Pakistan
Journal of Biological Sciences

Effect of Thermal Processing of Palm Sap on the Physico-Chemical Composition of Traditional Palm Sugar

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Abstract: During the production of palm sugar, the palm sap (*Arenga pinnata*) is heated up to 150°C. Besides the hydrolysis of carbohydrate to generate reducing sugars and degradation of amino acid, many physico-chemical changes produced at all these temperatures, having a significant impact on the overall quality of palm sugar. In this study, changes in physico-chemical properties of the palm sap due to heat processing were investigated. Analysis of colour, soluble solid, pH, temperature, sugar and amino acid concentration was determinant. The results showed clearly that the heating process at these high temperatures was necessary to create an environment which was rich in essential precursors for subsequent reactions such as Maillard reaction. Chemical compounds that showed drastic changes in concentration were polar side chain amino acids especially glutamine, asparagine and arginine as well as sucrose and pH value. Other quality characteristics of palm sugar based on colour and soluble solids (°Brix) shared an increase in concentration as a function of time.

Key words: Palm sap (*Arenga pinnata*), palm sugar, amino acids, thermal process, sucrose, Maillard reaction

INTRODUCTION

The well-known Maillard reaction results from an interaction between amino group derived, usually from amino acids or proteins and reducing carbohydrates (Bedinghaus and Ockerman, 1995). This reaction leads to the formation of compounds that, because of their volatility, influence the overall flavor of a product (Ames *et al.*, 1999). Since Ruckdeshel in 1914 reported aroma generation by Maillard pathway, the food industry has patented flavor formation processes from the heated aqueous mixtures of amino acids and reducing sugars. In the same way, thermal treatments of food as well as their basicity are favorable conditions for the generation of pyrazines compounds (Ho and Chen, 1999).

Palm sugar is one of the local delicacies widely consumed by Asians and used as ingredient in making cakes, desserts, food coating or drinks. Palm sugar is produced from sap derived from the tropical coconut tree (*Cocos nucifera*) or a palm tree called *Arenga pinnata* (Apriyantono *et al.*, 2002; Panyakul, 2001). During palm sugar production, the sap known as air nira is placed in a large wok on a wood fired stove and is cooked over 100°C for a few hours until it becomes concentrated. The exudates (palm sap liquid) are poured into bamboo moulds to form pure solid palm sugar. This product does not contain any type of additive or artificial colour but can

last for years when it is stored under the right conditions. Sucrose, the principal disaccharide in palm sap (*Arenga pinnata*). Kroh (1994) had reported that sucrose was hydrolyzed during the cooking process into its fractions glucose and fructose. The process is associated with the formation of aroma and deep colouration. The degree of aroma formation and the intensity of color are based on the structure of sugar presence in the sap whether mono, oligo or polysaccharide and the degree of interaction with the amino groups.

The Maillard reaction depends greatly on temperature with respect to which reaction route prevails and what pattern of intermediates and end products are formed. The relative proportions of these caramel compounds are dependent from the conditions favorable for the Maillard reaction. Those are the cooking temperature, heating time and the pH of the sap media.

The objective of the present study was to determine the changes in the physico-chemical properties of palm sap during the production of palm sugar according to the traditional method.

MATERIALS AND METHODS

Materials: The sweet liquid generated during the cooking of palm sugar, known as exudates, was collected from the

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traditional palm sugar producer in Kuala Pilah, Malaysia. Approximately 50 L of palm sap in a big wok was heated with an open process. Heated palm sap (200 mL) was sampled at intervals of 30 min throughout the 4 h heating process. The collected samples were cooled immediately by immersing in an ice-bath until the transport to the laboratory, where they were stored at -18°C for subsequent analysis.

Methods

Temperature: The temperature of the collected heated palm sap was measured using an infra-red thermometer (Quicktemp 826 T4, Testo, Vienna, Austria).

Moisture content: The samples were heated in the oven at 105°C for 24 h. The standard method for determining moisture in sugar rich food stuffs, is to mix the material with acid washed dry sand and filter by Whatman No. 1 filter paper.

Colour: Ten milliliter of each sample was diluted to a final volume of 100 mL with distilled water. The absorbance of each sample was read in a 1 cm quartz cell at 420 nm with a visible spectrophotometer (Spectronic 20, USA).

pH measurements: The pH value of the diluted samples (ten fold dilution) was determined with a pH meter (Schott pH meter, UK).

Amino acid analysis: Weighed samples of palm sap (1 g) were hydrolyzed with 10 mL of 6 N HCl in sealed Pyrex tubes under nitrogen atmosphere at 110°C for 24 h. The hydrolysate was diluted and the volume was brought to 100 mL in a volumetric flask with distilled water. The sample was spiked with 200 µL α-butyric amino acid (AABA) (Sigma Chemicals Co; St. Louis, MO, USA) as an internal standard.

As cysteine and methionine are partially destroyed by acid hydrolysis, a separate analysis for these was done. Both cysteine and methionine were oxidized at 4°C for 18 h with performic acid to cysteic acid and methionine sulphone, respectively. Then, the performic acid was removed from the samples at 80°C under vacuum on a rotary evaporator (Bucchi Laboratoriums, USA) prior to hydrolysis by 6N HCL. Tryptophan analysis was determined by hydrolyzing the samples with 10 mL of 4.2 N $Li(OH)_2$ in a sealed Pyrex tube in a nitrogen atmosphere at 120°C for 18 h.

The hydrolysate was diluted with distilled water; its pH was adjusted to 4.25 with acetic acid and the volume was made up to 100 mL with 0.1 N HCl. All hydrolyates passed through a 0.45 µm cellulose acetate filter prior to amino acid separation.

Standard Amino acids were obtained from Pierce Chemical Co, USA. Amino acid mixture supplemented with cysteic acid, methionine sulphone and tryptophan were obtained from Sigma Chemicals; St. Louis, MO, USA.

Amino acid determination: The Amino acids were separated by reversed-phase HPLC column (Waters, Milford, MA) controlled by a breeze system (Waters). The hydrolysates were injected in a pre-column derivatized with 6-Aminoquinolyl-N-Hydroxysuccinimidyl Carbamate (ACCQ) to determine primary amino acids. The separation of amino acids was performed on a Waters AccQ.Tag (3.9×150 mm). The mobile phases used were solvent A was 1:10 ratio (AccQ.Tag Eluent A Concentrate Commercial to deionized water) and solvent B was 60% (v/v) acetonitrile solutions. The injection volume was 100 µL and a flow rate of 1 mL min⁻¹ was used. For the determination of tryptophan, the hydrolysates were separated on a Waters C_{18} column (3.9×300 mm) using the same mobile phase.

The fluorescent detector was adjusted at excitation wavelength of 250 nm and emission wavelength of 395 nm. All reagents used were HPLC grade. Amino acids were quantified by comparing peak area of the samples with those of the internal standard, AABA.

HPLC separation of sugars: The sugars (1 g) were extracted with 50% aqueous acetonitrile and the volume was brought to 50 mL followed by mechanical stirring for 1 h. The extract was filtered through a 0.45 µm SEP-PAK C18 cartridge membrane filter. The filtrate was separated on an NH_2 HPLC column (4.6 mm ID×250 mm, 5 m particle size; Lichrosorb NH_2, Merck, Germany) thermostated at 30°C. The mobile phase consisted of acetonitrile-water 80:20 (v/v) at a flow rate of 1 mL min⁻¹. A Refractive Index (RI) detector was used for the detection of the sugar peaks.

Total soluble solids: Approximately 10 µL of each extract was used to measure Total Soluble Solids (TSS) using a refractometer (0-90%) (Atago, Japan).

RESULTS AND DISCUSSION

Moisture content and heat treatment: Figure 1 shows the temperature increments observed during the processing of palm sugar. Temperature after 30 min of cooking was 78.5°C and rose to 147.6°C at the end of cooking. During the cooking processing of palm sap by the traditional open method the evaporation of water reduced the moisture content from 84.66 to 12.11%. The relatively high percentage of moisture content (> 50%) for a long period (150 min) is of importance in generate
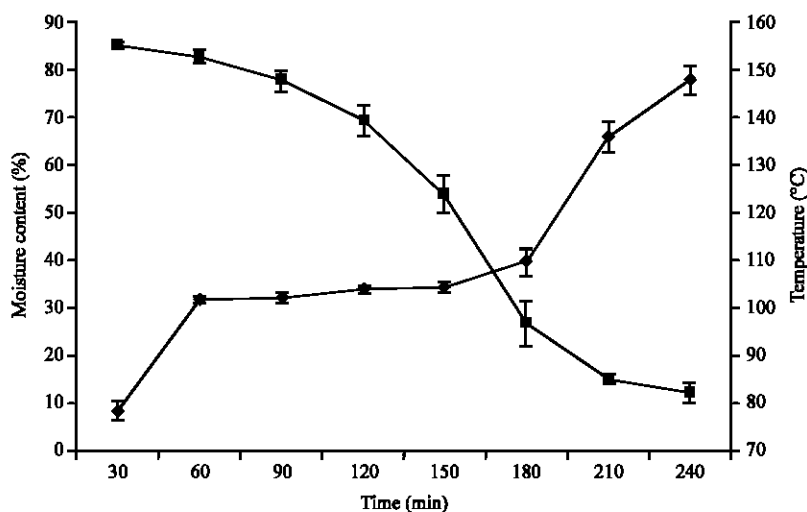
Fig. 1: Moisture content (■) and heating temperature (♦) of traditional palm sugar processing
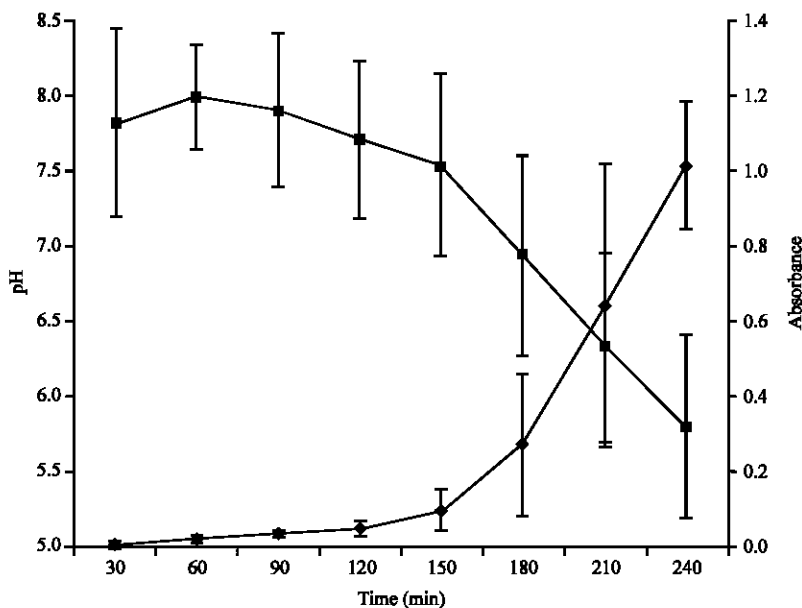

Fig. 2: Absorbance at 420 nm (♦) and pH (■) values of traditional palm sugar processing

essential precursor especially free ammonia or atom nitrogen and dicarbonyl compounds through retro-aldol and deamination reaction.

On the other hand, the higher moisture content of the system could be an unfavourable condition for Maillard reaction due to the reversible reaction of aldol-condensation; one of the main pathway for pyrazine volatile compounds formation. This interpretation is in agreement with the results obtained earlier by Ames *et al.* (2001). These investigators showed that in glycine/glucose model systems, the level of pyrazines classes decreased by approximately 3.5-fold in the presence of moisture. It is likely that the decrease moisture content during cooking of palm sap inhibited the

mobility of the reactants to interact but favoured the generation of the pyrazine volatile compounds. Therefore, it can be concluded that the moisture content played a different role at the different stages of Maillard reaction and the level of moisture content was greatly dependent on the combination between the temperature and the duration of time.

Colour and pH values: The colour of palm sugar liquid became darker as a function of time, with an increase in the absorbance from 0.01 at 30 min processing time to 1.01 at 240 min was associated with an inverse decrease in the pH values from 7.82 down to 5.80 throughout the process (Fig. 2). A slight increase in the pH value was

Table 1: Changes in amino acid (g/100 g) content of palm sugar during processing (N = 3)

	Heating time (min)							
Amino acid compound	30	60	90	120	150	180	210	240
Polar								
(i) Uncharged side chains	1.646	1.562	1.475	1.104	0.614	0.552	0.542	0.522
Asparagine (Asn)	0.325	0.312	0.300	0.289	0.201	0.181	0.169	0.165
Glutamine (Gln)	1.203	1.150	1.095	0.762	0.362	0.325	0.330	0.314
Serine (Ser)	0.046	0.033	0.031	0.017	0.015	0.014	0.013	0.014
Threonine (Thr)	0.072	0.067	0.049	0.036	0.036	0.032	0.030	0.029
(ii) Charged side chains	0.419	0.371	0.323	0.172	0.138	0.105	0.085	0.083
Arginine (Arg)	0.378	0.338	0.293	0.141	0.118	0.084	0.067	0.064
Lysine (Lys)	0.029	0.023	0.022	0.025	0.014	0.015	0.012	0.013
Histidine (His)	0.012	0.010	0.008	0.006	0.006	0.006	0.006	0.006
Non-polar side chains	0.178	0.166	0.158	0.129	0.104	0.087	0.087	0.085
Alanine (Ala)	0.031	0.029	0.026	0.020	0.013	0.013	0.013	0.015
Leucine (Leu)	0.007	0.006	0.005	0.004	0.003	0.002	0.003	0.002
Isoleucine (Ile)	0.016	0.013	0.011	0.007	0.005	0.004	0.004	0.003
Valine (Val)	0.011	0.010	0.010	0.009	0.010	0.007	0.008	0.008
Phenylalanine (Phe)	0.013	0.013	0.012	0.012	0.009	0.007	0.007	0.007
Tyrosine (Tyr)	0.015	0.010	0.009	0.007	0.007	0.005	0.004	0.004
Glycine (Gly)	0.059	0.057	0.054	0.039	0.035	0.031	0.030	0.028
Proline (Pro)	0.026	0.028	0.031	0.031	0.022	0.018	0.018	0.018
Total polar side chains	2.065	1.933	1.798	1.276	0.752	0.657	0.627	0.605
Total nonpolar side chains	0.178	0.166	0.158	0.129	0.104	0.087	0.087	0.085

observed at the first 30 min of cooking; obviously due to the decarboxylation and CO_2 evolution from reaction system during the initial stage of heating. The browning colour is usually directly correlated with the Maillard reaction based on the generation of free ammonia compounds from the degradation of amino acid which might be contributing to the brown colour. Based on the above mentioned findings, it can be concluded that Maillard reaction did take place in the cooking process of palm sugar.

The absorbance increments concomitant with the pH decrements are characteristics to Maillard reaction in the different systems (Apriyantono and Ames, 1993). The mechanism of brown colour formation is not fully understood and the structure of melanoidins is largely unknown. Studies by Cammerer and Kroh (1995) and Yaylayan and Kaminsky (1998) suggested that the melanoidin skeleton are mainly built up from sugar degradation products, formed in the early stages of the Maillard reaction, polymerized through aldol-type condensation and linked by amino compounds, such as amino acids. It has been reported that the non-enzymatic browning reaction was accelerated in a neutral or alkaline solution, since C_2 and C_3 fragments with high reactivity were substantially produced (Hayashi and Namiki, 1986). These statements best explained the changes in pH in palm sugar liquid in the early stage of processing. The decrease of pH in the earlier stage of Maillard reaction had been reported to be due to the loss of the basic amino groups (DeMan, 1999). However, in the later stages, after Amadori rearrangement, products with various acidities such as glyoxal, pyruvaldehyde, furfural and carboxylic

acid became distinguished and might influence the pH system. Carboxylic acid reduced the pH and slowed the reaction. Generally speaking, lower pH value favours the generation of non-nitrogen-containing compounds (Ho *et al.*, 1997). This phenomenon is apparent at higher temperature as temperature has a dramatic effect on the pK of buffers and thus buffer pH (Martins and Van Boekel, 2005). The high pH at 7.82 of palm sugar liquid (Fig. 2) probably decreased the protonation of nitrogen atom in the amino group or ammonia and increased the effective concentration of amino group or ammonia. The pH was reported to influence the colour and the overall aroma (Meynier and Mottram, 1995).

Both the reactivity of sugar fragmentation and availability of nitrogen atom sources in particular environment reaction medium are influenced by the pH value and of paramount importance in the Maillard reaction.

Amino acids patterns: Table 1 shows the patterns of the amino acid compounds and the AAB. The amino acids were categorized into three groups: Polar Uncharged Side-chain (PUS), Polar Charged Side-chain (PCS) and Non-Polar Side-chain (NPS) The relative concentrations, after the different heating times were used in terms of equivalents to the internal standard.

The NPS showed the least reactivity and differed significantly from the PUS and PCS group amino acids (Table 1). These amino acids might play a dominant role in Maillard reaction by providing nitrogen atom sources. The basic properties of those major amino acids were reported to have a stronger activity during the Maillard
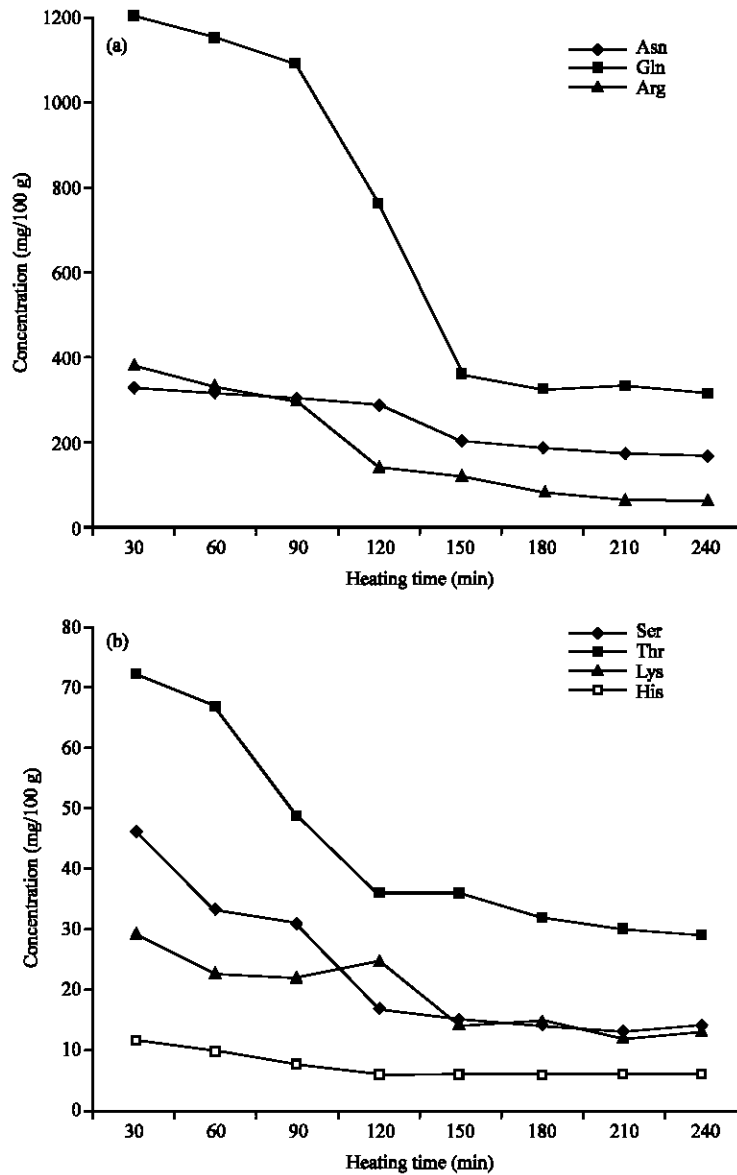
Fig. 3: Concentration of polar side chain group of amino acids (mg/100 g) (w/w) of traditional palm sugar processing

reaction if compared to the acidic and non-polar amino acid had the least activity (Piloty and Baltes, 1979). Arginine, an exception was reported to decrease the capability of glycine to produce pyrazines (Ho *et al.*, 1995).

The concentration of asparagine, glutamine and arginine were very high (> 1 g/100 g) at the beginning of the process and reduced significantly after 120 min (Fig. 3). The relatively high temperatures (Fig. 1) and the weak alkalinity (Fig. 2) of the samples could be the causative factors. Glutamine and asparagine are known to release more free ammonia during thermal reaction than other amino acids (Sohn and Ho, 1995). For glutamine and

asparagine, nitrogen may be released as ammonia by deamination (loss of the α-amino group) or by deamidation (loss of the amide group). However, deamidation appears to be the more important mechanism in oil media, with more ammonia being released from glutamine than asparagine (Ho and Kim, 1998). In addition, Sohn (1996) reported that only 1.3% of glutamic acid and more than 50% of asparagine, aspartic acid and glutamine were deamidated at pH 8.0.

A study by Ho and Chen (1999) showed that total pyrazines were generated more in glutamine-containing system than in serine and threonine-containing systems. Hence it is strongly believed that deamination reaction
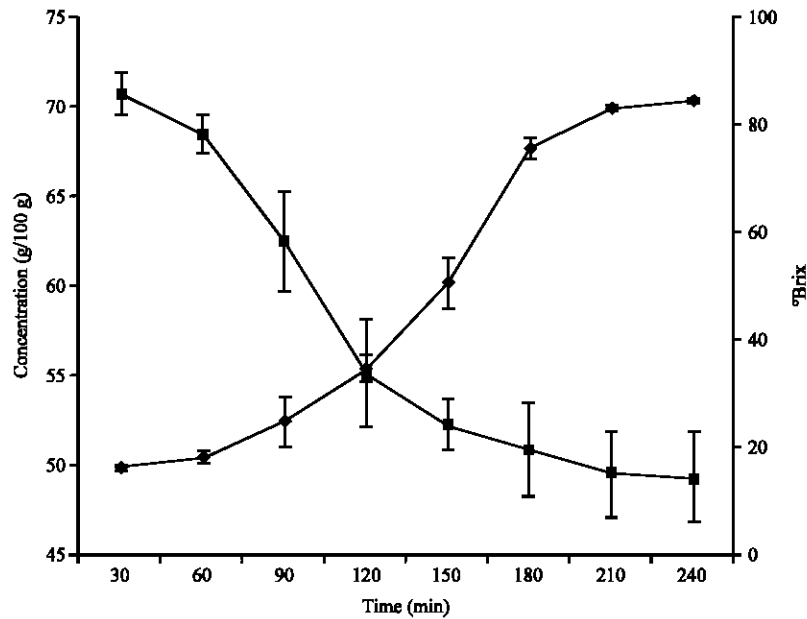
Fig. 4: Sucrose content (■) and soluble solid (♦) values in traditional palm sugar

was dominant and efficient in releasing more free ammonia from PUS amino acid in these aqueous base samples at the early stage of the heating process.

Unexpectedly, arginine amino acid consists of strong basic δ-guanidino group, which is actually unreactive and only very small fractions of the non-ionized formed are present at normal pH value (Creighton, 1984) displayed a significant changes in concentration in the current study (Fig. 4). It was assumed that a high fraction of the non-ionized amino group form was present at weak bases environment condition at the beginning of the heating process (Fig. 1) are potent nucleophiles, can easily catalyze sugar fragmentation and are involved in the later stage of Maillard reaction. On the other hand, lysine even with a small amount in this study is known to be the most reactive amino acid in the Maillard reaction (Miller *et al.*, 1984) due to its two reactive amino groups. It is believed that those amino acids containing nitrogen atoms in their side chains did have influence on providing nitrogen atom sources which was an important precursor in pyrazine formation.

Sugar: Figure 4 shows the relationship between soluble solid (°Brix) and sugar throughout the palm sugar cooking process. A drastic increment in soluble solid content was observed during the first 180 min followed by a lower rate of increase until 240 min. Sucrose concentration showed an opposite change compared to soluble solid where a drastic reduction occurred during the first 180 min and then almost constant concentration thereafter. Study by

Apriyantono *et al.* (2002) also showed a similar trend where sucrose and nitrogen decreased markedly with the increase in the intensity of browning during preparation of coconut sugar.

Cammerer *et al.* (2002) stated that hydro-thermolytic degradation of carbohydrates were favour under aqueous reaction conditions leading to the formation of more reactive monosaccharide. Surprisingly, these reactive monosaccharides were not detected in the present study. Hence, it was believed that due to the high reactivity of monosaccharide and aqueous condition at the initial stage of heating process at the present study. Monosaccharide such as glucose and fructose will participate at the early stage of Maillard reaction by forming an abundant pool of high reactive C2, C3 and C4 dicarbonyl compounds. Releasing of amino group compounds from amino acids at the early stage of Maillard reaction (Fig. 3) which were potent nucleophiles can easily catalyze sugar fragmentation (Fig. 4) further supported the phenomenon of no reducing sugar identified throughout the heating process of palm sap in our present study.

CONCLUSION

The traditional method used for the production of palm sugar lead to products of inconsistent quality, with Maillard compounds of variable proportion due to the high standard deviation of the variables in addition to the quality and age of raw material, in this case was palm sap (*Arenga pinnata*).

ACKNOWLEDGMENTS

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