Research Article

Characteristics and antioxidant activity of palm sugar syrup produced in Songkhla Province, Southern Thailand

Phisut Naknean[1] and Mutita Meenune[2]*

[1]Faculty of Agricultural Product Innovation and Technology, Srinakharinwirote University, Sukhumvit 23, Bangkok 10110, Thailand.

[2]Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Haad Yai, Songkhla, 90112, Thailand.

*Email: mutita_meenune@yahoo.co.uk

Abstract

Palm sugar syrup is obtained by heating fresh palm (*Borassus flabellifer*, Linn.) sap on a wood fire stove until it becomes a concentrate. The product properties vary extremely across the samples and within an individual local producer. Therefore, the objective of this study was to characterize the quality profile and antioxidant activity of palm sugar syrup produced in Songkhla Province. Twenty palm sugar syrup samples from primary producers in the province were analyzed for their quality and antioxidant activity. The results showed differences among the samples (P<0.05). The results also showed ranges in L*, a* and b* colour values of between 5.34 to 30.14, 21.13 to 36.14 and 8.19 to 56.12, respectively. The transmittance value ranged from 1.00% to 17.01%. The intermediate browning product (IBP) ranged from 0.42 to 1.25 and the browning intensity (BI) ranged from 0.57 to 1.52, respectively. The pH value varied between 4.49 and 5.42, while the total acidity varied from 0.20% to 0.52%. The total soluble solids ranged from 62.97°Brix to 72.57°Brix. The sucrose content ranged from 59.15% to 84.37%; the glucose and fructose content varied from 4.01% to 24.13% and 4.44% to 23.55%, respectively. The HMF content was found to vary between 20.13 mg/kg and 185.39 mg/kg. Iron content ranged from 3.27 μg/g to 59.43 μg/g and copper content varied from 1.53 μg/g to 3.97 μg/g, respectively. The total phenolic content ranged from 1.35 mg/g to 2.21 mg/g. DPPH radical scavenging activity varied from 13.27 μmol TE/g to 18.49 μmol TE/g. Ferric reducing antioxidant power ranged from 21.33 μmol TE/g to 30.62 μmol TE/g. Reducing power varied from 0.85 to 1.45. Therefore, these data can be used as a guideline to differentiate the quality of palm sugar syrup.

Keywords: *Borassus flabellifer,* quality, IBP, BI, TSS, HMF, FRAP, phenolic content.

Introduction

Palm (*Borassus flabellifer* Linn.) sap can be used as a raw material to produce palm sugar syrup. In the traditional production of palm sugar syrup, a large volume of filtered palm sap is heated on a wood-fired stove to above 100°C until its total soluble solid reaches 65°Brix [1]. During the heating process, physical and chemical changes occur which impart the unique colour and flavour features. The longer sap is boiled, the darker it becomes. Major reactions occur mainly during the process of heating the palm sap including the inversion reaction and nonenzymatic browning reactions (Maillard reaction and caramelisation). These reactions affect the properties of the palm sugar syrup. Moreover, Maillard reaction products (MRPs) and caramelisation products (CPs) have been found to exhibit antioxidative activity due to radical scavenging activity and reducing power [2]. Thus the antioxidant activity of palm sugar syrup was analyzed. Until now, scientific data has rarely been reported on the properties of palm sugar syrup in Thailand even though they are commercial products. Therefore, the aim of this work was to characterise the properties of palm sugar syrup produced in Songkhla Province, Thailand.

Materials and Methods

Raw materials
Twenty commercial palm sugar syrup samples were randomly purchased from local manufacturers located in Songkhla Province, southern Thailand. All samples were traditionally produced by thermally heating the palm sap on a wood fire stove. The samples were kept in bottles and analyzed immediately for physical, chemical and antioxidant activity after collection.

Determination of physical properties

Colour measurement
Colour measurements of samples were carried out using a Hunter Lab Colorflex colorimeter. Instrumental colour data was provided as CIE system in terms of L* (lightness), a*(redness and greenness) and b*(yellowness and blueness).

Measurement of clarity
The clarity of samples was estimated by measuring the transmittance at 650 nm using a spectrophotometer as described by Taiapaiboon [3] and expressed in terms of percentage.

Browning intensity
The intermediate browning product (IBP) and browning intensity (BI) of palm sugar syrup and palm sugar cake were determined by monitoring the absorbance at 280 and 420 nm, respectively. The absorbance was measured by using a UV-160 spectrophotometer (Shimadzu, Kyoto, Japan), as described by Takano [4]. Appropriate dilutions of palm sugar syrup were 8-fold for IBP and 4-fold for BI in order to obtain a reliable absorbance reading. Appropriate dilutions of palm sugar cake were 20-fold for IBP and 10-fold for BI in order to obtain a reliable absorbance reading.

Determination of chemical properties

Determination of pH
The pH value was measured at ambient temperature with a pH meter (Satorious, USA) which was calibrated at pH 4.0 and 7.0.

Determination of total acidity
The sample was diluted with distilled water and titrated with 0.01 N NaOH using a few drops of 1% phenolphthalein solution as an indicator. The sample was transferred as a measured quantity (1-3 ml or g) into approximately 10 ml of distilled water. The result was calculated as a percentage of lactic acid [5].

Determination of total soluble solid
The total soluble solid (TSS) content of palm sap and palm sugar syrup was determined as degree Brix using a hand refractometer.

Determination of moisture content
The moisture content of palm sugar syrup was measured using a vacuum oven. About 2-5 g of the sample was placed in a pre-dried aluminum dish and dried in a vacuum oven at $60^{o}C$ (pressure<70mmHg) for 6 h. The dried sample was placed in a dessicator and cooled for 0.5 h to room temperature. The weight was recorded and the percentage moisture based on the initial wet weight was calculated.

Determination of water activity
The water activity of palm sugar syrup was measured at room temperature using a water activity meter (Novasina, Thermostanter). The sample was inserted into a sample cup and another water activity measurement was made immediately to restrict moisture transfer from the air to the samples.

Determination of type and concentration of sugar
The type and concentration of sugar was determined using HPLC (Shimadzu, CR6A Chromatopac) with a Shim pack CLC NH2 column and refractive index detector. The mobile phase used was the solution of acetonitrile and water (85:15), pumped at a flow rate of 1.5 ml/min and injection volume 20 µl. The samples were prepared by making appropriate dilutions with distilled water. All sample solutions were passed through a 0.45 µm syringe filter (Nylon) to remove particulates prior to HPLC analysis. D-glucose, D-fructose and sucrose were used as external standards [6].

Determination of 5-hydroxymethylfurfural (HMF) content
Palm sugar syrup (5-10 g) was dissolved in deionized water and made up to 50 ml. After that it was centrifuged at 5,000 rpm for 15 min. The supernatant was used to measure HMF content. To determine the HMF content, 2 ml of supernatant was introduced into a tube. 2 ml of 12% trichloroacetic acid (TCA) and 2 ml of 0.025 M thiobabituric acid (TBA) were subsequently added and mixed thoroughly. The tube with sample was then placed in a water bath at $40^{o}C$. After incubating for 50 min, the tube was cooled immediately using water and the absorbance was measured at 443 nm. A calibration curve of HMF was utilized to quantify the HMF concentration [7].

Determination of protein content
Preparation of the dye
The dye solution was prepared monthly. Coomassie brilliant blue dye (100 mg) was dissolved in 50 ml of methanol followed by addition of 100 ml of phosphoric acid and made up to 1 litre with deionized water. The dye mixture was filtered twice through Whatman No. 1 filter paper.

Protein assay
Palm sugar syrup (2-5 g) was dissolved in deionized water and made up to 10 ml with deionized water. After that, 0.04 ml of sample was mixed with 2 ml of dye solution. The absorbance was measured at 595 nm. Bovine serum albumin was used as an external standard [8].

Determination of iron and copper content
Iron and copper contents were analyzed by an atomic absorption spectrophotometer. Palm sugar syrup of approximately 3 g was placed in a high form porcelain crucible. The furnace temperature was slowly increased from room temperature to 450°C in 1 h. The sample was ashed approximately 8 h until a white or grey ash residue was obtained. The residue was dissolved in 5 ml of HNO_3 (25% v/v). The solution was then transferred to a 10 ml volumetric flask and the volume made up. A standard solution of iron and copper was used as an external standard [9].

Determination of phenolic content
The appropriate dilution of palm sugar syrup (0.5 mL) was mixed with 0.5 mL of distilled water. Thereafter, 0.5 mL of Folin-Ciocalteu reagent (1:1 with water) and 2.5 mL of 2% sodium carbonate solution were added. The reaction mixture was mixed thoroughly and placed in the dark for 40 min and the absorbance was recorded at 725 nm. The total phenolic content was calculated from the standard curve of gallic acid and expressed as mg gallic acid per gram of dry sample after blank subtraction. Blank for each sample was prepared in the same manner, except that distilled water was used instead of Folin–Ciocalteu reagent [10].

Determination of DPPH radical scavenging activity
DPPH radical-scavenging activity was determined by DPPH assay, as described by Binsan, *et al* [11], with slight modification. A sample (1.5 ml) was added to 1.5 ml of 0.15 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol. The mixture was mixed vigorously and allowed to stand at room temperature in the dark for 60 min. The absorbance of the resulting solution was measured at 517 nm using a UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). The blank was prepared in the same manner, except that distilled water was used instead of the sample. A standard curve was prepared using Trolox in the range of 10–60 μM. The activity was expressed as μmol Trolox equivalents (TE)/g sample.

Determination of ferric reducing antioxidant power (FRAP)
Ferric reducing antioxidant power (FRAP) was assayed according to the method of Benzie and Strain [12]. Stock solutions included 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$. A working solution was prepared freshly by mixing 25 ml of acetate buffer, 2.5 ml of TPTZ solution and 2.5 ml of $FeCl_3.6H_2O$ solution. The mixed solution was incubated at 37°C for 30 min and was referred to as FRAP solution. A sample (150 μl) was mixed with 2850 μl of FRAP solution and kept for 30 min in the dark. The ferrous tripyridyltriazine complex (coloured product) was measured by reading the absorbance at 593 nm. The standard curve was prepared using Trolox ranging from 50 to 600 μM. The activity was expressed as μmol TE/g sample.

Determination of reducing power
The reducing power of a sample was measured as described by Matmaroh, *et al* [13]. Then 0.5 ml of each sample (appropriate dilution) was mixed with 0.5 ml of 0.2 M sodium phosphate buffer, pH 6.6 and 0.5 ml of potassium ferricyanide. The mixture was incubated at 50°C for 20 min and 0.5 ml of 10% (w/v) TCA was then added. Thereafter, 1 ml of distilled water and 200 μl of 0.1% (w/v) ferric chloride were added to the mixture. The absorbance was measured at 700 nm. Any increase in absorbance at 700 nm indicated an increased reducing power.

Statistical analysis

All analyses and measurements were performed in triplicate. The experimental design was a completely randomized design (CRD). Data was subjected to analysis of variance (ANOVA).

Comparison of means was carried out by Duncan's multiple-range test [14]. Analysis was performed using a SPSS package (SPSS for Windows, SPSS Inc, Chicago, IL). Principle Component Analysis (PCA) was applied to observe the relationships among all property indicators from ten palm sugar syrup samples by XLSTAT software (www.XLSTAT.com).

Results and Discussion

Physical properties
Table 1 shows the quality profile of twenty palm sugar syrup samples produced in Songkhla Province. All parameters were found to be different (P<0.05). The results showed a colour range of L*, a* and b* values between 5.34-30.14, 21.13-36.14 and 8.19-56.12, respectively. The heating process, such as heating temperature and time, could be a main factor affecting the variation of colour values. It affected the increase in a* and decrease in L* values of palm sugar syrup due to nonenzymatic browning reactions taking place. Generally, the rate of chemical reactions increase with increasing temperature and time [15]. The concentration processes that exposes palm sap to temperatures of 100°C or higher for prolonged periods can produce high reducing sugar content and cause dark colour and brown pigments. This corresponded to a decrease in L* value [7].

The turbidity of palm sugar syrup ranged from 1.00% to 17.01%. The turbidity of palm sugar syrup also depended greatly on its protein content and polyphenolic compounds, as well as the undissolved particles that were concentrated during heating process [1]. Moreover, the concentrated colloid particles from brown pigment during heating were also responsible for the clarity in palm sugar syrup. The IBP of all palm sugar syrup samples was found to vary from 0.42 to 1.25, while the BI of samples ranged from 0.57 to 1.52. The effects of the heating process, such as heating temperature and time taken for palm sugar syrup production, could also be main factors affecting the variations in IBP and BI [16].

Table 1. Quality profile of palm sugar syrup produced in Southern Thailand.

Quality	Ranges	Quality	Ranges
L*	5.34-30.14	Total soluble solid (°Brix)	62.97-72.57
a*	21.13-36.14	Fructose (%)	4.44-23.55
b*	8.19-56.12	Glucose (%)	4.01-24.13
Transmittance (%)	1.00-17.01	Sucrose (%)	59.15-84.37
IBP	0.42-1.25	Iron content (μg/g)	3.27-59.43
BI	0.57-1.52	Copper content (μg/g)	1.53-3.97
pH	4.49-5.42	DPPH (μmol TE/g sample)	13.27-18.49
Moisture content (%)	21.13-30.12	FRAP (μmol TE/g sample)	21.33-30.62
Water activity	0.79-0.81	Reducing power	0.85-1.45
Protein content (mg/g)	6.27-8.53	HMF content (mg/kg)	20.13-185.39
Total acidity (% as lactic acid)	0.20-0.52	Phenolic content (mg/g GAE)	1.35-2.21

Chemical properties

The twenty palm sugar syrup samples showed pH in a range of 4.49-5.42, while the total acidity ranged from 0.20% to 0.52%. These variations of pH and total acidity can be used for indicating food safety. An increase in acid concentration may be due to the effects of the sugar fermenting process, which is mainly based on activity caused by microorganisms. Microorganism contamination is responsible for the low pH and high total acidity of palm sugar syrup. Generally, palm sugar syrup that contains high sugar content is a very selective environment for the growth of microorganisms. During the evaporation process, microorganisms present in the palm sap are destroyed since the

syrup reaches a temperature 100°C or above at the terminal stage. However, during post-production, syrup could be contaminated by microorganisms from the air, equipment and packaging [17]. Microorganisms that survive after processing, such as osmophilic yeasts, can grow and produce organic acids yielding low pH and high total acidity. High temperatures during the summer season in southern Thailand favours the rapid growth of osmophilic yeasts (such as *Saccharomyces rouxii*). These microorganisms are normally found in syrup which is capable of growth at a low water activity value (<0.85) or high solute concentrations.

Brown colour of the palm sugar syrup will be promoted during a heating step as well as high acidity and low pH will accelerate the Maillard reaction. Therefore, the dark colour formation will be increased. The TSS of palm sugar syrup samples ranged from 62.97^{o}Brix to 72.57^{o}Brix. The definition of the Thai Industrial Standards Institute for palm sugar syrup is that it is syrup that is made by the evaporation of palm sap. It contains TSS that is at least 65°Brix in order to prevent the growth of microorganisms during storage under room temperature. From this definition, the TSS of four out of twenty samples did not meet this standard.

The moisture content (MC) of the twenty palm sugar syrup samples ranged from 21.13% to 30.12%. The MC of palm sugar syrup depends on the heating temperature, processing time and the TSS. The water activity (Aw) of all palm sugar syrup samples ranged from 0.79 to 0.81. Aw is an intrinsic product characteristic that most influences the microbial ecology of a sugar-rich product. Generally, osmophilic yeast can grow at low water activity (0.65-0.80) and may spoil products containing high concentrations of sugar. It may cause a decrease of the pH value in syrup during storage.

The results from HPLC show that all palm sugar syrup samples consisted of mainly sugars such as sucrose, glucose and fructose. The most abundant sugar found in the palm sugar syrup sample was sucrose, in a range from 59.15 to 84.37%. The glucose and fructose content ranged from 4.01% to 24.13% and 4.44% to 23.55%, respectively. The presence of fructose and glucose might be because they are present naturally and the inversion reaction took place during the heating process. The inversion reaction occurs when the glycosidic linkage of disaccharide is hydrolysed, releasing the monosaccharide units. Upon hydrolysis glucose and fructose are formed [18]. Reducing sugars act as a substrate of Maillard reaction occurring during the production of palm sugar syrup. High reducing sugar content present in palm sap also influences the browning colour of palm sugar syrup afterward, due to Maillard reaction.

HMF has been found to be a well known indicator of heating processing and/or the storage capacity of sugar-based products. The HMF was formed by the Maillard reaction and caramelisation taking place during heating palm sap to form palm sugar syrup. The HMF formation depends greatly on the processing method, degree of heating, acid condition and storage conditions. The HMF was found to vary from 20.13 mg/kg to 185.39 mg/kg. The HMF content of seven out of twenty samples was higher than the maximum limit as recommended by Codex Alimentarious (40 mg/kg, for sugar based products) [19].

The phenolic content of all palm sugar syrup samples was found in a range of 1.35-2.21 mg/g GAE. The presence of the phenolic compound was due to it occurring naturally in the palm sap itself and it dissolved from *Kiam* wood and was added during the palm sap collection [1]. The DPPH radical scavenging activity of all samples ranged from 13.27-18.49 μmol TE/g sample. The FRAP varied between 21.33 and 30.62 μmol TE/g. The reducing power was found in a range of 0.85-1.45. The presence of antioxidant activity in palm sugar syrup was probably due to the presence of phenolic compounds and the formation of Maillard reaction products (MRPs) and caramelisation products (CPs) during heating process. The antioxidant activity of phenolic compounds is clearly related to

free radical-scavenging and hydrogen-donation ability. The MRPs and CPs could function as electron donors. The hydroxyl groups of MRPs or CPs play an important role in reducing activity. Additionally, the intermediate reductone compounds of the MRPs were reported to break the radical chain by donation of hydrogen atoms [20, 21].

Relationships among samples

Since the palm sugar syrup samples contained a large variation of properties, scores for all properties were significantly different (P<0.05). The data measured from twenty two physical and chemical properties and antioxidant activity from twenty palm sugar syrup samples were also analyzed using multivariate technique-Principal Component Analysis (PCA). A principal component analysis (PCA) was used to explore relationships among data. Two principal components (PC1 and PC2) were calculated. They accounted for 59.44% of the variability in the original data as can be seen in Figure 1.

The graphical PCA illustrated positive correlation of a*, IBP and BI. It indicates that nonenzymatic browning took place, resulting in the promotion of browning pigment formation. The pH value and sucrose content correlated well and showed a negative relationship with reducing sugar as well as fructose and glucose contents. This indicates an inversion reaction that induced the increment in fructose and glucose contents and a decrease in sucrose content because this reaction usually occurs in acid conditions. The negative correlation between L* value with HMF content, a* value and BI suggests accumulation of brown pigment from nonenzymatic browning occurred in palm sugar syrup.
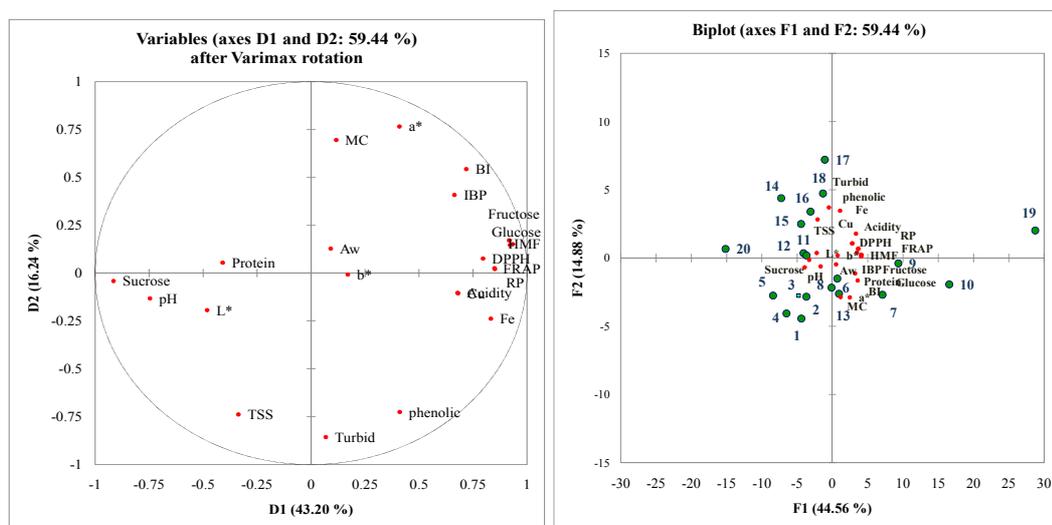

Figure 1. Biplot PC1 and PC2 of the properties of twenty palm sugar syrup samples.

Conclusion

The 20 palm sugar syrup samples contained large variations in all qualities. This is mainly due to the experiences of each producer. The quality of palm sap, temperature and time during evaporating process are of major concern. An increase in antioxidant activity is highly correlated with the amount of increases in MRPs, CPs and phenolic content. The intensity of brown colour, sweet taste, thickness and viscosity of palm sugar syrup is influenced by the heating process. In addition, good practices such as hygiene, sanitary facilities and equipment could greatly contribute to extend this products shelf-life. Therefore, the quality data from this characterization can be used to indicate the standard and grade of palm sugar syrup.

References

1.   Phaichammnan, M., Posri, W. and Meenune, M. (2010). Quality profile of palm sugar concentrate produced in Songkhla province, Southern Thailand. *International Food Research Journal,* 17:425-32.

2.   Marales, F.J. and Jimenez-Perez, S. (2001). Free radical scavenging capacity of Maillard reaction products as related to colour and fluoresences. *Trends in Food Science and Technology,* 11:119-25.

3.   Taiapaiboon, S. (2004). Effect of high pressure and heat treatment on quality of palm sap. Masters Thesis. Prince of Songkla University.

4.   Takano, H. (2005). Investigation of chemical and physical properties of southwestern Wisconsin maple syrup. Masters Thesis. University of Wisconsin-Stout.

5.   Ranganna, S. (1986). Handbook of analysis and quality control for fruit and vegetable products. New Delhi: McGraw-Hill.

6.   Stuckel, G.J. and Low, H.K. (1996). The chemical composition of 80 pure maple syrup samples produced in North America. *Food Research International,* 29: 373-79.

7.   Rattanathanalerk, M., Chiewchan, N. and Srichumpoung, W. (2005). Effect of thermal processing on the quality loss of pineapple juice. *Journal of Food Engineering,* 66:259-65.

8.   Boyes, S., Strubi, P. and Dawes, H. (1997). Measurement of protein content in fruit juices, wine and plant extracts in the presence of endogenous organic compounds. *LWT-Food Science and Technology,* 30:778-85.

9.   Tuzen, M., Silici, S., Mendil, D. and Soylak, M. (2007). Trace element levels in honey from different region of Turkey. *Food Chemistry,* 103:325-30.

10. Balange, A. and Benjakul, S. (2009). Use of kiam wood extract as gel enhancer for mackerel (*Rastrelliger kanagurta*) surimi. *International Journal of Food Science and Technology,* 44:1661-9.

11. Binsan, W., Benjakul, S., Visessanguan, W., Roytrakul, S., Tanaka, M. and Kishimura, H.(2008). Antioxidative activity of Mungoong, an extract paste, from the cephalothorax of white shrimp (*Litopenaeus vannamei*). *Food Chemistry,* 106:185-93.

12. Benzie, I.F.F. and Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. *Analytical Biochemistry,* 239:70-6.

13. Matmaroh, K., Benjakul, S. and Tanaka, M. (2006). Effect of reactant concentrations on Maillard reaction in a fructose-glycine model system and the inhibition of black tiger shrimp polyphenoloxidase. *Food Chemistry,* 98:1-8.

14.   Steel, R.G.D. and Torrie, J. (1980). Principle and procedures of statistics: A biometrical approach. New York: McGraw-Hill.

15.   Martins, S.I.F.S., Jongen, W.M.F. and Van Boekel, M.A.J.S. (2001). A review of Maillard reaction in food and implications to kinetic modeling. *Trends in Food Science and Technology,* 11:364-73.

16.   Naknean, P., Meenune, M. and Roudaut, G. (2009). Changes in physical and chemical properties during the production of palm sugar syrup by open pan and vacuum evaporator. *Asian Journal of Food and Agro-Industry,* 2:448-56.

17.   Dumont, J., Saucier, L., Allard, G.B. and Aurouze, B. (1993). Microbiological, physicochemical and sensory quality of maple aseptically packaged in paper-based laminated. *International Journal of Food Science and Technology,* 28:83-93.

18.   Wienen, W.J. and Shallenberger, R.S. (1988). Influence of acid and temperature on the rate of inversion of sucrose. *Food Chemistry,* 29:51-5.

19.   Turhan, I., Tetik, N., Karhan, M., Gurel, F. and Tavukcuoglu, R.H. (2008). Quality of honeys influenced by thermal treatment. *LWT-Food Science and Technology,* 41:1396-9.

20.   Rufian-Henares, J.A. and Morales, F.J. (2007). Functional properties of melanoidins: *In vitro* antioxidant, antimicrobial and antihypertensive activities. *Food Research International,* 40:995-1002.

21.   Kim. J. and Lee, Y. (2009). Antioxidative activity of Maillard reaction products derived from aqueous glucose/glycine, diglycine and triglycine model systems as a function of heating time. *Food Chemistry,* 116:227-32.