International Food Research Journal 17: 461-468 (2010)

Effect of steam blanching on lycopene and total phenolics in pink guava puree industry by-products

[1]Kong, K. W., [1]Emmy, H. K. I., [1]Azizah, O., [1*]Amin, I. and [2]Tan, C. P.

[1]*Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.*
[2]*Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.*

Abstract: Lycopene and total phenolics of pink guava puree industry by-products (refiner, siever and decanter) were evaluated after steam blanching at selected temperatures and times. Lycopene content was in the order of decanter > siever > refiner (7.3, 6.3 and 1.5 mg/100 g, respectively), and the content of total phenolics was in the order of refiner > siever > decanter (4434.1, 2881.3 and 1529.3 mg GAE/100 g, respectively). Regression coefficients for temperatures (x_1) and times (x_2) from multiple linear regression models of siever and decanter showed significant ($p<0.05$) negative relationships with lycopene (y_1) and total phenolics (y_2). Nevertheless, lycopene content in decanter increased significantly about 13% from the control when steam blanching at 60 °C for 20-60 min. More than 27% of total phenolics were lost in blanched refiner. Regression analysis revealed the increasing loss of lycopene and total phenolics during steam blanching could occur when increasing in temperature and time.

Keywords: Steam blanching, lycopene, phenolics, Psidium guajava, by-products

Introduction

By-products of fruits and vegetables industry were reported have potential sources of functional food ingredients or nutraceuticals (Schieber *et al.*, 2001). Numerous studies have showed that by-products are rich in antioxidant compounds such as grape seeds (Bozan *et al.*, 2008), yam peels (Chung *et al.*, 2008) and pomegranate peels (Li *et al.*, 2006).

Pink guava (*Psidium guajava*) is a tropical fruit high in lycopene (Padula and Rodriguez-Amaya, 1986) and polyphenols (Thaipong *et al.*, 2006). The Malaysian common name for pink guava is "jambu batu merah" or "jambu merah". The only pink guava estate that commercially cultivates the fruits in Malaysia is located in Sitiawan, Perak. About 25% of industrial by-products are discarded during the production of pink guava puree. The by-products are derived during the crushing, refining and sieving steps of the processing line were known as refiner, siever and decanter, respectively. These by-products have been investigated for their potential as antioxidant sources. A study by Amin and Mukhirah (2006) reported that these by-products possessed high antioxidant properties. However, little information on the antioxidant components of these by-products was reported.

Kinetic studies demonstrated that the stability and extraction of lycopene and phenolics compounds in tomato puree and black berry juice were influenced by temperature and time (Shi *et al.*, 2003; Wang and Xu, 2007). Thermal treatment has been reported to increase extractable lycopene by breaking down the cell walls and weakening the interaction between the compound and its tissue matrix (Shi *et al.*, 2008). Besides, liberation of bound phenolics from other components by thermal treatment has also been reported (Xu *et al.*, 2007).

There has been no study on the effect of thermal treatment on lycopene and phenolic contents in pink guava by-products. Thus, present work was aimed to determine the effects of steam blanching treatment on the studied compounds. Furthermore, findings from this study could provide useful information for the industry to identify the potential sources for lycopene

*Corresponding author.
Email: *amin@medic.upm.edu.my*
Tel: +603-8946-2435; Fax: +603-8942-6769

and phenolics compounds. Moreover, information on the heating stability of the compounds could help the industry in monitoring suitable heating conditions for better production.

Materials and Methods

Sample preparation

Pink guava (*Psidium guajava* var. Sungkai Beaumont) by-products produced from puree industry were obtained from Golden Hope Food and Beverages Sdn. Bhd., Sitiawan, Perak, Malaysia.

Blanching treatment

A 2.5 kg homogenized sample was used and 120 g of the sample was weighed into falcon tubes with 30 g each. The uncovered falcon tube was steam blanched in the water bath (W350, Memmert, Schwabach, Germany) at different temperatures (50, 60, 70, 80, 90 °C) and times (20, 40, 60 min). Then, the sample was freeze-dried and ground into fine particles. The resultant powder was sieved through 0.25 mm laboratory sieve to obtain uniform particle size. Freeze-dried sample without blanching treatment was used as the control. All samples were kept in airtight container and stored at -20 ºC until further analysis.

Determination of lycopene content

Determination of lycopene content was done according to the method of Fish, Perkins-Veazie and Collins (2002) with modifications. A sample (0.6 g) was weighed into a test tube. A mixture of 5 ml of pure acetone containing 0.05% butylated hydroxytoluene (BHT), 5 ml of 95% ethanol and 10 ml of hexane was added into sample and vortexed for 1 min. Then, the test tube was shaked in ice bath at 200 rpm by orbital shaker (Heidolph Unimax 1010, Schwabach, Germany) for 20 min. After that, 3 ml of deionized water was added and shaked again at 200 rpm for another 5 min. Finally, the mixture was allowed for phase separation for about 5 min. Hexane layer was read in 1 cm path length quartz cuvette using UV-VIS spectrophotometer (UV-1601, Shimadzu Corporation, Victoria, Australia) at 503 nm with hexane as the blank. Lycopene content was estimated as mg/100 g dry matter of pink guava by-products using molar extinction coefficient for lycopene in hexane which is $17.2\times10^{4}M^{-1}\times$cm (Zechmeister *et al.*, 1943) and the molecular weight 536.9 da.

Determination of total phenolics

Determination of total phenolics was done according to the method of Singleton and Rossi (1965). A 0.2 g sample was extracted with 2 ml of 80% aqueous methanol containing 1% hydrochloric acid for 2 h at 50 °C using an orbital shaker at 200 rpm. The mixture was filtered and 0.2 ml of filtrate was mixed with 1.5 ml of Folin–Ciocalteu reagent. The mixture was allowed to stand at room temperature for 5 min. After that, 1.5 ml of sodium bicarbonate solution (0.566 M) was added to the mixture. Absorbance was read at 725 nm using UV-VIS spectrophotometer after 90 min reaction time. Results were expressed as mg gallic acid equivalents (GAE)/100 g dry matter pink guava by-products. The concentration of gallic acid used was in the range between 0.02-0.10 mg/ml.

Statistical analysis

Data were expressed as mean ± standard deviation of three measurements. Data were statistically analysed using statistical software, SPSS version 15 for Windows (SPSS Inc, Chicago, Illinois, USA). One-way analysis of variance (ANOVA) was used to compare means between groups. Multiple linear regression analysis was done to determine the relationship between the independent variables (x_1: temperature and x_2: time) and the dependent variables (y_1: lycopene and y_2: total phenolics). The level of significance was set at $p<0.05$. Regression model generated as followed:

$$y=b_0+b_1x_1+b_2x_2$$

Where: y is the dependent variables (y_1: lycopene content; y_2: total phenolics); x is the independent variables (x_1: temperature; x_2: time); b_0 is a constant value; b_1 and b_2 are the linear coefficients for the independent variables.

Results and Discussion

Lycopene and total phenolics of pink guava by-products

Lycopene was the most abundant carotenoid found in pink guava which made up more than 80% of the total carotenoids (Padula and Rodriguez-Amaya, 1986). Moreover, pink guava was also rich in polyphenols (Misra and Seshadri, 1968). Present results showed that lycopene content of the studied by-products was in descending order of decanter > siever > refiner (7.3, 6.3 and 1.5 mg/100 g, respectively) (Figure 1). The highest lycopene content in decanter

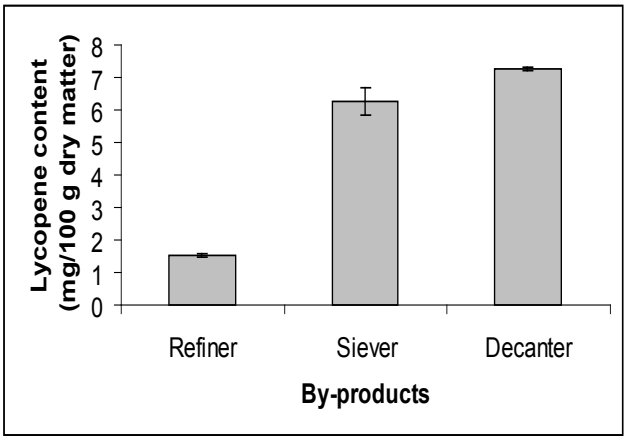

Figure 1. Lycopene content of pink guava by-products. Values are expressed as mean ± standard deviation (n=3). All results are significantly different at the level $p<0.05$.

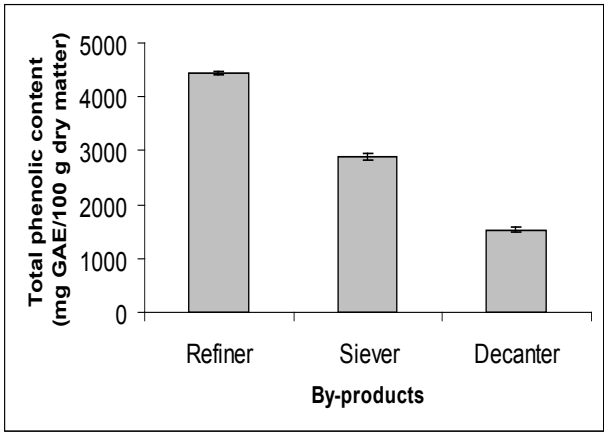

Figure 2. Total phenolic content of pink guava by-products. Values are expressed as mean ± standard deviation (n=3). All results are significantly different at the level $p<0.05$.

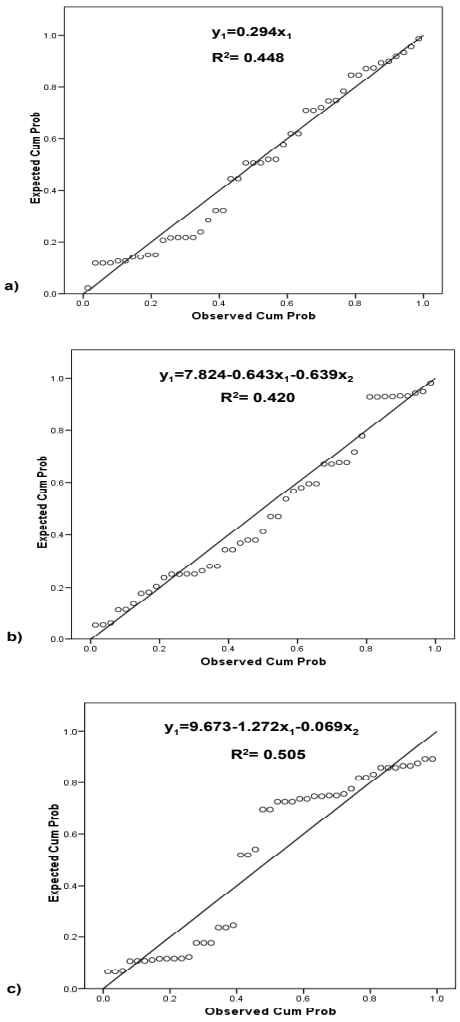

Figure 3. Multiple linear regression model and normal P-P plot of regression standardized residual for lycopene in a) refiner, b) siever and c) decanter.

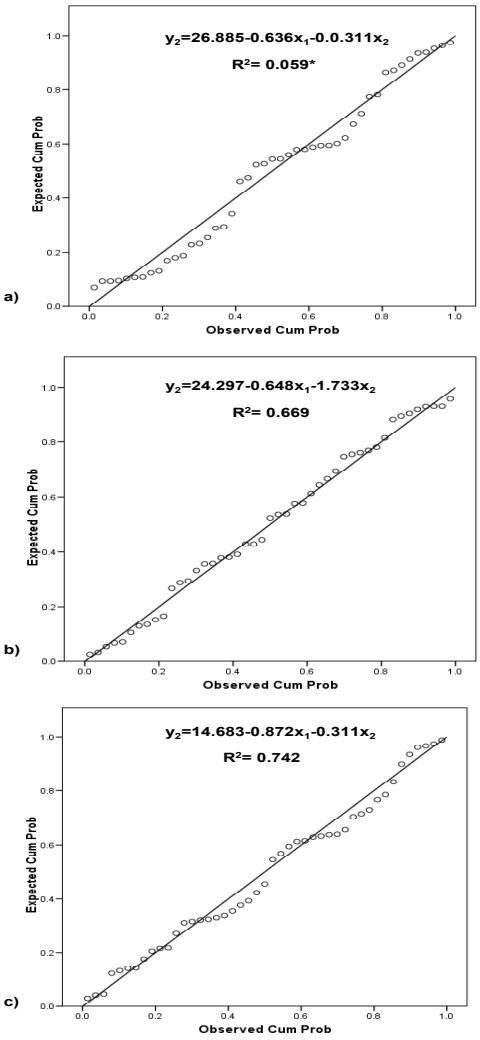

Figure 4. Multiple linear regression model and normal P-P plot of regression standardized residual for total phenolics in a) refiner, b) siever and c) decanter. '*' showed no significant for the regression model.

Table 1. Effect of different steam blanching treatments on lycopene content

Sample	Blanching time (min)	Lycopene content (mg/100 g dry matter)				
		Blanching temperatures (°C)				
		50	60	70	80	90
Refiner	20	0.66 ± 0.03	0.83 ± 0.00	0.68 ± 0.09	1.32 ± 0.48	1.61 ± 0.09
	40	1.14 ± 0.18	0.68 ± 0.18	0.45 ± 0.21	0.94 ± 0.09	1.14 ± 0.18
	60	0.85 ± 0.03	1.06 ± 0.30	0.92 ± 0.03	0.92 ± 0.03	1.20 ± 0.18
Siever	20	6.52 ± 0.12	5.23 ± 0.33	5.55 ± 0.03	6.53 ± 0.06	6.57 ± 0.06
	40	5.06 ± 0.03	5.96 ± 0.24	5.25 ± 0.09	5.89 ± 0.12	5.55 ± 0.06
	60	4.63 ± 0.18	4.35 ± 0.42	3.41 ± 0.30	5.46 ± 0.45	5.49 ± 0.06
Decanter	20	7.04 ± 0.03	8.25 ± 0.42	5.91 ± 0.06	6.47 ± 0.15	6.01 ± 0.12
	40	6.55 ± 0.00	8.23 ± 0.03	7.70 ± 0.00	5.79 ± 0.30	8.20 ± 0.06
	60	5.43 ± 0.03	8.22 ± 0.09	6.76 ± 0.00	6.60 ± 0.09	6.38 ± 0.12

Values are expressed as mean ± standard deviation (n=3).

Table 2. Effect of different steam blanching treatments on total phenolic content

Sample	Blanching time (min)	Total phenolic content (mg GAE/100 g dry matter)				
		Blanching temperatures (°C)				
		50	60	70	80	90
Refiner	20	2624.22 ± 10.87	2173.17 ± 76.17	1944.26 ± 42.84	2691.88 ± 164.56	2499.06 ± 235.99
	40	3243.29 ± 80.22	2130.32 ± 79.50	2537.40 ± 17.03	2813.66 ± 304.26	2252.10 ± 144.73
	60	2875.68 ± 83.16	1966.81 ± 17.58	2078.45 ± 161.35	2561.08 ± 265.86	2139.34 ± 171.13
Siever	20	2197.98 ± 48.83	2318.63 ± 17.58	2097.62 ± 168.48	1900.28 ± 121.52	1975.83 ± 45.43
	40	2020.94 ± 76.25	1797.67 ± 59.69	1702.95 ± 8.95	1715.35 ± 47.88	1846.16 ± 81.21
	60	1843.90 ± 29.56	1884.50 ± 36.17	1671.37 ± 48.12	1792.03 ± 27.55	1565.38 ± 64.57
Decanter	20	1306.47 ± 34.95	1285.05 ± 40.08	1236.56 ± 47.97	1141.84 ± 25.33	961.98 ± 6.40
	40	1183.68 ± 7.79	1232.05 ± 32.14	1215.14 ± 81.26	1016.11 ± 56.62	968.18 ± 30.87
	60	1301.40 ± 19.21	1354.40 ± 17.61	1074.75 ± 82.10	961.42 ± 6.10	929.28 ± 35.20

Values are expressed as mean ± standard deviation (n=3).

could be indicated by the high reddish flesh content compared to other studied by-products. The reddish flesh of pink guava has been previously reported to be rich in lycopene (Padula and Rodriguez-Amaya, 1986).

The studied by-products had higher content of total phenolics compared to lycopene (Figure 1 & 2). Refiner exhibited the highest total phenolic content followed by siever and decanter (Figure 2). It was observed mostly consisted of seeds and peels of the guava fruits. As by-products of food processing, seeds and peels are reported to have higher phenolics compounds than the flesh (Bozan *et al.*, 2008; Chung *et al.*, 2008; Jiménez-Escrig *et al*., 2001). Additionally, non-peeled guava also demonstrated a higher phenolic content as compared to the peeled off guava (Lim *et al.*, 2007). Thus, instead of the flesh, peels and seeds from the fruits could contribute a significant amount of the phenolic content.

Effect of steam blanching on lycopene and phenolic contents

The temperature selected for steam blanching was less than 100 °C. Degradation could be observed in lycopene and phenolics compounds of tomato puree after heating at more than 100 °C as reported by Shi *et al.* (2003) and Xu *et al.* (2007). In this study, the multiple linear regression models obtained was demonstrated about 40-50% of the relationships between steam blanching conditions and lycopene. These were represented as R^2 values of 0.448, 0.420 and 0.505 for refiner, siever and decanter, respectively (Figure 3). The reduction of lycopene content was occurred during the increment of steam blanching temperature and time. The degradation was revealed by the significant linear coefficient of the regression model as follows:

$$\text{Refiner: } y_1 = 0.294x_1$$

$$\text{Siever: } y_1 = 7.824 - 0.643x_1 - 0.639x_2$$

$$\text{Decanter: } y_1 = 9.673 - 1.272x_1 - 0.069x_2$$

Table 1 showed decanter exhibited the highest lycopene content after steam blanching at 60 °C for 20, 40, 60 min (8.3, 8.2, 8.2 mg/100 g, respectively) followed by 90 °C for 40 min (8.2 mg/100 g) and 70 °C for 40 min (7.7 mg/100 g). Interestingly, an increment of lycopene content about 13% was found mostly at 60 °C for 20-60 min as compared to the fresh decanter (control). A similar finding was observed by Mayer-Miebach and Spieß (2003) who found 15% increased in lycopene availability when carrots were blanched at 90 °C for 15 min. Lycopene is naturally protected by the cellular structure and bind to lipoprotein components (Deruère *et al.*, 1994). The increment of extractable lycopene could be due to thermal denaturation of protein and breakdowns of cell walls that might enhance the releasing of the bioactive compounds (Rodriguez-Amaya and Kimura, 2004).

Furthermore, there were significant reductions in total phenolics in all samples. The significant ($p<0.05$) regression models were obtained for siever and decanter as follow (Figure 4):

$$\text{Siever: } y_2 = 24.297 - 0.648x_1 - 1.733x_2$$

$$\text{Decanter: } y_2 = 14.683 - 0.872x_1 - 0.311x_2$$

The regression models indicate that negative correlation was found between total phenolics and effect of steam blanching conditions (temperature and time). About 67-74% of the effect from steam blanching conditions can be explained by the regression models. Unfortunately, no statistically significant was found from regression model for total phenolics and effect of steam blanching conditions on refiner. However, present results were in agreement with Chen and Lin (2007) that phenolics content in cooked yams prepared at different temperatures (50-100 °C) was lower compared to the raw ones.

Although, high total phenolics (3243.3 mg GAE/100 g) was obtained in refiner after steam blanching at 50 °C for 40 min, but at least 27% of the total phenolics were lost after the treatment (Table 2). This result was in line with Chung *et al.* (2008) that more than 40% of phenolic content in yam peels were lost after blanching at 85 °C for 30 sec. Phenolic compounds can be found either in free, conjugated and bound forms. Although, an increase in free phenolic acids were reported after heat treatment, but generally there was a decline in total phenolic content (Xu *et al.*, 2007). Nonetheless, many other factors may affect the loss of lycopene and phenolic compounds such as storage stability, enzymatic and non enzymatic oxidation and isomerization (Rodriguez-Amaya and Kimura, 2004; Wang and Xu, 2007).

Conclusion

Decanter and refiner are the most potential pink guava puree industry by-products that can be used as sources for lycopene and phenolic compounds. Based on the multiple linear regression models, lycopene and total phenolics contents in siever and decanter had reduced when the temperature and time of steam

blanching increased. Interestingly, steam blanching at 60 °C for 20-60 min had significantly increased the lycopene content in decanter about 13%. This treatment may help the industry in increasing their productivity. However, similar treatment should be considered in refiner due to more than 27% lost of the total phenolics content.

Acknowledgements

The financial assistance was provided by the Ministry of Science, Technology and Innovation of Malaysia (ScienceFund 03-01-04-SF0011). Samples were provided by Golden Hope Food and Beverages (M) Sdn. Bhd. Malaysia.

References

Amin, I. and Mukhrizah, O. 2006. Antioxidant capacity of methanolic and water extracts prepared from food-processing by-products. Journal of the Science of Food and Agriculture 86 (5): 778-784.

Bozan, B., Tosun, G. and Ö̈zcan, D. 2008. Study of polyphenol content in the seeds of red grape (*Vitis vinifera* L.) varieties cultivated in Turkey and their antiradical activity. Food Chemistry 109 (2): 426-430.

Chen, Y.-T. and Lin, K.-W. 2007. Effects of heating temperature on the total phenolic compound, antioxidative ability and the stability of dioscorin of various yam cultivars. Food Chemistry 101 (3): 955-963.

Chung, Y.-C., Chiang, B.-H., Wei, J.-H., Wang, C.-K., Chen, P.-C. and Hsu, C.-K. 2008. Effects of blanching, drying and extraction processes on the antioxidant activity of yam (*Dioscorea alata*). International Journal of Food Science and Technology 43 (5): 859-864.

Deruère, J., Römer, S., d'Harlingue, A., Backhaus, R. A., Kuntz, M. and Camara, B. 1994. Fibril assembly and carotenoid overaccumulation in chromoplasts: A model for supramolecular lipoprotein structures. Plant Cell 6 (1): 119-133.

Fish, W.W., Perkins-Veazie, P. and Collins, J.K. 2002. A quantitative assay for lycopene that utilizes reduced volumes of organic solvents. Journal of Food Composition and Analysis 15 (3): 309-317.

Jiménez-Escrig, A., Rincón, M., Pulido, R. and Saura-Calixto, F. 2001 Guava fruit (*Psidium guajava* L.) as a new source of antioxidant dietary fiber. Journal of Agricultural and Food Chemistry 49 (11): 5489-5493.

Kirca, A., Ö̈zkan, M. and Cemeroğlu, B. 2007. Effects of temperature, solid content and pH on the stability of black carrot anthocyanins. Food Chemistry 101 (1): 212-218.

Li, Y., Guo, C., Yang, J., Wei, J., Xu, J. and Cheng, S. 2006. Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. Food Chemistry 96 (2): 254-260.

Lim, Y.Y., Lim, T.T., and Tee, J.J. 2007. Antioxidant properties of several tropical fruits: A comparative study. Food Chemistry 103 (3): 1003-1008.

Mayer-Miebach, E. and Spieß, W.E.L. 2003. Influence of cold storage and blanching on the carotenoid content of Kintoki carrots. Journal of Food Engineering 56 (2-3): 211-213.

Misra, K. and Seshadri, T.R. 1968. Chemical components of the fruits of Psidium guajava. Phytochemistry 7 (4): 641-645.

Padula, M. and Rodriguez-Amaya, D.B. 1986. Characterisation of the carotenoids and assessment of the vitamin A value of Brasilian guavas (*Psidium guajava* L.). Food Chemistry 20 (1): 11-19.

Rodriguez-Amaya, D.B. and Kimura, M. 2004. Carotenoids in foods. In Rodriguez-Amaya, D.B. and Kimura, M., Harvestplus Handbook for Carotenoid Analysis, p. 2-7. Washington: HarvestPlus.

Schieber, A., Stintzing, F.C. and Carle, R. 2001. By-products of plant food processing as a source of functional compounds-recent developments. Trends in Food Science and Technology 12 (11): 401-413.

Shi, J., Dai, Y., Kakuda, Y., Mittal, G. and Xue, S.J. 2008. Effect of heating and exposure to light on the stability of lycopene in tomato puree. Food Control 19 (5): 514-520.

Shi, J., Le Maguer, M., Bryan, M. and Kakuda, Y. 2003. Kinetics of lycopene degradation in tomato puree by heat and light irradiation. Journal of Food Process Engineering 25 (6): 485-498.

Singleton, V.L. and Rossi, J.A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture 16 (3): 144-158.

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L. and Byrne, D. H. 2006. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of Food Composition and Analysis 19 (6-7): 669-675.

Xu, G., Ye, X., Chen, J. and Liu, D. 2007. Effect of heat treatment on the phenolic compounds and antioxidant capacity of citrus peel extract. Journal of Agricultural and Food Chemistry 55 (2): 330-335.

Wang, W.D. and Xu, S.Y. 2007. Degradation kinetics of anthocyanins in blackberry juice and concentrate. Journal of Food Engineering 82 (3): 271–275.

Zechmeister, L., Lerosen, A.L., Schroeder, W.A., Polgar, A. and Pauling, L. 1943. Spectral characteristics and configuration of some stereo isomeric carotenoids including prolycopene and pro-γ-carotene. Journal of the American Chemical Society 65 (10): 1940-1951.