Antioxidative Activities of Palm Sugar-Like Flavouring

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Abstract: Maillard reaction products have been considered to have antioxidant capacities due to the reductones compound formations which contribute to antioxidant activity. Besides caramelisation, Maillard reaction takes place during the production of palm sugar. This paper reports the antioxidative activity of palm sugar-like flavourings and the commercial palm sugar (CPS) which was obtained from Pahang by using different types of methods. Formulation of palm sugar-like flavourings which had been optimised was obtained from the previous studies. Palm sugar-like flavourings (PSLFs) were prepared from sucrose and selected amino acids (Asparagine, Glutamine, Arginine and Lysine) at various ratios with buffer solutions (pH 7.86), heated at 143°C for 116 minutes. The results revealed that the PSLF (C) showed a significant difference ($p<0.05$) in reducing power, thiobarbituric acid (TBA) test, and radical scavenging activity (DPPH) compared to other formulations of PSLF and commercial palm sugar.

Keywords: Palm sugar-like flavouring, antioxidative activity, radical scavenging activity (DPPH), reducing power, thiobarbituric acid (TBA).

INTRODUCTION

Antioxidant has become a very prominent topic in health consumption. Research on antioxidant in common food products have proven that disease fighting properties may be found in almost all fruits and vegetables or even processed food. As long as food had been cooked, Maillard reaction has played a very crucial role in giving good appearance and taste [1] and has been shown to produce antioxidant components as well [2].

Production of palm sugar-like flavourings is also related to the Maillard reaction process. The Maillard reaction involved in the formation of brown pigments due to the condensation between the carbonyl groups of reducing sugar and aldehydes and ketones, the free amino groups of lysine and/or other free amino acids (such as amino acids, peptides and proteins) or any nitrogenous compound [3, 4]. A lot of work has been carried out in investigating the antioxidant activity of Maillard reaction product in a model system and also in other foods, such as in beer [5] and coffee [6]. The development of a brown colour called "melanoidins" was an extremely important and obvious feature of the extent of the advanced Maillard reaction [7]. It had been reported to have antioxidant activity through scavenging oxygen radicals or chelating metals [8]. Through *in vivo* studies, it has been shown that Maillard reaction products were capable in contributing as reducing agents [9], metal chelators [10] and radical scavengers [11].

Besides improving the flavour, colour and texture of the food product, Maillard reaction products also substantially contribute to the shelf-life of heat-treated foods [12]. Maillard reaction products have been used to prevent lipid oxidation in many products and exhibit antioxidative activity in meat products [13, 14]. The oxidation of singlet oxygen generated by exposing methylene blue to light was strongly inhibited by fructose-tryptophan Maillard reaction products and by tryptophan and this suggests that the scavenging of active oxygen species by Maillard reaction products is an important mechanism of the antioxidant [15]. Bedinghaus & Ockerman [14] found that reducing sugars and free amino acids generated antioxidant compounds in cooked ground pork patties.

The present study was conducted to investigate the antioxidant activity of various formulations of palm sugar-like flavouring (PSLF) and commercial palm sugar (CPS) using different types of methodology.

MATERIALS AND METHODOLOGY

Preparation of Palm Sugar-Like Flavouring

Five samples of palm sugar like-flavourings were prepared according to the formulations and parameters which had been optimised by Ho *et al.* [16]. Commercial palm sugar was obtained from Pahang, Malaysia.The reactant materials (Table **1**) were dissolved in 100 ml of 0.2 M phosphate buffer (pH 7.8) solution. The mixture was then heated in half covered reaction vessel (500 ml) which was immersed in 1 L of silicon oil at 143°C for 116 minutes. The samples were kept in 25 ml screw cap bottles and stored at -18°C.

Measurement of pH

pH values were measured by using a pH meter (Radiometer Analytical, model PHM 210). Approximately

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Table 1. Formulation Used to Prepare Palm-Sugar Like Flavouring

Reactant Materials	Samples (mmol)				
	A	B	C	D	E
Sucrose	12.75	12.65	12.68	12.68	12.77
Asparagine	2.55	2.53	2.55	2.55	2.53
Glutamine	2.99	2.59	2.23	2.23	2.26
Arginine	0.62	0.62	0.62	0.62	0.61
Lysine	0.65	0.48	0.48	1.01	0.95

0.5 gram of each sample was dissolved in 10 ml of distilled water and the analysis was made in triplicates.

Extraction of Antioxidant Maillard Reaction Products

The extraction of Maillard reaction products (MRP) from PSLF was carried out according to the method of Yusuf and Romeo [8]. The sample was dissolved in distilled water (40ml/g) using vortex. The mixture was centrifuged at 26,000g for 30 minutes at 4°C. Supernatant was collected and kept at 4°C for further analysis. Each sample was prepared in triplicates.

Determination of Browning Intensity

Browning intensity of PSLF samples was measured according to Ajandouz *et al.* [17] with slight modifications. Appropriate dilution (sample extract/ distilled water) (1: 9, v/v) was prepared using distilled water and the absorbance was measured at 420 nm using UV-Vis spectrophotometer (Shimadzu, model UV-2450). The dilutions were made in order to obtain optical density of less than 1.5 [8].

Determination of DPPH Radical Scavenging Activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was determined according to the method of Wittayachai, *et al.* [18]. Approximately, 80µl of PSLF extract was added with 320µl distilled water and 2 ml of 0.12 mM DPPH in methanol. The mixtures were then mixed vigorously and allowed to stand at room temperature in the dark for 30 minutes. The absorbance of the sample mixture was then measured at 517 nm using the UV-Vis spectrophotometer (Shimadzu, model UV-2450). The control sample was prepared in the same manner as the preparation of sample mixtures except that deionised water was used instead of the PSLF samples. The blank sample was handled in the same manner but deionised water was used instead of a DPPH solution.

Percentage of DPPH radical scavenging activity =

$$1-\left[\frac{\text{Absorbance of blank}}{\text{Absorbance of samples}}\right]\times 100$$

Determination of Reducing Activity

The reducing power of PSLF was determined according to the method of Wittayachai *et al.* [18]. Approximately, 1 ml of 0.2 M phosphate buffer (pH 6.6) and 1 ml of 1% (w/v) potassium ferricyanide were added to 1 ml of PSLF extract sample. The reaction mixture was then incubated at 50°C in a water bath for 20 minutes. Subsequently, 1 ml of 10% acid trichloroacetic acid was added to the mixture. The mixtures were centrifuged at 750*g* using centrifuge (KUBOTA, model 2450). Supernatant (1 ml) was mixed with distilled water (1 ml) and 200µl of ferric chloride. The absorbance of the mixture was measured at 700nm.

Ferric Thiocyanate Method

The ferric thiocyanate method (FTC) was carried out according to Mohd Zin *et al.* [19]. Samples of 4 mg were dissolved in 99.5% ethanol (4.1 ml) and mixed with 2.5% linoleic acid, 0.05M phosphate buffer (pH 7.0, 8 ml) and distilled water (3.9 ml) and kept in screw cap bottles and incubated in a temperature controlled water bath at 45°C, overnight. Subsequently, 9.7 ml of 75% ethanol and 0.1 ml of 30 % ammonium thiocyanate were added into 0.1 ml of the incubated sample, followed by 0.02 M of ferrous chloride in 3.5% hydrochloric acid. The mixture was left for 3 minutes. The absorbance was measured at 500 nm using the UV-Vis spectrophotometer (Shimadzu, model UV-2450).

Thiobarbituric Acid Test (TBA)

The test was conducted according to the method of Kikozaki & Nakatani [20]. The same samples prepared for the FTC method were used. A sample solution (1 ml) was mixed with 20% trichloroacetic acid (2 ml) and thiobarbituric acid solution (2 ml). The mixture was then placed in a boiling water bath for 10 minutes and cooled before it was centrifuged at 3000 rpm for 20 mins. Absorbance of supernatant was measured at 532 nm using the UV-Vis spectrophotometer (Shimadzu, model UV-2450).

Percentage of TBA test =

$$1-\left[\frac{\text{Absorbance of blank}}{\text{Absorbance of samples}}\right]\times 100$$

The Statistical Analysis

All experiments were conducted in triplicate and statistical analysis was done according to the SAS (1990) User's Guide. Descriptive statistical analysis was performed by using analysis of variance. Duncan's multiple range tests were used to determine significant differences between the means. Graphs were made by using the Microsoft Excel (Microsoft Corporation). The data were expressed as means ± S.D. The group differences were evaluated by using t-tests with $p < 0.05$ considered as indicating a statistically significant difference.

Table 2. The Initial and Final pH Value of Palm Sugar-Like Flavouring and Commercial Palm Sugar

Samples	pH Value	
	Initial	Final
A	7.92 ± 0.03	6.31 ± 0.04
B	8.02 ± 0.02	6.91 ± 0.02
C	8.00 ± 0.08	6.15 ± 0.01
D	8.08 ± 0.03	6.99 ± 0.06
E	8.74 ± 0.08	6.90 ± 0.01
Commercial Palm Sugar (CPS)	N/A	5.61 ± 0.11

RESULTS AND DISCUSSION

Analysis of pH

The pHs of all PSLF with different initial pHs decreased significantly ($p<0.05$) from their initial values within 116 minutes (Table **2**). The interaction between sucrose and amino acids throughout the Maillard reactions was prone to lowering the pHs value. The reactivity of sugars and amino acids was highly influenced by the pH. The initial pH value of the model system was considered as a crucial part in the Maillard reaction [21]. The initial pH of all PSLF samples was more than pH 7. At higher pH, the open chain form of sugar and unprotonated form of amino group, were considered to be the reactive forms of Maillard reactions [22].

The reduction of pH in Maillard reaction was due to the formation of organic acids such as formic and acetic acids from the degradation of fructose and glucose [23]. This had contributed to the present of H^+ ion in the system. The lower the pH, the more protonated amino group was present in the mixture. The decreasing level of pH was in agreement with Apriyantono and Ames [24], Morales and Jimenez-Perez [25] and Benjakul *et al.* [26] who found that during the Maillard reaction, pH frequently decreased as the heating time increased.

In Table **2**, the lowest final pH depicted in CPS was probably caused by the pH value and the composition of palm sap derived from the tropical palm tree [27], and enviromental factors, such as weather, geographic area of plantation, the fertility of soil and the age of palm tree. The uncontrolled temperature and duration of cooking time also influenced the yield of palm sugar.

Analysis of Browning Intensity

PSLF C in Fig. (**1**) showed the highest browning intensity of 0.257 ± 0.03 and was significantly different as compared to other PSLFs (A,B,D and E) and CPS ($p<0.05$). The degree of browning was usually measured *via* the absorbance at 420 nm which was used analytically to assess the extent of the Maillard reaction that has taken place in samples.

Apart from the Maillard reaction, the caramelisation of glucose which contributed to non-enzymatic browning reactions also took place at the same time [26, 28]. However, previous reports [29], showed that the type of amino compounds and reducing sugars, as well as the pH of the medium, are the factors that mainly affect the rate of the Maillard reaction.

The highest concentration of sucrose in PSLF C rendered the high browning intensity. Sucrose, which is a carbohydrate consist of 1 molecule of glucose and 1 molecule of fructose contributed to the caramelisation process at the early stage of PSLF production. These two reducing sugars would react stimultaneously with amino

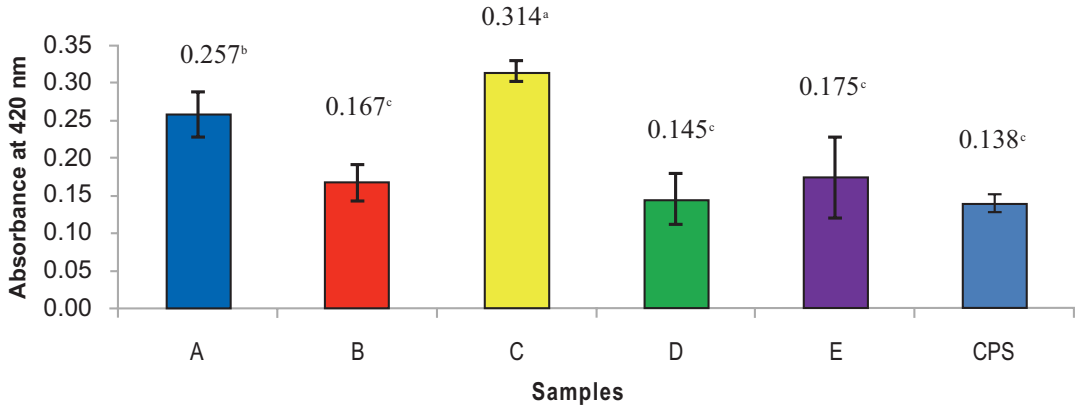

Fig. (1). Browning of Various Formulations of Palm Sugar-Like Flavouring and Commercial Palm Sugar (CPS).

*Values are the means of triplicate analysis.

*Values followed by a different superscript letter are significantly different ($p < 0.05$).

acids and in the following Maillard reaction. The browning intensity was found to be dependent on sugar concentration. The browning rate was influenced by the type of reducing sugar involved in the reaction. The reactivity of reducing sugar was reported to decrease in the following order: aldopentose> aldohexoses> ketohexoses> disaccharide [30]. A small amount of reducing sugar was sufficient to cause considerable browning [31]. A previous study reported that the isomerisation and degradation reaction of sugar played an important role [32].

A study on fructose in the Maillard reaction showed that the level of browning intensity was high in fructose as it had many chain structured to give a condensation product, N-glycosylamine [33] which rearranged to form Amadori rearrangement products (ARPs). This reaction step was very important as it could be initiated under mild conditions. Food that had been kept at room temperature may eventually turn brown due to the polymerisation of the degradation products of ARPs. At high temperatures, ARPs can be formed within hours and degrade or react with other food components to produce the characteristic of aroma and the brown colour [34]. Fructose was sensitive neither in the present of nor in the absence of oxygen at the early stage of the Maillard reaction. Hence, the reaction was more extensive in ARP reactions due to the formation of more N-glycosylamine structures. The Amadori form which was decomposed, formed a lot of compounds, including reductones which were very reactive and able to produce florescent compounds which are possible precursor for browning pigment, melanoidins [35]. Brandis *et al.* [36] found that fructose experience a higher browning intensity as compared to other reducing sugars when it is heated for 60 mins at 120ºC. In the advanced stage of the Maillard reaction, a range of reactions took place, including cyclisations, dehydrations, retroaldolisations, rearrangements, isomerisations and further condensations which ultimately to the formation of melanoidins [23].

According to Ashoor and Zent [37], common amino acids and amides were classified into the Maillard browning produced when they were heated with common reducing sugar. They were high browning producing amino acids, such as lysine, glycine, tryptophan and tyrosine, intermediate browning producing amino acids, like proline, leucine, isoleucine, alanine, hydroxyproline, phenylalanine, methionine, valine, glutamine and asparagine, and low browning producing amino acids of histidine, threonine, aspartic acid, arginine, glutamic acid and cysteine.

Lyisne, a high browning producing amino acid was used in all samples of PSLF. It served as a source of reactive amino groups for the Maillard reactions as it has two α- and β- amino groups, it may have higher reactivity than other amino acids [38]. Although, lysine was high in the formulation of PLSF samples D and E, the interactions from intermediate browning producing amino acids (asparagine and glutamine) would render a higher absorbance in other formulations.

Analysis of DPPH Radical Scavenging Activity

Fig. (**2**) showed the DPPH radical scavenging activity of PSLF and CPS. Subsequently, there was a significante difference in PSLF and CPS ($p<0.05$).

The DPPH-scavenging activity was due to the presence of a compound capable of producing hydrogen atoms [39]. DPPH radical had been scavenged by the Maillard reaction product through the hydrogen process to form stable DPPH-H molecules [40]. The decrease in absorbance value indicated the transfer of hydrogen ion to DPPH radicals. PSLF C depicted a higher percentage, parallel with the higher contribution of H^+ ion. The colour of the solution would change from purple to yellow when DPPH had received the H^+ ion [39].

The Maillard reaction, has been known to be associated with the formation of compounds with pronounced antioxidant activity [41]. This could be due to the reductone structure which had been formed as a result of heating. An outcome that is consistent with Yen and Hsieh [42], Murakamie *et al.* [43], Morales and Jimenez-Perez [25] and Benjakul *et al.* [26] who also found that Maillard reaction products had DPPH radical scavenging activity.

According to Murakami *et al.* [43], the radical scavenging activity in the late stage of the Maillard reaction

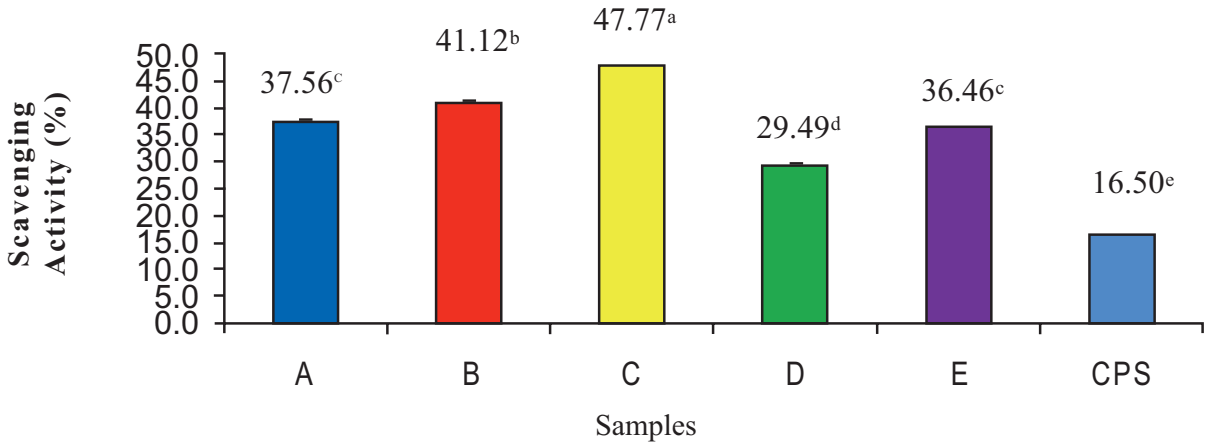

Fig. (2). Percentage (%) of DPPH Radical-Scavenging Activities of Various Formulations of Palm Sugar-Like Flavouring and Commercial Palm Sugar (CPS).

*Values are the means of triplicate analysis.

*Values followed by a different superscript letter are significantly different ($p < 0.05$).

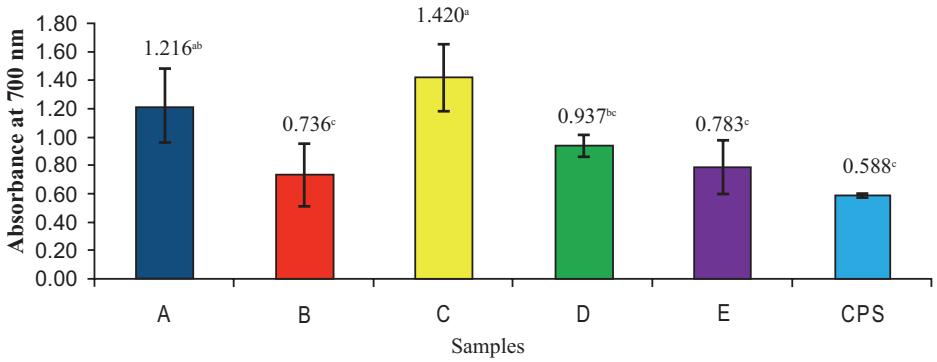

Fig. (3). Reducing Power of Various Formulations of Palm Sugar-Like Flavouring and Commercial Palm Sugar (CPS).

*Values are the means of triplicate analysis.

*Values followed by a different superscript letter are significantly different ($p < 0.05$).

was derived from the brown pigments. Hayase *et al*. [44] speculated that melanoidins showed a greater rate of scavenging hydroxyl radicals as a result of the presence of reductones, enamides or pyrrole-like structure in the melanoidins. In addition, melanoidins were reported to have relatively stable free radicals in their molecules. The free radicals also were considered to be important for scavenging the radicals.

Analysis of Reducing Power

The reducing power activity of PSLF and the CPS is depicted in Fig. (**3**). There was a significant difference between PSLF C and the CPS. Reductones from the thermolysis of Amadori products in the primary stage of the Maillard reaction were mainly responsible for the production of reducing power in the reaction. The higher reducing power of the reaction mixture was determined by the higher absorbance [45].

The Fe^{3+} ions which were added to the Maillard reaction product would occur the electron transfer process and the ions would be reduced to Fe^{2+}. The Fe^{2+} ions which were formed in the acidic solution, where a stable red coloured complex solution was produced. It is possible for Fe^{2+} ions to generate the radicals' protons or by redox processes with suitable Maillard reaction products, such as sugar, reductones or phenols which involved the reduction of the Fe^{3+} ions themselves to Fe^{2+} ions [46]. Reductones from PSLF may occur in the electron transfer process, involving the reduction of Fe^{3+} ions to Fe^{2+} ions. PSLF formulation containing the highest level of sugar (formulation C) had a greater reducing power than other formulations. The result was inconsistent with Benjakul, *et al*. [28] which reported that the Maillard reaction product which had the highest concentration and the most reactive reducing sugar, possessed greater reducing powers. The result revealed that Maillard reaction products from PSLF could function as electron donors. Yoshimura *et al*. [47] reported that Maillard reaction products from a glucose-amino acid mixture had a higher reducing power, especially when the heating time was increased. Hydroxyl group of MRP plays a role in the reducing activity.

Analysis of TBA

From Fig. (**4**), all formulations of PSLF were able to inhibit the lipid oxidation. Formulation C had the highest antioxidant activity (28.18%). However, there was no

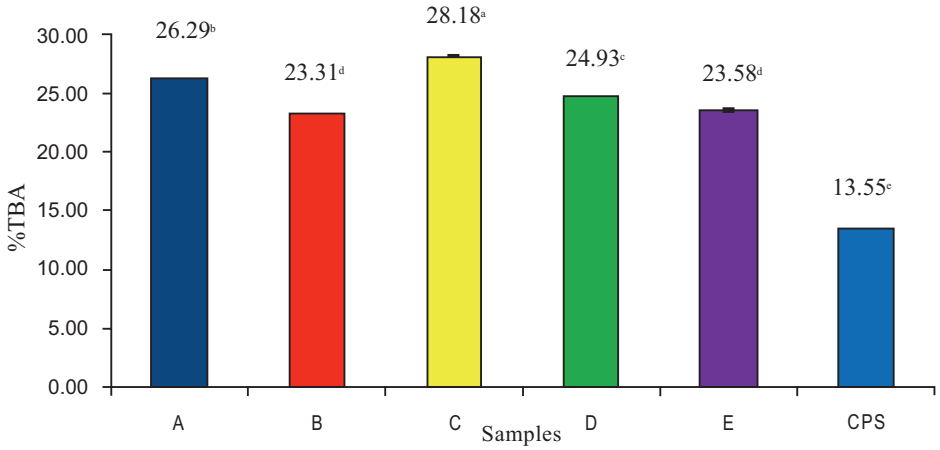

Fig. (4). Percentage (%) of Thiobarbituric Acid (TBA) test of Various Formulations of Palm Sugar-Like Flavouring and Commercial Palm Sugar (CPS).

*Values are the means of triplicate analysis.

*Values followed by a different superscript letter are significantly different ($p < 0.05$).

significant difference between all samples of PSLF ($p>0.05$) and lipid oxidation was enhanced by heat, light, heavy metals and the presence of pigments. A model lipid system which contained linoleic acid in ethanol had been used to determine the antioxidant activity ability of MRP which measures the inhibition of linoleic acid oxidation. Antioxidants exert their effect by donating electrons or hydrogen atoms to free radicals containing lipids and by forming antioxidant-lipid complexes [48].

The antioxidants from MRP, inactive reactive radicals at the initial steps of the autooxidation of the linoleic acid system, thus, avoid the propagation of the radical chain reaction [49]. Most Maillard reaction products had an antioxidant effect on the TBA test and some MRP had even greater antioxidant effects than others. According to Bedinghaus and Ockerman [14], the antioxidant potential of MRP showed that materials from natural precursors, for example, histidine, lysine and tryptophan can be used in cooked product, such as meat.

From Norimasa *et al.* [50], the isolated reductones from the reaction mixture of aldohexoses and secondary amines were effective for inhibiting autooxidation of several kinds of vegetable oils. The study had been conducted by using the Maillard reaction product, where the effect of browning reaction solution on TBA value of linoleic acid was compared to the effect on peroxide values, where the TBA value gave a decreasing effect as well as peroxide value. Subsequently, this indicated that Maillard reaction products inhibited the formation of peroxides or/and carbonyl compounds. Accordingly, the peroxide value decreasing effect of the Maillard reaction products was considered to be responsible for the antioxidant activity [51].

Although, the antioxidative mechanism of the Maillard reaction product is still unclear, it is thought that the reductone structures, the electron donor property, the chelating properties of melanoidins against transition metals, as well as the scavenging activity of Amadori compounds and melanoidins against reactive species are important factors in promoting the inhibition of lipid and phenolic oxidation reactions [52].

CONCLUSION

The pH of PSLF showed the pronounced effect on the Maillard reaction. The Maillard reaction product prepared by heating the mixture of PSLF with lower pHs showed a higher antioxidant activity. Basically, the antioxidant activity moved proportionally with the increasing absorbance of browning intensity at 420 nm. Concentration of sucrose in the mixture plays an important role as it contributed to the formation of the browning process. PSLF with formulation C (Sucrose 12.96 mmol, Asparagine 2.53 mmol, Glutamine 2.59 mmol, Arginine 0.62 mmol and lysine 0.48 mmol) depicted the highest antioxidant activity as compared to other formulations and CPS.

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