresh weight reduction of total mesophilic aerobes, *E. coli* and mold comparing to non-treated control spears stored at 25°C, respectively, after seven days of 4°C cold storage (Table 5). Thus, both heat-shock and 4°C cold storage are useful approach for controlling the microbial proliferation on stored green asparagus spears. However, a combination of heat-shock treatment and 4°C cold storage is a desirable choice for further reducing microbial load on green asparagus spears.

Conclusion

In the present study, significant differences in physical and chemical parameters including the fresh-weight loss, toughness, the contents of lignin, cellulose and H_2O_2, and the activities of PAL, CAD and POD were found between the control and the heat (48°C)-shocked green asparagus spears that were stored at 25 or 4°C for seven days. The heat-shocked green asparagus spears tended to show less fresh-weight loss, show less toughness increase, accumulate less lignin, cellulose and H_2O_2, and exhibit less PAL, CAD and POD activities than the control spears. Heat-shock treatment also exhibited significant reductions in the proliferations of total mesophilic aerobes, *E. coli* and mold within seven days of storage. Cold (4°C) storage effectively preserved the quality of green asparagus spears by inducing significant declines in lignin, cellulose and H_2O_2 accumulations, decreasing the activities of PAL, CAD and POD, and decreasing the proliferations of total mesophilic aerobes, *E. coli* and mold. It appears that the 48°C heat-shock treatment can be considered for maintaining the quality of fresh-cut green asparagus spears to some extent. A combination of 48°C heat-shock and 4°C cold storage can further improve the quality of stored green asparagus spears.

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