International Journal of Agricultural Technology 2016 Vol. 12(6): 1153-1164
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

Changes of Anthocyanins, Total Phenolic Contents and Antioxidant Activity in Black Plum (*Syzygium Cumini* Skeels) Juice, During Local Can Processing

Rawinipa Srimoon*

Department of Applied Science and Biotechnology, Faculty of Agro-Industrial Technology, Rajamangala University of Technology Tawan-OK, Chanthaburi Campus, Thailand 22210

Rawinipa Srimoon (2016). Changes of anthocyanins, total phenolic contents and antioxidant activity in black plum (*Syzygium cumini* Skeels) juice, during local can processing. International Journal of Agricultural Technology 12(6): 1153-1164.

Changes in anthocyanins, total phenolic content and antioxidant activity in black plum (*Syzygium cumini* Skeels) juice during local can processing were investigated. Juice samples from four key processing steps (P1: pressing, P2: boiling and filtering the pulps, P3: mixing with sucrose syrup in a ratio of 40:60 (juice:syrup) to produce 40% black plum juice and P4: filtering and pasteurizing) were evaluated total anthocyanins content in term of cyanidin-3-glucoside (Cyd-3-glu), malvidin-3-glucoside (Mvd-3-glu) and pelagonidin-3-glucoside (Pgd-3-glu) by the pH differential method, total phenolic content using Folin-Ciocalteu phenol reagent and antioxidant activity by DPPH assay. The results showed that after black plums were processed, about 88%, 27%, 60% and 154% of total anthocyanins, total phenolics, TEAC and EC_{50}, respectively, were significantly lost ($P < 0.05$). It could be due to the degradation of anthocyanins during thermal processing, both a long treatment time in boiling step and a short period of time in pasteurization step. Other two anthocyanins; Mvd-3-glu and Pgd-3-glu; were also decreased. In contrast, high temperature treatment increased total phenolic content due to more disruption of cellular membranes and the formation of Maillard reaction products. In addition, there was no significant difference ($P > 0.05$) of antioxidant activity during the first thermal treatment. However, antioxidant activity significantly decreased at the end of the pasteurization step ($P < 0.05$). Major loss of all compounds was found in the procedure of juice dilution with sucrose syrup to produce 40% juice. The losses were proportional to their dilution ratio. It was indicated that concentrated juice has more health benefits than diluted juice. So, percentage of juice dilution should be the most important factor for juice consumption. The changes of total anthocyanins content was more correlated with antioxidant activity ($r^2=0.9708$ and 0.9111 for TEAC and EC_{50}, respectively). It could be suggested that anthocyanins played an important role in antioxidant properties in black plum juice more than other phenolic compounds.

Keywords: Black plum, Anthocyanins, Can processing

***Corresponding author**: Rawinipa Srimoon; **E-mail**: rawinipa.srimoon@gmail.com

Introduction

Black plum (*Syzygium cumini* (L.) Skeels with belonging to Myrtaceae Family is one of the most important commercial plant species that is commonly found in Southeast Asia, particularly in Thailand. Several previous reports showed that *S. cumini* was used to apply for a medicinal and health benefits for a long time. It could treat sore throat, chronic diarrhea, antidiabetic, antidiuretic and reduce DNA damage in human peripheral blood (Bhandary, 1995; Jagetia and Baliga, 2002; Veigas *et al*., 2007; Gopu *et al*., 2015). This is because it is composed of numerous important antioxidants, especially anthocyanins and polyphenolic compounds, such as gallic acid, tannins, and oxalic acid.

Dominant bioactive compounds founded in black plum and its juice are anthocyanins. They are characterized into the groups of flavonoids (a type of polyphenols), generally found in the form of glycosides. Major six anthocyanins found in plants are cyanidin, pelagonidin, delphinidin, malvidin, peonidin and petunidin (Lee *et al*., 2008). There were a lot of studies reported that anthocyanins possessed antioxidants, antibacterial, anti-inflammatory and also anticacinogenic properties (Corrales *et al*., 2008; Tiwari *et al*., 2010; Oancea and Oprean, 2011). For these reasons, consumption of black plum and its processing products containing high concentration of anthocyanins is health benefits. Unfortunately, anthocyanins are very unstable and easy to degrade during processing. Pressing, heating, additive and clarification agents adding, and pasteurizing, can destroy anthocyanins and other bioactive compounds (Vallverdú-Queralt *et al*., 2012). It showed that these processes decrease total anthocyanins and antioxidant activity in fruit juice product. However, there is no report focused on can processing affected on the anthocyanins and other antioxidants in black plum juice. Therefore, in this study, the changes of anthocyanins content, polyphenolic concentration and antioxidant activity in black plum juice during local can processing were investigated. This study could be not only used as a guideline for the canning processes but also sustained the nutritional values of black plum juice before reaching the consumers.

Materials and methods

Juice samples

Samples of black plum juice were collected by the anonymous local fruit juice cannery factory in Chanthaburi province, Thailand. Juice was then produced by using frozen fruits stored in refrigerator (-4 ^{o}C). Four key steps (P1 – P4) in juice processing procedures were illustrated as shown in fig 1.

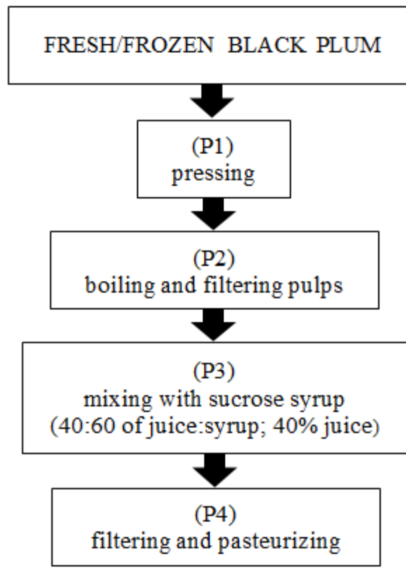

Fig 1. Four key steps (P1 – P4) in black plum juice processing.

Juice samples in each step were evaluated the total anthocyanins contents by the pH differential method, total phenolic content using Folin-Ciocalteu phenol reagent and antioxidant activity by DPPH assay. Chemical reagents used in the study were analytical grade: Potassium chloride (KCl, Univar Ajax Finechem Australia), Acetic acid (CH_3COOH, Univar Ajax Finechem Australia), Sodium acetate (CH_3COONa, Univar Ajax Finechem Australia), Hydrochloric acid (HCl, Merck Germany), Folin-Ciocalteu phenol reagent (Loba Chemie India), Sodium carbonate (Na_2CO_3, Univar Ajax Finechem New Zealand), Gallic acid (Sigma - Aldrich USA), DPPH (2,2-diphenyl-1-picryl hydrazyl, Sigma-Aldrich USA), Trolox (Sigma- Aldrich USA), and Methanol (Merck Germany).

Determination of total anthocyanins content

Total anthocyanins contents were quantified in triplicate using pH differential method, according to Golmohamadi *et al*. (2013). Two aliquots of 1.0 ml of fruit juice were well mixed 4.0 ml of KCl (pH 1.0), and the others with 4.0 ml of acetate buffer (pH 4.5). After incubation for one hour at room temperature, the absorbances were measured spectrophotometrically (Libra S22 Biochrom) at 510 and 700 nm. Total anthocyanins were expressed as mg anthocyanins [cyanidin-3-glucoside (Cyd-3-glu), malvidin-3-glucoside (Mvd-3-glu) and pelagonidin-3-glucoside (Pgd-3-glu)] per ml of sample. Molar extinction coefficient and molecular mass of Cyd-3-glu, Mvd-3-glu and Pgd-3-glu were shown in Table 1.

Table 1 Molar extinction coefficient and molecular mass of three investigated anthocyanins.

Anthocyanins	Molar extinction coefficient ($l.cm^{-1}.mol^{-1}$)	molecular mass ($g.mol^{-1}$)
cyanidin-3-glucoside (Cyd-3-glu)	26,900	449.2
malvidin-3-glucoside (Mvd-3-glu)	28,000	463.3
pelagonidin-3-glucoside (Pgd-3-glu)	22,400	433.2

Determination of total phenolic content

Total phenolic content was performed in triplicate using a Folin-Ciocalteu method, slightly adapted from Wong *et al.* (2006). An aliquot of 2 ml of fruit juice was mixed with 5.0 ml of 10% Folin-Ciocalteu phenol reagent, and then 2.0 ml of 7.5% Na_2CO_3 was added and shaken. After that the solution was left in the dark at room temperature for one hour. The absorbance was measured at 765 nm using gallic acid (10 – 100 mg/l) as a standard. Total phenolic content was expressed as mg gallic acid equivalent per ml of sample.

Antioxidant activity assay

Antioxidant activity was determined in triplicate using DPPH assay as described by Shimada *et al*. (1992). An aliquot of 50 – 200 µl of fruit juice was mixed with 4.5 ml of DPPH (40 mg/l) and then diluted to 5 ml with double-distilled water. After sitting for 30 minutes at room temperature, the absorbance was measured at 515 nm using a spectrophotometer. Antioxidant activity was expressed as EC_{50} (effective concentration of antioxidants that caused 50% reduction in DPPH concentration) and TEAC (Trolox equivalent antioxidant capacity) against the standard curve performed between 1-4 mg/l of Trolox.

Statistical analysis

The data were expressed as mean of triplicate±standard deviation. Analysis of variance (ANOVA) was used to estimate the changes of anthocyanins, total phenolic content and antioxidant activity during canning processes. The differences among fruit juice samples were analyzed by using t-test at a level of $P<0.05$ of significance.

Results and discussions

Changes in total anthocyanins (in term of cyanidin-3-glucoside, Cyd-3-glu), total phenolic content (in term of gallic acid equivalent, GAE) and antioxidant activity (in term of TEAC and EC_{50}) in black plum juice samples were shown in Table 2 and Fig 2.

Table 2 Changes in total anthocyanins, total phenolic content and antioxidant activity in black plum juice samples during can processing* (n = 3).

processing steps	Total anthocyanins (mg Cyd-3-glu/ml)	Total phenolics (mg GAE/ml)	TEAC ** (mg Trolox/ml)	EC_{50} (μl)
P1	0.557 ± 0.0006^{a}	2.103 ± 0.0075^{a}	0.263 ± 0.0026^{a}	36.33 ± 0.363^{a}
P2	0.547 ± 0.0025^{b}	3.347 ± 0.0068^{b}	0.263 ± 0.0028^{a}	36.33 ± 0.391^{a}
P3	0.130 ± 0.0004^{c}	1.214 ± 0.0029^{c}	0.085 ± 0.0002^{b}	111.71 ± 0.285^{b}
P4	0.069 ± 0.0011^{d}	1.531 ± 0.0021^{d}	0.104 ± 0.0009^{c}	92.18 ± 0.762^{c}

* Different superscript letters (a, b, c,…) in the same columns represented the significant differences ($P<0.05$).

** EC_{50} of Trolox = 0.00191 ± 0.000 mg/ml

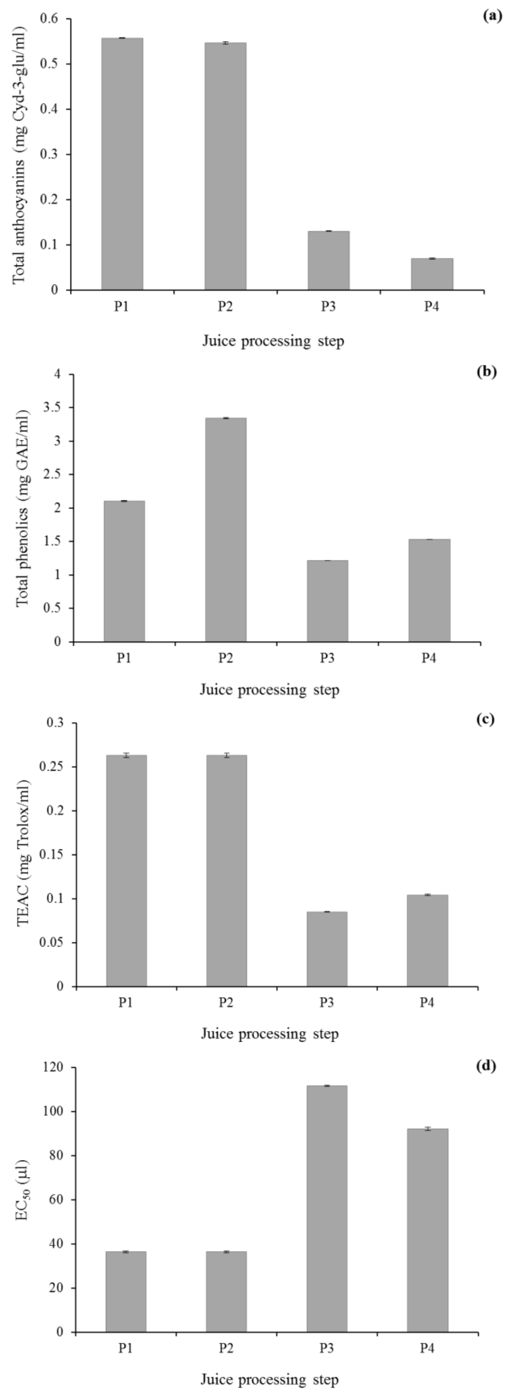

Fig 2. Changing profiles of (a) total anthocyanins, (b) total phenolics, (c) TEAC and (d) EC_{50} in black plum juice samples during can processing (P1 – P4)

The results showed that total anthocyanins concentration significantly decreased during can processing ($P<0.05$). After pressing the raw materials (P1), juice was boiled and filtered (P2) in order to inhibit the polyphenol oxidase enzymes (brownish-color forming enzymes) and to primarily inactivate the pathogens. Total anthocyanins decreased from 0.557±0.0006 mg Cyd-3-glu/ml in P1 to 0.547±0.0025 mg Cyd-3-glu/ml in P2 due to high temperature degradation (nearly 100 °C, about 30 minutes). Generally, anthocyanins degrade easily, especially caused by temperature, light, oxygen and pH (Tiwari *et al*., 2010; Oancea and Oprean, 2011). So, anthocyanins concentration retained in processed products from fruits and vegetables might lower than that of fresh materials. According to the study of Moldovan *et al*. (2012), anthocyanins stability depended on temperature and the degradation rate of them increased with increasing temperature. Patras *et al*. (2009) also found in the same way when the impact of traditional thermal processing on anthocyanins was investigated in strawberry and blackberry purées compared with the fresh fruits.

After the procedure of P2, juice was diluted with sucrose syrup in the ratio of 40:60 (juice:syrup) to produce 40% black plum juice (P3). There was the significant loss ($P<0.05$) of anthocyanins by 76%. It was found that the decreasing of anthocyanins was proportional to their dilution ratio. The more pure juice was diluted, the more anthocyanins were decreased. These suggested that consuming concentrated juice was better than diluted juice; although they lost some bioactive compounds from the boiling step. After P3 process, filtered juice was filled into the cans and pasteurized at 77 °C for 1 minute (P4). The results showed that about half of anthocyanins concentration was significantly lost ($P<0.05$) due to high temperature; although the treatment time was very short. To sustain the health beneficial compounds in juice during the pasteurization process, non-thermal technologies such as pulsed-electric field and ultrasound treatment were suggested to be used. These methods had a little effect on the concentration of bioactive compounds in food samples, such as juice, milk and wine (Cocito *et al.*, 1995; Tiwari *et al.*, 2010; Panyamuangjai *et al*, 2012; Bourneow and Santimalai, 2015).

Not only the changes in total anthocyanins in term of cyanidin-3-glucoside (Cyd-3-glu) were analyzed, but those of malvidin-3-glucoside (Mvd-3-glu) and pelagonidin-3-glucoside (Pgd-3-glu) were also calculated. Changes of them were shown in Table 3 and Fig 3. In average, anthocyanins decreased by 88% after can processing.

Table 3 Changes in three investigated anthocyanins in black plum juice samples during can processing* (n = 3).

Processing steps	Anthocyanins (mg/ml)		
	Cyd-3-glu	Mvd-3-glu	Pgd-3-glu
P1	0.557 ± 0.0006[a]	0.552 ± 0.0006[a]	0.645 ± 0.0007[a]
P2	0.547 ± 0.0025[b]	0.541 ± 0.0025[b]	0.632 ± 0.0029[b]
P3	0.130 ± 0.0005[c]	0.129 ± 0.0005[c]	0.151 ± 0.0006[c]
P4	0.069 ± 0.0011[d]	0.069 ± 0.0011[d]	0.080 ± 0.0013[d]

* Different superscript letters (a, b, c,…) in the same columns represented the significant differences (P<0.05).

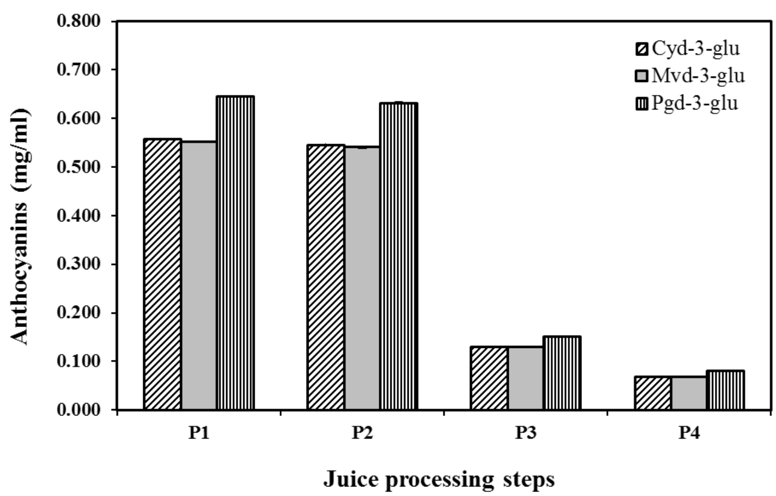

Fig 3. Changing profiles of three investigated anthocyanins during can processing.

Unlike anthocyanins, total phenolic content significantly increased (P<0.05) after the procedure of thermal treatment in P2 step. Although juice was subjected to high temperature, some phenolic compounds, such as phenolic acid, flavonoids, lignins and tannins, could be altered to the secondary metabolites, enhancing the concentration of total phenolic compounds. It was called the Maillard reaction products. Moreover, the disruption of cellular membranes during heating could also increase the concentration of polyphenolics. (Cohen *et al.*, 2001). The similar results were observed in the study of Dini *et al*. (2013). They found that total phenolic content in steamed pumpkin was higher than that of boiled and raw pumpkins, respectively. This increasing was caused by the occurrence of Mailard reaction products. In step of dilution with sucrose syrup (P3), total phenolic content was 64% decreased. It could be explained in the same way as the decreasing of anthocyanins. Afterwards, total phenolic content significantly increased in the rest procedure.

There was no significant difference of antioxidant activity ($P>0.05$), expressed in term of TEAC and EC_{50}, during P1 and P2 step. Similar to anthocyanins and total phenolics, the major loss of antioxidant activity was observed in the dilution step. TEAC was decreased while EC_{50} was increased. However, antioxidant activity significantly decreased after ending the pasteurization step ($P<0.05$).

With reference to Table 4 and Fig 4, total anthocyanins content (Cyd-3-glu) showed a good relationship with TEAC (r^2=0.9708) and EC_{50} (r^2= 0.9111), although they were not the same changing profile. Total phenolic content showed fair correlation with TEAC (r^2=0.7029) and EC_{50} (r^2= 0.7071), but they had the same trend. To sum up, it could be suggested that anthocyanins played an important role in antioxidant activities in black plum juices more than other phenolic compounds.

Table 4 Regression coefficients between the investigated parameters (n = 3).

Parameters	Total phenolics	TEAC	EC_{50}
Total anthocyanins	0.6466	0.9708	0.9111
Total phenolics	-	0.7029	0.7071

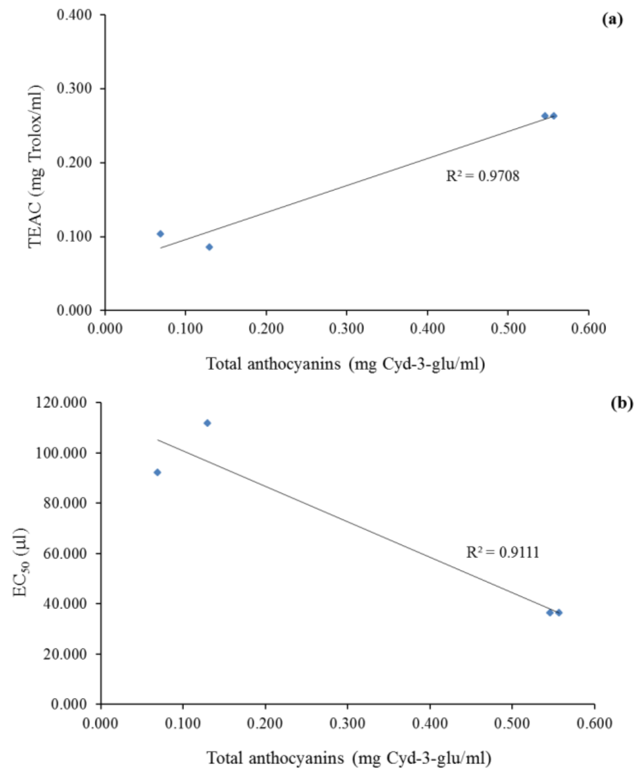

Fig 4. Correlations between total anthocyanins and (a) TEAC (b) EC_{50}.

From the results of this study, about 88% of anthocyanins, 27% of total phenolic, 60% of TEAC and 154% of EC_{50} were lost after processing. Boiling and pasteurizing decreased anthocyanins content because anthocyanins were less stable in high temperature. In contrast, heating both long and short time increased total phenolic content due to more disruption of cellular membranes and the formation of Maillard reaction products. Major loss of all compounds was found in the step of dilution with sucrose syrup to produce 40% juice. This loss was proportional to the dilution ratio. Antioxidant activity had the same changing profiles as total phenolic compounds, but it was more correlated with total anthocyanins concentration. It could be indicated that anthocyanins played a major role in antioxidant capacity in black plum juice because they showed good relationships with TEAC and EC_{50}.

In conclusions, black plum contains high concentration of antioxidants, especially anthocyanins. Unfortunately, this fruit is seasonal limited; it cannot consume freshly through the year. Processing into ready-to-eat products is more comfortable for consumers; even its antioxidative values may be changed during processing. To retain the healthy juice, improvements the fruit processing are important. For examples, enzymatic-assisted can be used in pressing step. Non-thermal treatment, such as pulsed-electric field or ultrasonication, may be used to enhance juice yields or inactivate the pathogens. Moreover, the results of this research indicated that concentrated juice has more health benefits than diluted juice. So, the percentage of juice dilution is a very important decision factor for selecting juice.

Acknowledgement

The author is thankful to Rajamangala University of Technology Tawan-OK, Thailand for the financial support. The author would like to offer particular thanks to an anonymous local fruit juice cannery factory in Chanthaburi province, Thailand, for black plum juice samples donation.

References

Bhandary, M.J., Chandrashekar, K.R. and Kaveriappa, K.M. (1995). Medical ethnobotany of the Siddis of Uttara Kannada district, Karnataka, India. Journal of Ethnophamacology 47:149-158.

Bourneow, C. and Santimalai, S. (2015). Process optimization for microbial reduction in Durian juice by using pulsed electric field. Proceedings of The Seventh International Conference on Science. Nakhon Pathom and Petchaburi, Thailand 237-243.

Cocito, C., Gaetano, G. and Delfini, C. (1995). Rapid extraction of aroma compounds in must and wine by means of ultrasound. Food Chemistry 52:311 – 320.

Cohen, M.F., Sakihama, Y. and Yamasaki, H. (2001). Roles of plant flavonoids in interactions with microbes: from protection against pathogens to mediation of mutualism. In S.G. Pandalai (Ed.). Recent research developments in plant physiology 2:157-173.

Corrales, M., Toepfl, S., Butz, P., Knorr, D. and Tauscher, B. (2008). Extraction of anthocyanins from grape by-products assisted by ultrasonics, high hydrostatic pressure or pulsed electric fields: a comparison. Innovative Food Science and Emerging Technologies 9:85-91.

Dini, I., Tenore, G.C. and Dini, A. (2013). Effect of industrial and domestic processing on antioxidant properties of pumpkin pulp. LWT-Food Science and Technology 53:382-385.

Golmohamadi, A., Möller, G., Powers, J. and Nindo, C. (2013). Effect of ultrasound frequency on antioxidant activity, total phenolic and anthocyanin content of red raspberry puree. Ultrasonics Sonochemistry 20:1316-1323.

Gopu, V., Kothandapani, S. and Shetty, P.H. (2015). Quorum quenching activity of *Syzygium cumini* (L.) Skeels and its anthocyanin malvidin against *Klebsiella pneumonia*. Microbial Pathogenesis 79:61-69.

Jagetia, G.C. and Baliga, M.S. (2002). *Syzygium cumini* (Janum) reduces the radiation-induced DNA damage in the cultured human peripheral blood lymphocytes: a preliminary study. Toxicology Letters 132:19-25.

Lee, J., Rennaker, C. and Wrolstad, R.E. (2008). Correlation of two anthocyanin quantification methods: HPLC and spectrophotometric methods. Food Chemistry 110:782-786.

Moldovan, B., David, L., Chişbora, C. and Cimpoiu, C. (2012). Degradation kinetics of anthocyanins from European cranberry bush (*Viburnum opulus L.*) fruit extracts, effects of temperature, pH and storage solvent. Molecules 17:11655-11666.

Oancea, S. and Oprean, L. (2011). Anthocyanins, from biosynthesis in plants to human health benefits-a review. Acta Universitatis Cibiniensis Series E: Food Technology 15(1):3-16.

Panyamuangjai, V., Janthara, S., Kusuya, R., Yawootti, R. and Intra, P. (2012). Application of pulsed electric field for milk pasteurization. KMUTT Research and Development Journal 35(4):469-484.

Patras, A., Brunton, N.P., Da Pieve, S. and Butler, F. (2009). Impact of high pressure processing on total antioxidant activity, phenolic, ascorbic acid, anthocyanin content and colour of strawberry and blackberry purées. Innovative Food Science and Emerging Technologies 10:308-313.

Shimada, K., Fujikawa, K., Yahara, K. and Nakamura, T. (1992). Antioxidative properties of xanthans on the autoxidation of soybean oil in cyclodextrin emulsion. Journal of Agricultural and Food Chemistry 40:945-948.

Tiwari, B.K., Patras, A., Brunton, N., Cullen, P.J. and O'Donnell, C.P. (2010). Effect of ultrasound processing on anthocyanins and color of red grape juice. Ultrasonics Sonochemistry 17:598-604.

Vallverdú-Queralt, A., Medina-Remón, A., Casals-Ribes, I., Andres-Lacueva, C., Waterhouse, A.L. and Lamuela-Raventos, R.M. (2012). Effect of tomato industrial processing on phenolic profile and hydrophilic antioxidant capacity. LWT-Food Science and Technology 47:154-160.

Veigas, J.M., Narayan, M.S., Laxman, P.M. and Neelwarne, B. (2007). Chemical nature, stability and bioefficacies of anthocyanins from fruit peel of *Syzygium cumini* Skeels. Food Chemistry 105:619-627.

Wong, S.P., Leong, L.P. and Koh, J.H.W. (2006). Antioxidant activities of aqueous extracts of selected plants. Food Chemistry 99:775-783.

(Received 25 September 2016, accepted 31 October 2016)