PAPER

PHYSICOCHEMICAL, MICROBIAL AND SENSORY QUALITY OF FRESH-CUT RED BEETROOTS IN RELATION TO SANITIZATION METHOD AND STORAGE DURATION

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ABSTRACT

Effects of sanitization and storage on fresh-cut beetroots (*Beta vulgaris* L.) were evaluated following sanitation – peeling - cutting (SPC), peeling – sanitation – cutting (PSC) and peeling – cutting – sanitation (PCS) methods with (Cl), or without (TW), 100 ppm chlorine solution, then packaged in polyethylene bag and stored at 5°C for up to 14 days. Chroma values of fresh-cut beetroots significantly declined whereas whiteness index and titratable acidity values increased, however, texture and total soluble solid contents showed no significant variation. Betalain contents decreased gradually and total phenol content showed inconsistence trend. PCS-Cl treated samples accounted for higher betalains decline and received lower visual quality scores despite its lower total aerobic bacterial count. Minimum microbial population was observed in PSC-Cl methods along with higher levels of betalain contents. Considering pigment retention, microbial and visual qualities, beetroots sanitized with chlorine water following PSC method was the best processing way for fresh-cut beetroots and therefore, PSC-Cl treatment could commercially be used for processing of fresh-cut beetroots.

- Keywords: betalain, microbial population, quality, red beetroot, sanitation, total phenolic -

INTRODUCTION

Beet is a root vegetable of the Chenopodiaceae family whose edible part is its tuberous root. Its purple-red color is due to the presence of betalain pigments. These pigments are similar to anthocyanins and flavonoids, which were wrongly termed in the old literature as anthocyanins containing nitrogen. Betalains are water-soluble, vacuolar pigments and are found only in ten families of the *Centrospermae* group and are divided into two classes: red betacyanin and yellow betaxanthin (FENENA, 1995). In addition to their color, betalains possess several desirable biological activities including antioxidant, anti-inflammatory, hepatoprotective, and antitumor properties (ESCRIBANO *et al.*, 1998; WINKLER *et al.*, 2005). Although some studies have indicated their potential as antioxidant pigments, betalains have not been much explored because of their relative scarceness in nature. The bioavailability of betalains was reported to be high in humans, and they remain stable in the gastrointestinal tract without any significant loss in antioxidative properties, which increases their value as health food additives (FRANK *et al.*, 2005; PAVLOV *et al.*, 2005). Among other compounds with antioxidant properties, phenolics are believed to act as antioxidant, anti-carcinogenic, anti-microbial, anti-mutagenic and anti-inflammatory, as well as in the reduction of cardiovascular diseases (YANG *et al.*, 2001; KIM *et al.*, 2003; VALI *et al.*, 2007). Because of these nutritional importances of plant phenolics, there has been an increasing interest in the evaluation of their changes with postharvest treatment (CHAUDRY *et al.*, 1998; LEWIS *et al.*, 1999). However, literatures are scanty on phenolic content of plant foods, especially of roots vegetables, which are important constituents of diets in many countries. Storage and processing can reduce the content of phenolic compounds as some of them are easily oxidized, while others are more stable. Processing in the form of simple peeling of fruit and vegetables can remove a major portion of the phenols, as the concentrations of these substances are often higher in the outer than the inner parts (MANACH *et al.*, 2004). Pigment distribution in beetroots, on the other hand, also varied substantially among the outer, middle and center tissues, the former contained much higher pigments than the later tissues (GAERTNER and GOLDMAN, 2005).

Beet was traditionally used as a vegetable boiled in stews, baked in tarts, roasted as a whole and cut into salads. This vegetable has recently been using as a minimally processed food in many countries. However, the main technological problems of processing fresh-cut beet roots are the significant discoloration and dehydration of the minimally processed material. Washing and rinsing operations carried out after slicing have favored the loss of betacyanin and betaxanthin, since these pigments are soluble in water (NILSON, 1970). Moreover, minimally processed produces are more perishable than their whole counterparts, due to undergoing severe physical stresses especially during peeling and cutting procedures. Minimal processing comprises selection, washing, peeling and cutting procedures that are aimed at producing a fresh and convenient product to prepare and consume (BURNS, 1995). The quality of fresh-cut produce depends on many factors, such as the state of the original plant (variety, plant part and maturation stage), harvesting date and storage, environmental factors and processing techniques (MOURE *et al.*, 2001). However, rapid quality deterioration and shorter shelf-life are major problems facing the industry for maintaining the quality and safety of fresh-cut produces. Several studies have shown that fresh-cut produces are particularly susceptible to microbial growth owing to the removal of plant protective tissues and the release of cellular fluids from cutting (CARLIN *et al.*, 1989; HEARD, 2002). The cut slices of beetroot become vulnerable to microbial attack, moisture loss and dehydration because of their large surface area. To ensure the microbial safety, use of proper sanitizing agents along the processing line is an important step in fresh-cut produce processing. Chlorine has been used for sanitation purposes in food processing industry for several decades and perhaps the most widely used sanitizer in food industry. It is inexpensive, convenient to use and works against many food borne pathogens. Liquid chlorine and hypochlorite generally used in the 50 to 200 mg L^{-1} concentration range with a contact time of 1 to 2 min. ADAMS *et al.* (1989) reported that washing lettuce leaves with 100 mg L^{-1} free chlorine can reduce population of aerobic mesophiles by more than 98% as compared to 92% reduction in tap water without chlorine.

The use of beetroots as a fresh-cut produce is relatively new, especially in Asian countries. The information on various quality parameters of fresh-cut beetroots processed with different sanitization methods is limited. Hence, the objectives of this study were to find out the best processing and sanitation way for maintaining some physicochemical, microbial and sensory qualities, especially pigment and phenolic contents of fresh-cut beetroots during storage at 5°C.

MATERIALS AND METHODS

Preparation of sample

Beetroots (*Beta vulgaris* L. var. Udan) samples were harvested from a commercial farm in Jeju island, Republic of Korea, and were transported to our laboratory within two days. Samples were thoroughly washed in running tap water and subjected to different ways of sani-

tation treatments such as sanitation – peeling - cutting (SPC), peeling – sanitation – cutting (PSC) and peeling – cutting – sanitation (PCS) with (Cl), or without (TW), 100 ppm chlorinated solution. Chlorinated solution was prepared with NaOCl (100 ppm free chlorine, pH 7.0) as a standard industrial disinfection treatment for fresh-cut produces. After peeling out, beetroot samples were cut into small pieces (ca. 4 × 5 × 1 cm) excluding the top and bottom most portions. Sanitation treatments lasted for 2 min. To remove the excess surface water, all sanitized samples were spread over a sieve like plastic tray (40 × 50 cm) previously washed and disinfected with the same sanitizing solution and allowed at room temperature. Fresh-cut beetroots sample of about 300 g were packaged in 80 μm nylon polyethylene bag (25 × 20 cm) and thermally sealed. Four replicates of each bag per treatment and storage duration (0, 3, 7, 10 and 14 days of storage) were prepared and stored in a dark cold room at 5°C. On each evaluation day, outer and inner tissues were separated from the fresh-cut red beetroots slices discarding the middle portion and were stored at -80°C until needed for biochemical analyses.

Color and texture measurement

Using a chromameter (Minolta CR-400, Minolta, Osaka, Japan), color readings were taken from the middle portion of both sides of sliced red beetroots on each evaluation day. Three pieces of sliced beetroot from each pouch were randomly selected and six readings (from both sides of each piece) were taken from each replicate and a total of 24 readings were averaged from each treatment on the measurement day. The chromameter was calibrated using the manufacturer's standard white plate (Y 93.5, x 0.3155, y 0.3320). Color changes were quantified in the L^*, a^*, b^* color space. L^* refers to the lightness and ranges from black = 0 to white = 100. A negative value of a^* indicates green, while a positive number indicates red color. Positive and negative b^* indicate yellow and blue color, respectively. The color values were further converted into Chroma value (OZTURK *et al.*, 2009) and 'Whiteness Index' (WI) (BOLIN and HUXSOLL, 1991) and were computed by the formulae:

$$\text{Chroma} = (a^{*2} + b^{*2})^{1/2};$$
$$\text{WI} = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$$

The texture of fresh-cut red beetroot was measure in term of force required to make a puncture hole horizontally on the sliced beetroots. The test was conducted through destructive puncture test performance using a texture analyzer (TA Plus, Lloyd Instruments, Ametek Inc., UK) following the method of HAMPSHIRE *et al.* (1987). Each sliced beetroots sample was placed horizontally on the stationary platform of the analyzer for the test. Tests were carried out on the middle part of each sliced beetroots with a 4 mm diameter stainless steel cylindrical probe. The movement of the probe was adjusted to 5, 2 and 10 mm/s as the pre-test, test and post-test speed, respectively. With a running load cell of 100 N, the probe was attached to a creep meter equipped with the software (NEXYGEN™MT v 4.5, Lloyd Instruments, Ametek Inc., UK) for automatic analysis using a computer. The maximum amount of force (N) needed to make a puncture hole on the sliced beetroot was recorded. Five pieces of sliced beetroots from each pouch were tested and a total of 20 data from four replicates were averaged from each treatment. All measurements were taken at room temperature (20°±2°C).

Determination of total soluble solid, pH, and titratable acidity

Total soluble solid (TSS), pH and titratable acidity (TA) were measured according to the AOAC (1980) procedures. On each evaluation day, about 5 pieces of sliced beetroot from each bag were cut into small pieces and wrapped with 2 layers of cotton cloth and placed in a Juice maker (Fru-X80, GooJung Chromatech Inc., Korea) attached to an air supplier (EvaA–0300) of the same company. Then pressure was created by the air supplier to obtain a homogenized solution of beetroots sample. TSS of the resultant cleared juice was measured in terms of °Brix using a refractometer (PAL–1, Atago Co. Ltd, Tokyo, Japan). The pH was determined using a pH meter (D-55122, Schott Instruments GmbH, Germany) with a glass electrode. Titratable acidity was measured by potentiometric titration with 0.1 N NaOH up to pH 8.2 using 10 mL juice and the results were expressed as percentage of citric acid.

Determination of betalains (betacyanin and betaxanthin) and total phenolic contents

The methodology used for the determination of betacyanin and betaxanthin were adopted from VON ELBE (2001). Five gram of previously frozen samples from the outer and inner parts of sliced beetroots were macerated separately in 15 mL distilled water using an Ultra-Turrax tissue homogenizer (T 25 B, Ika Works Sdn. Bhd, Malaysia) at a moderate speed for about 1 min. The homogenate was transferred to a volumetric flask filtered through Whatman no. 1 filter paper placed on a glass funnel. The filter cake was washed several times with distilled water until the extract became colorless. The extract volume was adjusted with distilled water. The resulting beetroots juice or tissue extract was diluted with 0.05 M phosphate buffer, pH 6.5 such that the absorbance of beetroots juice at

538 nm was in between 0.4 and 0.5 AU. Finally, the absorbance of beetroots juice was measured at 476, 538 and 600 nm with an UV-VIS recording spectrophotometer (DU 650, Beckman Coulter™, USA) and 0.05 M phosphate buffer, pH 6.5 was used as the blank. Values of betacyanin and betaxanthin amounts were obtained through the equation:

$$x = 1.095 \times (a - c),\ y = b - z - x/3.1,\ z = a - x$$

where a = sample reading at 538 nm; b = sample reading at 476 nm; c = sample reading at 600 nm; x = betacyanin absorption; y = betaxanthin absorption; z = impurities absorption (VON ELBE, 2001).

Total phenolic compound was determined based on the method described by SINGLETON and ROSSI (1965) with few modifications. Five gram previously frozen sample from the outer and inner parts of fresh-cut red beetroots slices were separately homogenized with 80% ethanol using an Ultra-Turrax tissue homogenizer (T 25 B, Ika Works Sdn. Bhd, Malaysia) at a moderate speed for about 1 min. The homogenate was incubated at 60°C water bath for 30 min and centrifuged at 15,000 × *g* for 15 min at 20°C and then the supernatant was collected in a volumetric flask. The homogenized tissue was re-extracted with 80% ethanol at the same way and the resulting supernatants were mixed together and carefully made known volume with 80% ethanol. For the determination, Folin-Ciocalteu reagent was diluted with distilled water to make 1 N phenol reagent. In a test tube, 1 mL supernatant was diluted with 8 mL distilled water and 1 mL of 1 N phenol reagent was added followed by mixing. After 5 min, 1 mL 15% sodium carbonate solution was added, mixed well and allowed the mixture at room temperature (~20°C) for 2 h. The absorbance was read at 725 nm using an UV-VIS recording spectrophotometer (DU 650, Beckman Coulter™, USA). The concentration of total phenol was calculated using standard curve of gallic acid and expressed as gallic acid equivalents in mg 100 g^{-1} fresh weight.

Monitoring of microbial population

Microbiological counts were carried out on every sampling day including washing day. Fresh-cut beetroots pouches were aseptically opened using a sterilized scissors dipped in 95% ethanol and then ignited in the flame of a Bunsen burner. Twenty grams beetroots sample was weighed out from each pouch and placed in a stomacher bag (Masher-bag P-LTS, BAC-cT®, NBT, Japan). The beetroots sample was diluted 1:9 in double distilled autoclaved water and homogenized in a stomacher (Seward Stomacher 400C, Brinkmann, USA) for 1 min at 230 rpm. A 10-fold serial dilution was also made from the homogenate and 1 mL of homogenate solution was inoculated onto total aerobic bacterial (TAB) count Petrifilm™ (3M Microbiology Products, St. Paul, Minn., USA). The plates were then incubated at 35°C for 48 h and the developing colonies (about 25 – 250) were counted with the assistance of a 3M microbial colony counter (same company as of plate) and reported as colony forming units (CFU) per gram of sample. Similarly, 1 mL homogenate was plated onto 3M Petrifilm™ yeast and mold (YM) count plates (same company) and incubated for 72 h at 25°C. After incubation, yeast and mold colonies were counted manually according to the instruction guide of the company. Colonization data were then converted to log CFU per gram of fresh sample.

Sensory evaluation

The sensory analysis of fresh-cut beetroots sample was carried out by an 8-member (aged 24 - 48) expert panel. The members of the panel were trained to recognize and score off-odor and overall visual quality of fresh-cut beetroots prior to the test. Off-odor was evaluated immediately after opening the packages and scored on a 5-point scale in which 0 = none, 1 = slight, 2 = moderate, 3 = strong, and 4 = extremely strong (LOPEZ-GALVEZ *et al.*, 1997); a score of 3 was considered non-acceptable. Overall visual quality was evaluated by using 9-point scale (9 = excellent, 7 = good, 5 = fair, 3 = poor and 1 = unusable) (GONZALEZ-AGUILAR *et al.*, 1999). A score of 6 was considered as the limit of marketability.

Statistical analysis

The experiment was conducted with four replications per treatment. Statistical analyses of the data were carried out using SAS software (SAS Institute, Cary, NC, USA). The level of significance was calculated from the *F* value of ANOVA. Mean comparison was achieved by Duncan's multiple range test. Prior to the final experiment, two preliminary experiments were conducted with limited replications that resulted similar trend.

RESULTS AND DISCUSSION

Texture, total soluble solids, pH and titratable acidity

The effects of different washing methods and storage duration on the textural properties of fresh-cut red beetroots are presented in Table 1. The forces were almost similar for all the sanitization methods, except for an insignificant ($P>0.05$) lower value was recorded in PSC-Cl treatment on washing day. However, the values were slightly increased to an

Table 1 - Changes in some physicochemical qualities of fresh-cut redbeet root during storage at 5°C after processed with different sanitization methods.

Parameter/storage day	Sanitization method					
	SPC-TW	SPC-Cl	PSC-TW	PSC-Cl	PCS-TW	PCS-Cl
Texture (N)						
0	52.58aA	52.22aA	49.92aA	47.61aA	51.13aA	52.49aA
7	52.83aA	53.80aA	51.40aA	49.51aA	52.47aA	53.85aA
14	53.43aA	54.97aA	53.39aA	50.36aA	54.29aA	55.36aA
°Brix						
0	8.50aA	8.67aA	7.83aA	8.70aA	8.33aA	8.23aA
7	8.23aA	8.60aA	8.37aA	8.03aA	8.57aA	8.53aA
14	7.90aA	8.67aA	8.03aA	8.27aA	8.43aA	8.37aA
pH						
0	6.28aA	6.25aAB	6.24bAB	6.28aA	6.22aAB	6.16cB
7	6.42aAB	6.38aBC	6.38aBC	6.30aC	6.35aBC	6.50aA
14	6.26aA	6.28aA	6.21bA	6.30aA	6.21aA	6.36bA
TA (% citric acid)						
0	0.10bA	0.10bA	0.10bA	0.12aA	0.09bA	0.09bA
7	0.09bAB	0.08bB	0.08bB	0.10aAB	0.11bA	0.10bAB
14	0.17aA	0.15aA	0.15aA	0.16aA	0.18aA	0.14aA

SPC = sanitation - peeling - cutting, PSC = peeling - sanitation - cutting, PCS = peeling - cutting - sanitation, Cl = sanitation with 100 ppm chlorinated water, TW = sanitation with tap water.
Values represent the mean of four replicates. Means under the same heading in each column or row with different small or capital letters, respectively are significantly different according to the Duncan test (P<0.05).

insignificant level at the end of 14 days storage. PCS-Cl treated sample exhibited the highest value among the treatments at the end of storage. Maintaining texture of fresh-cut produces is one the main criteria which is affected by morphology, cell turgor, cells wall-middle lamella structure, water content, biochemical components and also by the genetic background of plant species (HARKER *et al.*, 1997). Peeling and cutting of vegetable exposes the interior tissues and drastically increase the rate of evaporation of water. However, we observed less than 1% moisture loss of the original weight at the end of storage (data not shown). Apart from water loss, the slight increase in texture value during storage of fresh-cut beetroots in our study might be due to the lignification of cells that rendered harder tissues and as a result of wound healing at cut surfaces. Packaging fresh-cut produces with suitable polyethylene film and selecting appropriate storage temperature have shown to preserve quality. The insignificant increases in texture of fresh-cut beetroots indicate that our packaging film and storage temperature were appropriate.

There was no significant difference (P>0.05) found in total soluble solid (TSS) content in fresh-cut beetroots regardless of sanitization treatment and storage duration, whereas slight variations were observed in pH and titratable acidity (TA) values among the treatments and different storage durations (Table 1). These reflect that although TSS was maintained throughout the storage, TA values increased significantly at the end of the storage that resulted smaller decline in pH values. The lowest TSS (7.83°Brix) was found in PSC-TW treated sample on washing day while the highest value (8.70°Brix) was recorded in PSC-Cl treated sample on the same day. It was reported that TSS of fresh-cut beetroots are varied depending on the cut type (KLUGE *et al.*, 2006), sanitization period (VITTI *et al.*, 2011) and storage temperature (VITTI *et al.*, 2005). Although our TSS data were little higher than that of VITTI *et al.* (2011) and VITTI *et al.* (2005) studies with fresh-cut beetroots, we found both similar amount and changing pattern of TSS during storage as reported by KLUGE *et al.* (2006). The differences in TSS in this study and other studies might be due to the differences in soil condition, cultivar and sowing time (FELLER and FINK, 2004). The pH and TA values ranges from 6.16 to 6.50 and 0.08 to 0.18, respectively among the processing methods and throughout the storage (Table 1). In general, pH values slightly increased in the middle of storage and decreased later to their initial values. TA values expressed as percentage of citric acid, on the other hand, remained unchanged until the middle of storage and then increased about 1.5 fold at the end of storage to their initial levels. Among the treatments, PCS-Cl treated sam-

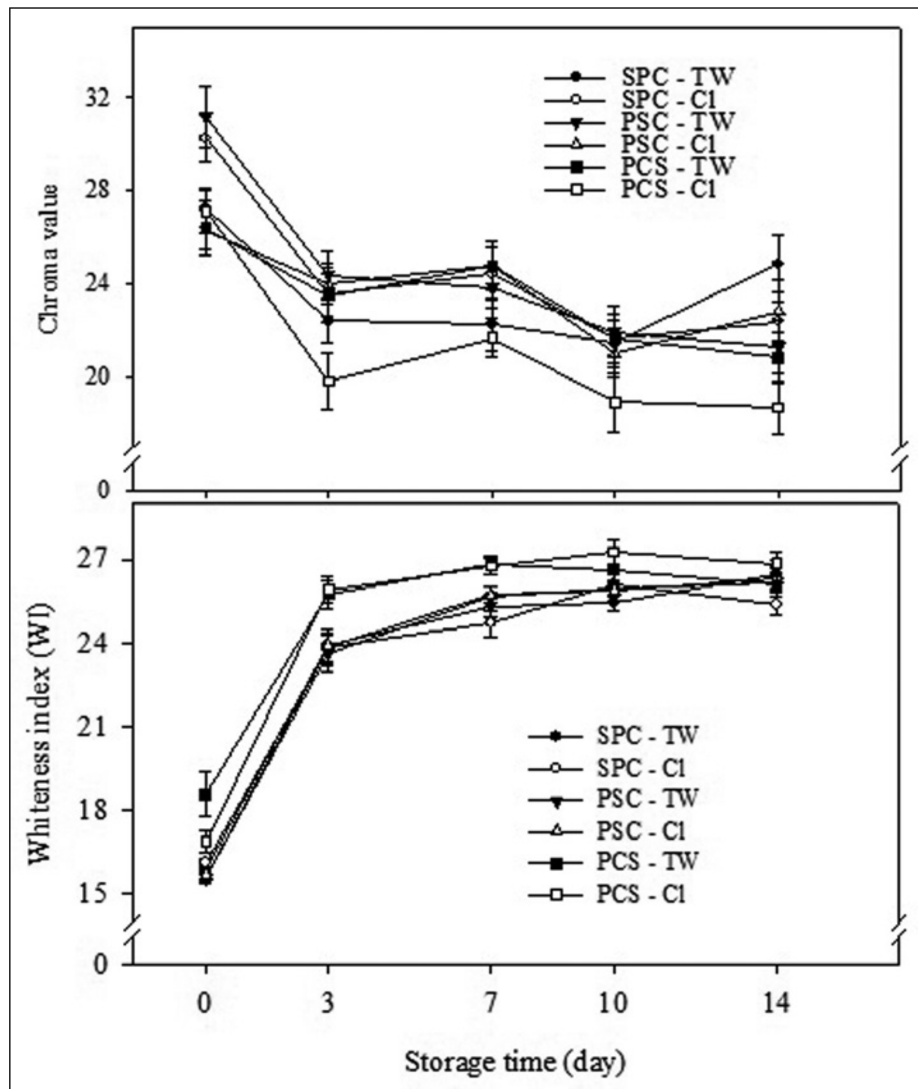

Fig. 1 - Changes in chroma value (above), whiteness index (below) of fresh-cut red beetroot after processing with different sanitation methods and during storage at 5°C. Legends: SPC = sanitation–peeling-cutting, PSC = peeling–sanitation–cutting, PCS = peeling–cutting–sanitation, Cl = sanitation with 100 ppm chlorinated water, TW = sanitation with tap water.

ple showed the lowest values of pH and TA on washing day and 14 days of storage, respectively. In agreement with our results, LOPEZ OSORNIO and CHAVES (1998) found no variation in pH values over a 7-day storage period of raw grated beetroots at 4°C and packaged in trays wrapped with polyvinylchloride film. However, they found steadily increased values of TA throughout the storage. Again, PILON *et al.* (2006) reported that the contents of titratable acidity were not affected by the storage period in minimally processed carrot while the pH values decreased at the beginning of storage and increased thereafter to their initial levels at the end of 3-week storage period. The smaller range of changes in citric acid content during storage of fresh-cut beetroots might be accounted for lower respiratory activity, which is the indicator of retardation of overall metabolic activities.

Color parameters

Produce color is one of the most important quality factors that directly affect consumers' choice. In this study, the effects of different sanitization methods on the color parameters of fresh-cut beetroots were measured in term of chroma value as well as whiteness index and presented in Fig. 1. Among the treatments, SPC-Cl and PSC-TW showed significantly higher ($P<0.05$) chroma value on washing day. However, chroma values declined gradually in all treatments when storage progressed except few fluctuations on 7 and 14 days of storage. There was a significant ($P<0.05$) decline in chroma value in PCS-Cl treatment when storage progressed exhibiting the lowest values of chroma throughout the storage among the treatments. This result implies that sanitation after cutting yielded higher loss of pigment in beetroots slices there-

by decreased the intensity of color. Between the other two sanitation treatments using chlorine water, PSC-Cl showed no significant decline in chroma value until the end of storage except on 10-day while SPC-Cl showed gradual decrease until 10-day followed by a slight increase at the end of storage (Fig. 1). The decrease in color is a consequence of loss of betalains, the main pigments of red beetroots (VON ELBE, 2001). Therefore, we found significant variation and decrease in betalain contents both on washing day and subsequent storage of fresh-cut beetroots in this study (Table 2 and later discussion). In consistence with our result, gradual decrease in color index of fresh-cut beetroots was reported previously (VITTI *et al.*, 2005; KLUGE *et al.*, 2006; VITTI *et al.*, 2011). Since chroma represents color saturation, which varies from dull (low value) to vivid color (high value), we used chroma value as an indicator of freshness and purity of the color of beetroots slices. However, LOPEZ OSORNIO and CHAVES (1998) found significant increase in chroma values during storage of grated beetroots. This might be attributed to the differences in processing, subsequent packaging and storage condition of beetroots in the present study with their study. Whiteness index (WI) on the other hand, sharply increased on the third day of storage in all samples (Fig. 1). Both of the sanitized and water washed samples of peeling – cutting - sanitation (PCS) method exhibited significantly ($P<0.05$) higher values of WI from the washing day to 10 days of storage. This result also indicates that higher pigment loss was occurred when beetroots samples were subjected to sanitize after cutting. Other two methods showed similar values of WI both on processing day and throughout the storage. At the end of the storage, WI values reached nearly similar level for all samples. Although WI was first measured for lignin formation on the surface of fresh-cut carrot slices (BOLIN and HUXSOLL, 1991), LOPEZ OSORNIO and CHAVES (1998) reported that the whitish substances in grated beetroots are a protective lignin biochemically synthesized after peeling. It was also reported that WI is the most sensitive and easily measured indicator of sensory quality of fresh-cut carrot.

Betalains (betacyanin and betaxanthin) content

Significantly higher betalain contents were found in the outer tissues than in the inner tissues of fresh-cut beetroots slices (Table 2). Beetroots samples processed with peeling – cutting - sanitation (PCS) method exhibited significantly ($P<0.05$) lower amount of betalain (betacyanin and betaxanthin) contents on washing day compared to other methods. In general, sanitation – peeling – cutting (SPC) method ensured higher amount of betalain contents than other methods whereas the betalain contents

Table 2 - Changes in betalain (betacyanin and betaxanthin) content in the outer and inner tissues of fresh-cut red beetroots processed with different sanitization methods and during storage at 5°C.

Treatment	Betalain content/storage day														
	Betacyanin (mg 100g^{-1} FW)					Betaxanthin (mg 100g^{-1} FW)					Total betalain (mg 100g^{-1} FW)				
	0	3	7	10	14	0	3	7	10	14	0	3	7	10	14
Outer tissue															
SPC-TW	74.65aA	65.48aAB	55.79aBC	54.57aBC	49.41aC	60.54aA	54.26aA	51.64aB	47.33aBC	42.95aC	135.19aA	119.74aAB	107.43aBC	101.90aBC	92.36aC
SPC-Cl	72.85abA	62.08aAB	60.37aAB	59.70aAB	51.91aB	56.30aA	54.53aA	51.15aAB	49.12aAB	44.13aB	129.15abA	116.61aAB	111.52aAB	108.82aAB	96.04aB
PSC-TW	65.35abA	55.80aA	53.31aA	52.49aA	49.03aA	52.50aA	51.21aA	47.35aA	41.62aA	42.49aA	117.85abA	107.01aA	100.66aA	94.11aA	91.52aA
PSC-Cl	66.72abA	55.50aA	53.36aA	54.96aA	54.95aA	50.91aA	45.60aA	45.49aA	43.42aA	41.88aA	117.63abA	101.10aA	98.85aA	98.38aA	96.83aA
PCS-TW	58.06bA	53.75aA	52.47aA	49.13aA	47.92aA	46.46aA	41.78aA	39.78aA	44.37aA	39.74aA	104.52bA	95.53aA	92.25aA	93.50aA	87.66aA
PCS-Cl	57.65bA	51.52aA	48.42aA	45.02aA	42.80aA	47.87aA	43.86aA	45.17aA	40.34aA	39.25aA	105.52bA	95.38aA	93.59aA	85.36aA	82.05aA
Inner tissue															
SPC-TW	37.45abA	34.18aAB	34.78aAB	30.65aAB	25.38aB	29.82abA	28.31aA	28.60aA	23.74aAB	18.72aB	67.27abA	62.49aA	63.38aA	54.39aAB	44.10aB
SPC-Cl	39.88aA	31.61aAB	32.46abAB	29.43aB	29.24aB	32.30aA	23.91aBC	26.88aAB	21.41aBC	18.08aC	72.18aA	55.52aB	59.34abAB	50.84aB	47.32aB
PSC-TW	30.88bcA	30.53aA	30.89abA	26.03aA	25.27aA	25.31abA	22.80aA	24.09aA	19.34aA	18.93aA	56.19bcA	53.33aA	54.98abA	45.37aA	44.20aA
PSC-Cl	28.50cA	27.71aA	31.93abA	26.76aA	28.15aA	25.93abA	21.19aAB	21.04aAB	20.76aAB	18.35aB	54.43bcA	48.90aA	52.97abA	47.52aA	46.50aA
PCS-TW	28.06cA	26.88 aA	24.03bA	24.72aA	25.29aA	22.19bA	20.33aA	21.52aA	19.68aA	17.96aA	50.25cA	47.21aA	45.55bA	44.40aA	43.25aA
PCS-Cl	25.48cA	25.79 aA	24.39abA	26.18aA	23.37aA	22.17bA	22.98aA	21.75aA	19.76aA	18.45aA	47.65cA	48.77aA	46.14abA	45.94aA	41.82aA

SPC = sanitation – peeling - cutting, PSC = peeling – sanitation – cutting, PCS = peeling – cutting – sanitation, Cl = sanitation with 100 ppm chlorinated water, TW = sanitation with tap water.
Values represent the mean of four replicates. Means under the same heading in each column or row with different small or capital letters, respectively are significantly different according to the Duncan test ($P<0.05$).

of peeling – sanitation – cutting (PSC) methods were in between the betalain contents levels of SPC and PCS methods. However, betalain contents gradually decreased when storage progressed in all washing/sanitation treatments. The rate of betalain decline during storage was higher in SPC sanitization method compared to other two methods thereby significant ($P<0.05$) declines were observed in all components of both tissues at the end of storage (Table 2). The highest (34.4%) decline of total betalain was found in both of SPC-TW and SPC-Cl treated samples of inner tissues while the lowest (12.2%) decline was observed in PCS-Cl treated sample of same portion of sliced beetroots. Overall, the decline in betacyanin was little higher than betaxanthin in the outer tissues whereas opposite trend was found in the inner tissues. At the end of the storage, total betalain content was almost similar in SPC-TW and PSC-TW treated samples as well as in SPC-Cl and PSC-Cl treated samples of both tissues (Table 2). Samples of PCS treatments showed the lowest amount of total betalain contents at the end of storage in both tissues. It appears that although the declining rate of betalain was lower in PCS method than other methods, the total amount of betalain content was comparatively lower than other methods at the end of storage probably due to the higher loss of betalain in this method on washing day. In this study, sanitization and washing favor larger pigment losses of beetroots slices owing to their exposure to water or sanitized solution and therefore, we observed significant variation in betalain contents on washing day among the processing/sanitization methods. Similar to our results, the variations and decreases in betalains were also found in few studies (VITTI *et al.*, 2005; KLUGE *et al.*, 2006; VITTI *et al.*, 2011). In contrast our pigment decline rate, LOPEZ OSORNIO and CHAVES (1998) found about 40-50% decreases in betalain amount in grated beetroot after 7 days of storage at 0°C, whereas at 4°C the decreases were greater. However, these authors did not measure the pigment contents at different tissues of beetroots, which we did in the present study. Betalains accumulate in cell vacuoles of the leaves, flowers and fruits of the plants that synthesize them, mainly in epidermal and/or subepidermal tissues (JACKMAN and SMITH, 1996). Moreover, it was reported that the total phenolic contents and the main betacyanin present in red beetroots distributed mostly towards the outer parts of the root and decreasing in the order peel, crown and flesh (KUJALA *et al.*, 2000). This localized distribution of betalains was also found in our study. However, due to large number of samples, we did not use the middle part of beetroots tissues and only the outer and inner tissues were used for biochemical measurement. Nevertheless, we assume that our fresh-cut beetroots slices were larger in size than that of grated beetroot which might prevented higher decline of pigments compared to that of LOPEZ OSORNIO and CHAVES (1998) findings. NILSON (1973) observed the contents of betacyanin and betaxanthin is cultivar dependent and the levels of these pigments are around 45 to 210 and 20 to 140 mg 100 g^{-1}, respectively.

Total phenolic contents

Total phenolic contents of sliced beetroots (Fig. 2) followed similar distribution trend at different tissues that we found in betalain contents and is supported by the results of KUJALA *et al.* (2000). On the washing day, total phenolic contents also followed similar pattern as we observed for betalains contents among the treatments. The amount of total phenolic contents in the outer and inner tissues of beetroots samples ranged from 102.4 to 116.4 mg GAE 100 g^{-1} FW and 52.1 to 80.0 mg GAE 100 g^{-1} FW, respectively on washing day (Fig. 2). Higher amount of total phenolic content was observed in SPC method followed by PSC and PCS methods. However, we did not find any specific trend in total phenolic content of sliced beetroots during storage at low temperature. In the outer tissue, total phenolic contents declined on the third day of storage and then slightly increased the content or keep constant throughout the storage. At the end of the storage, total phenolic contents of this portion slightly increased ($P>0.05$) than the levels observed on washing day. PCS-TW treated sample showed the lowest amount of total phenolic contents both on washing day and throughout the storage. At the end of storage, SPC-TW treated sample showed the highest amount of total phenolic content (118.1 mg GAE 100 g^{-1} FW) followed by PSC-Cl treated sample (116.8 mg GAE 100 g^{-1} FW). Variations in total phenolic contents were also observed in the inner tissues on washing day and the contents either increased or decreased or maintained its level after fluctuating in between the storage. Although the highest amount of total phenol (79.9 mg GAE 100 g^{-1} FW) was determined in SPC-TW treated inner tissues' sample on washing day, PSC-Cl treated sample retained the highest amount (71.3 mg GAE 100 g^{-1} FW) among the treatments at the end of storage (Fig. 2). KUJALA *et al.* (2000) observed decreased amount of total phenolic content until 63 days and the amount remained almost unchanged until 196 days of storage of raw beetroots. The effects of different storage temperatures on the total phenolic content in plants have been studied and, like our study, both increases and decreases in phenolic contents have been reported. For example, LEWIS *et al.* (1999) observed an increase in total phenolic contents of colored potato tubers during storage at 4°C, whereas CORDENUNSI *et al.* (2005) found that total phenolic contents of strawberry remained

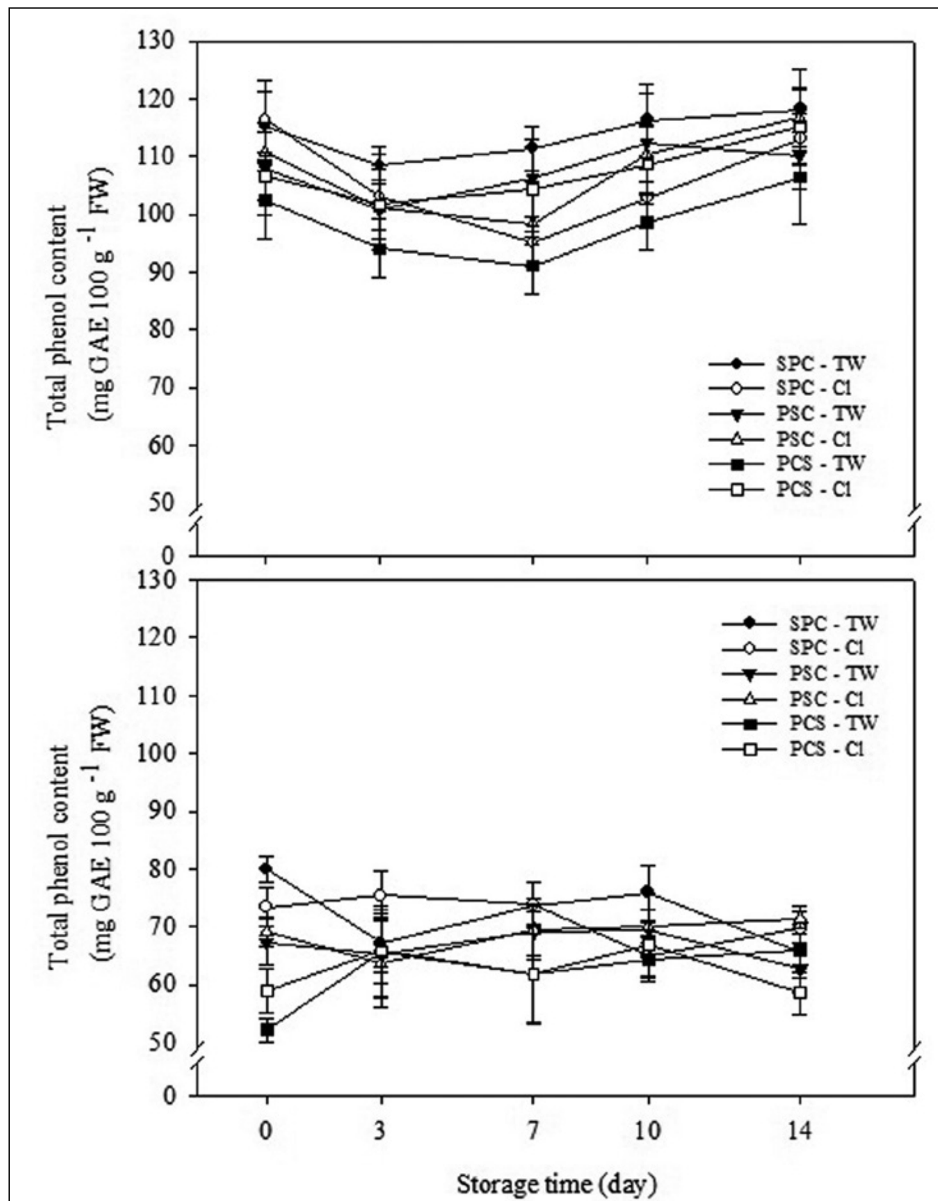

Fig. 2 - Total phenol content of outer (above) and inner (below) tissues of fresh-cut red beetroots after processing with different sanitation methods and during storage at 5°C. Legends: same as shown in Fig. 1.

constant or even slightly decreased during storage at different temperatures.

Microbial quality

Figure 3 shows the changes in total aerobic bacteria (TAB) count and yeast and mold (YM) count of fresh-cut red beetroots on washing day and during successive storage at low temperature. Significant variation ($P<0.05$) was found in TAB among the washing treatments both on washing day and throughout the storage. The range of TAB was found 2.6 log CFU g^{-1} in PSC-Cl to 3.4 log CFU g^{-1} in SPC-TW treated samples on washing day. This result indicates that sanitation of beetroots with 100 ppm chlorinated water ensured a significant reduction in TAB both in PSC and PCS methods. In order to reduce the microbial population, chlorine solution has been used as sanitizer in many fresh-cut vegetables including beetroots (LOPEZ OSORNIO and CHAVES, 1997; VITTI *et al.*, 2011) and sweet potatoes (ERTURK and PICHA, 2006). However, studies on microbial safety or monitoring of microbial population in fresh cut beetroots are still limited as compared to other fresh produces. Since beetroots contain several bioactive compounds, its use as fresh-cut produce is promising where microbial safety should be addressed and ensured. Beetroots washed with tap water either before or after peeling had nearly similar number of TAB which indicates the necessity of sanitation of fresh-cut beetroots with appropriate sanitizer. Sanitation with chlorinated water be-

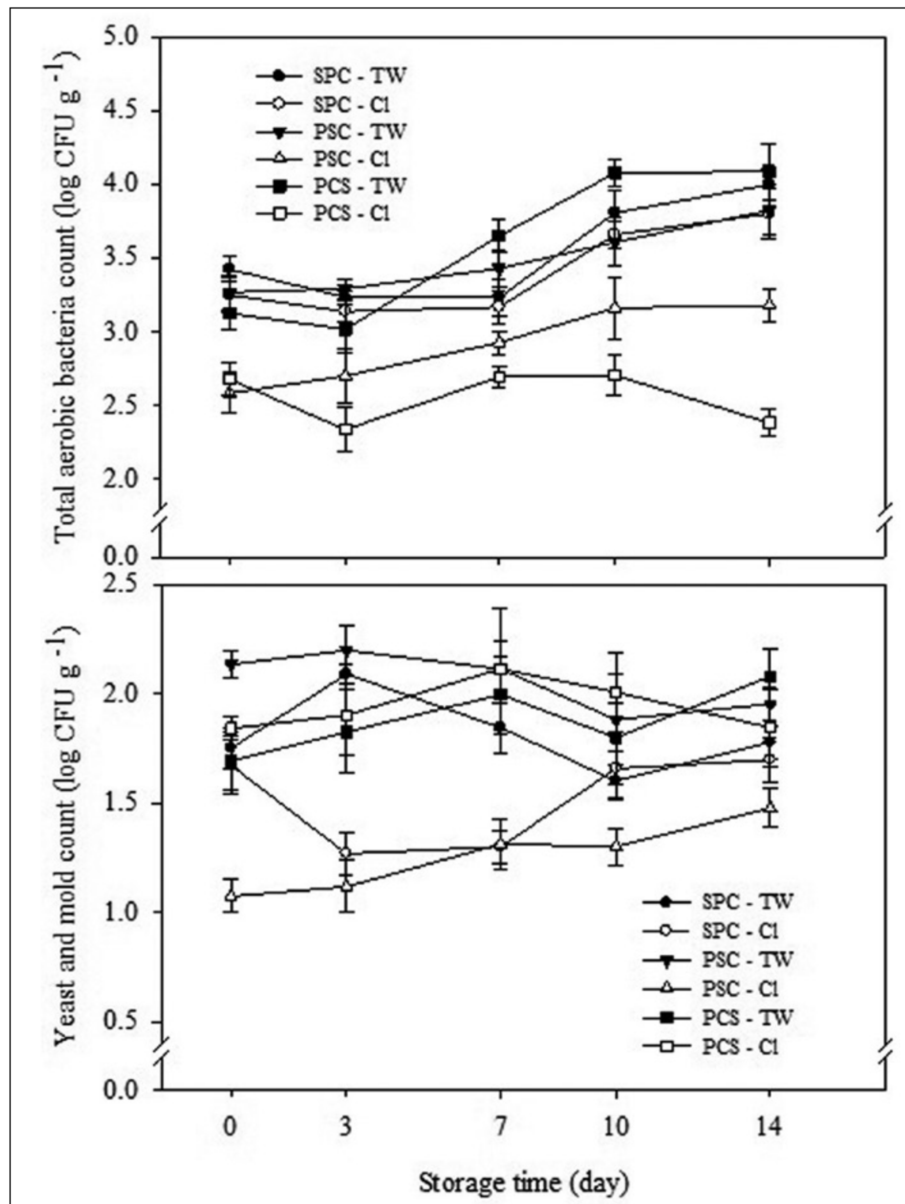

Fig. 3 - Effects of different sanitation methods on total aerobic bacteria (TAB) count and yeast and mold (YM) count of fresh-cut red beetroots during storage at 5°C. Legends: same as shown in Fig. 1.

fore peeling showed almost no effect in reducing TAB as compared to after peeling or after slicing sanitation. ERTURK and PICHA (2006) reported that chlorination of sweet potatoes before slicing could not ensure acceptable microbiological quality of fresh-cut sweet potatoes. However, in our study we found significant reduction in TAB when beetroot samples were sanitized after peeling but before slicing though the numbers of TAB were significantly lower in after slicing sanitation throughout the storage (Fig. 3). LOPEZ OSORNIO and CHAVES (1997) found significant reduction in yeast and total aerobic count in grated beetroots after washing treatment with chlorinated water. The number of TAB gradually increased until the end of storage with few fluctuations. Among the treatments, PCS-Cl treated sample showed the lowest numbers of TAB throughout the storage followed by PSC-Cl treated sample (Fig. 3). However, we found higher losses of betalain (Table 1), lower level of total phenol contents especially in inner tissues (Fig. 2) as well as lower visual quality score (later discussion) in PCS-Cl treated samples which limited the potentiality of this treatment. All tap water washed samples regardless of processing methods showed similar pattern of changes in TAB. Sanitation of beetroot with 100 ppm chlorine water following SPC method exhibited similar number of TAB until 7 days of storage and the number increased thereafter to reach the similar number with PSC-TW treatment.

Yeast and mold (YM) count, on the other hand, showed different patterns of changes that we ob-

served in TAB (Fig. 3). Among the treatments, PSC-Cl treatment ensured the lowest number of YM count on the washing day (1.1 log CFU g^{-1}) and throughout the storage. This result indicates that about 1.1 log CFU g^{-1} reduction in YM count was occurred in PSC-Cl treatment on the washing day as compared to PSC-TW treatment in which the highest YM count (2.2 log CFU g^{-1}) was observed on that day. Unlike TAB count, there was no remarkable increase found in YM count when storage progressed. It appeared that survival and growth pattern of aerobic bacteria as well as yeast and mold are different in fresh cut beetroots. The decontamination effect of chlorinated water depends on dipping time, concentration of active chlorine, water temperature and the type of produce used (ERTURK and PICHA, 2006; VITTI *et al.*, 2011). In agreement with our results, ERTURK and PICHA (2006) found no significant reduction in yeast and mold count in fresh-cut sweet potato among control, chlorination of peeled whole roots and chlorination after slicing the roots regardless of water temperatures and concentration of chlorine, except for a significant reduction in chlorination after slicing at 20°C using 200 ppm chlorine solution. However, these authors found significant reduction in initial mesophilic population using both 100 and 200 ppm chlorine water washing of peeled whole sweet potato roots. LOPEZ OSORNIO and CHAVES (1997), on the other hand, found significant decrease in the initial yeast counts in grated beetroots after washing in 252 ppm active chlorine solution at 8°C. These findings may suggest that higher concentration of active chlorine solution might be effective in reducing yeast and mold count in root vegetables. However, higher concentration of chlorine and longer dipping time caused a substantial decline in pigment contents in beetroots (VITTI *et al.*, 2011). Our YM count result suggests that higher surface area exposed to washing treatment were more susceptible to YM contamination and thereby unlikely TAB count, we found significantly ($P<0.05$) higher levels of YM count in PCS-Cl treatment (Fig. 3). Among the three sanitation methods used in our study, peeling-sanitation-cutting (PSC) was the best method for reducing YM count both on washing day and throughout the storage.

Sensory quality

Visual quality of sliced beetroot decreased gradually when storage time elapsed (Fig. 4). However, we did not notice off-odor in any sample until the end of storage (data not shown). Although all samples retained marketable limit until 7 days of storage, samples processed only with SPC and PSC methods retained their marketable limit which was set at 6 in a 9-point scale until 10 days of storage. In a previous study, VITTI *et al.* (2005) also reported that minimally processed beetroots could be stored until 10 days at low temperature and the produce quality drastically reduced corresponding with the increases in storage temperatures. Due to

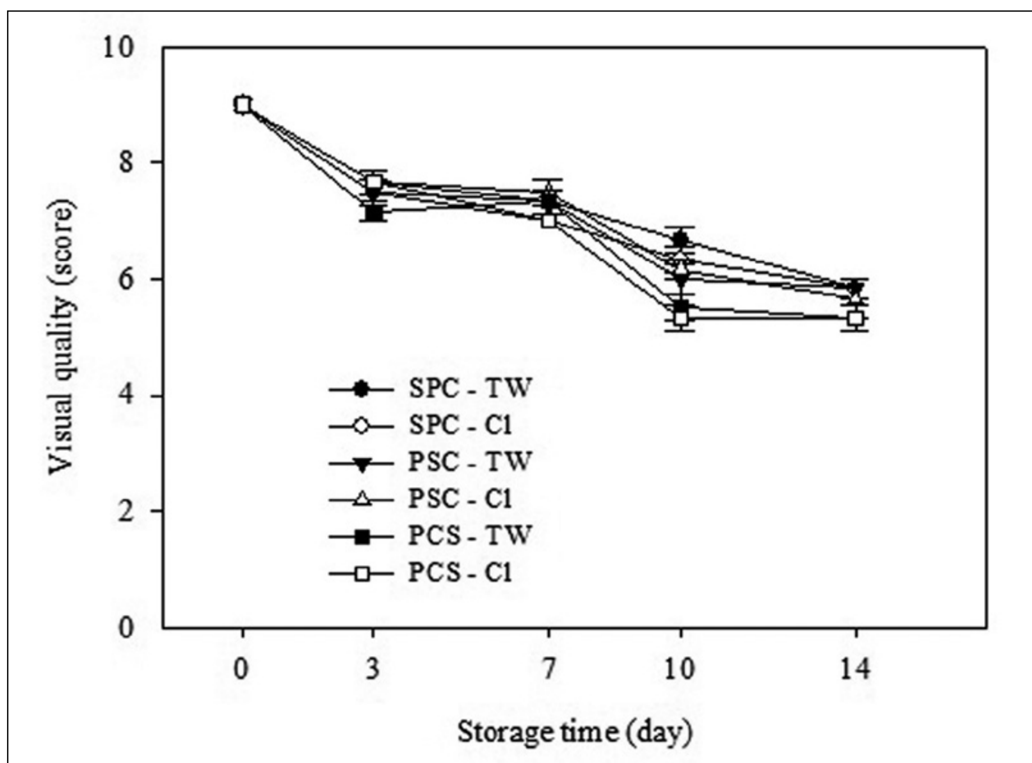

Fig. 4 - Visual quality scores of fresh-cut red beetroots during storage at 5°C after processing with different sanitation methods. Legends: same as shown in Fig. 1.

the earlier development of whitish substances on the surface of beetroots slices, samples processed with PCS method received lower visual quality scores from 7 days of storage. Moreover, we found higher losses of betalain and as a consequence higher values of whiteness index in samples treated with this method (Table 2 and Fig. 1). However, the chlorine treated sample of PCS methods showed the lowest number of TAB among the treatments (Fig. 3). This result indicates that maintenance of visual quality of fresh-cut beetroots may not depend on microbial load at least until certain limit. In general, consumer can only assess the sensory appearance. Hence, there is an increasing demand for the development of improved methods that guarantee a high produce quality and safety until the end of shelf life, especially for fresh-cut produces. At the end of storage, samples of all treatments exhibited the lower values of marketable limit. Overall our results indicate that fresh-cut beetroots processed with SPC and PSC methods could be marketable until 10 days of storage at low temperature whereas samples processed with PCS could have only 7 days of marketable life.

CONCLUSIONS

Significantly lower values of chroma and higher values of whiteness index were found in samples processed with peeling – cutting –sanitation (PCS) method, especially in PCS-Cl treatment. Although PCS-Cl treatment showed the lowest values of TAB throughout the storage, higher betalain decline along with lower level of total phenol and visual quality score limited the storability of beetroots in this treatment only until 7 days. Beetroots processed with SPC method exhibited the highest amount of betalain and total phenol contents, but showed higher number of microbial population. Samples of PSC method exhibited intermediate levels of betalain and PSC-Cl treatment ensured minimum number of microbial population. Based on measured parameters, we conclude that PSC would be the most suitable method for processing of fresh-cut beetroots and PSC-Cl would be the best treatment for ensuring minimum number of microbial population and maintaining betalain and total phenolic contents. Therefore, PSC-Cl treatment could commercially be use for processing and storing of fresh-cut beetroots at low temperature.

ACKNOWLEDGEMENTS

The first author gratefully acknowledges the financial support from the National Institute of Horticultural and Herbal Science (NIHHS), Rural Development Administration (RDA), Republic of Korea received through the Postdoctoral Fellowship Program for foreign researcher.

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Paper Received June 10, 2014 Accepted August 14, 2014