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Antioxidative activity of caramelisation products and their preventive effect on lipid oxidation in fish mince

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Abstract

The antioxidative activity of caramelisation products (CPs) from different sugars (glucose, fructose, ribose and xylose) prepared by heating the sugar solutions at 100 °C at pH 7 and 10 was investigated. Browning and intermediate degradation products increased with increasing heating time. The development was dependent upon the type of sugar and was generally higher at alkaline pH than neutral pH. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and reducing power increased with the increase in browning and intermediate formation. Among all sugars tested, fructose exhibited the highest antioxidant activity as evidenced by the greatest scavenging effect and reducing power. Fructose CPs showed ferrous iron chelating potential. The formation of thiobarbituric acid reactive substances (TBARS) in a comminuted saury model system was retarded with the addition of fructose CPs during iced storage. Therefore, CPs of sugars can be a promising novel antioxidant to prevent lipid oxidation in food systems.

Keywords: Antioxidant; Browning; Caramelisation; Fish; Lipid oxidation; Thermal degradation products

1. Introduction

Non-enzymatic browning reactions occurring in food during processing and storage are known to contribute to food quality and acceptability. Those reactions, including the Maillard reaction, caramelisation, chemical oxidation of phenols and maderisation are favored by heat treatments (Manzocco, Calligaris, Mastrocola, Nicoli, & Lerici, 2001). Among non-enzymatic browning reactions, the Maillard reaction has been the most intensively studied. Maillard reaction products (MRPs) have been found to exhibit antioxidative activity due to radical scavenging activity (Marales & Jimenez-Perez, 2001; Yen & Hsieh, 1995), metal chelating activity (Wijewickreme, Kitts, & Durance, 1997), scavenging of active oxygen species (Yoshimura, Iijima, Watanabe, & Nakazawa, 1997), as well as decomposition of hydroperoxide (Tanaka, Chiu, Nagashima, & Taguchi, 1992). MRPs can be used to prevent lipid oxidation in food systems. Chiu, Tanaka, Nagashima, and Taguchi (1991) reported that MRPs prepared from fructose-tryptophan effectively inhibited the oxidation of sardine lipid.

During the development of brown color caused by the Maillard reaction, caramelisation can occur simultaneously (Buera, Chirife, Resnik, & Wetzler, 1987a). Caramelisation reactions contribute to overall nonenzymatic browning, especially in the alkaline pH ranges, leading to an overestimation of the Maillard reaction in foods (Ajandouz & Puigserver, 1999; Ajandouz, Tchiakpe, DalleOre, Benajiba, & Puigserver, 2001). Acetone extracts from glucose caramelisation have been reported to prevent the oxidation of soybean oil (Rhee & Kim, 1975). However, no information regarding the antioxidant potency of caramelisation products (CPs) from different single sugars has been reported.

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Lipid oxidation of seafoods can occur easily due to the presence of polyunsaturated fatty acids (Harris & Tall, 1994). This reaction can be initiated and accelerated by various mechanisms, including the production of singlet oxygen, enzymatic and non-enzymatic generation of free radicals and active oxygen (Kubow, 1992). To prevent the quality changes caused by lipid oxidation in foods, various synthetic antioxidants have been used. Owing to the increasing demand for food devoid of synthetic additives, much effort has been paid to the discovery of new natural antioxidants. Some plants extracts have been used successfully as natural antioxidants (Shahidi, 1997). However, fundamental studies on the antioxidative activity of CPs and their use to prevent lipid oxidation are lacking. The objectives of this study were to study the antioxidative activity and mode of action of CPs as well as to examine their effect on prevention of lipid oxidation in comminuted fish model systems.

2. Materials and methods

2.1. Chemicals

D-glucose, D-fructose, D-ribose, ferric chloride, potassium ferricyanide, thiobarbituric acid, ferric chloride and trichloroacetic acid were purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). 2,2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Sigma (St. Louis, Mo, USA). D-xylose and malonaldehyde were purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan).

2.2. Preparation of caramelisation products (CPs)

Solutions of D-glucose, D-fructose, D-ribose and D-xylose were prepared at a concentration of 0.05 M and at two different pH (7 and 10). Sugars were dissolved in 0.05 M sodium phosphate buffer, pH 7 and 0.05 sodium carbonate buffer, pH 10. 10 ml of each sugar solution was transferred to a screw-capped test tube and subjected to heating in a silicone oil bath at 100 °C for 0, 5, 10, 20, 30, 60, 90, 120 and 150 min. At the heating time designated, the samples were taken and cooled in iced water immediately. The solutions obtained were referred to as "caramelisation products or CPs". The samples were stored at 4 °C until used for analysis.

2.3. Physical and chemical analysis

2.3.1. UV-absorbance and browning determination

The formation of intermediate products was monitored by their absorbance at peak values. UV-absorbance was determined at wavelengths of 205 and 285 nm for the samples prepared at pH 7 and 270 and 285 nm for those prepared at pH 10 using a UV-160 spectrophotometer (Shimadzu, Kyoto, Japan). The browning intensity was measured at a wavelength of 420 nm. Prior to UV-absorbance determination, samples prepared at pH 7 and 10 were diluted to 5 and 50 times, respectively, using distilled water as a diluent. For browning measurements, a five-fold dilution was made for samples prepared at pH 10. The absorbance ratio (A_{205}/A_{420}, A_{270}/A_{420} and A_{285}/A_{420}) was also calculated to monitor the transformation of UV-absorbing compounds into brown polymers.

2.3.2. Determination of reducing sugars

Reducing sugars in CPs were measured according to the method of Nelson–Somogyi as described by Chaplin and Kennedy (1994). Fifty-fold dilution was made for all samples before analysis. Standard curves were prepared using the individual sugars. The changes in reducing sugar were expressed as the relative concentration (%) in comparison with the original content.

2.3.3. Determination of DPPH radical scavenging

DPPH radical scavenging activity was determined according to the method of Yen and Hsieh (1995) with a slight modification. CPs (400 μl) was added with 2 ml of DPPH solution (0.12 mM in 95% methanol). Prior to analysis, four-fold and twenty-fold dilution was made for samples prepared at pH 7 and 10, respectively, using distilled water as the diluent. The reaction mixture was mixed well and incubated at room temperature for 30 min. The absorbance of the resulting solutions were determined at 517 nm against a blank of distilled water. The radical scavenging activity was measured as a decrease in the absorbance of DPPH-sample mixture and calculated using the following equation

$$\text{Scavenging activity} = [1 - A_{\text{sample}}/\text{A}_{\text{blank}}] \times 100.$$

2.3.4. Determination of reducing power

Reducing power of CPs was measured as described by Oyaizu (1986) with a slight modification. 0.5 ml of each sample (two-fold and ten-fold dilution for CPs prepared at pH 7 and 10, respectively), was mixed with 0.5 ml of 0.2 M sodium phosphate buffer, pH 6.6 and 0.5 ml of potassium ferricyanide. The reaction mixture was incubated at 50 °C for 20 min and 0.5 ml of 10% (w/v) TCA was then added. Thereafter, 2 ml of distilled water and 400 μl of 0.1% (w/v) ferric chloride were added to the mixture (2 ml). The absorbance was measured at 700 nm using a UV-160 spectrophotometer. Any increase in absorbance at 700 nm indicated an increased reducing power.

2.3.5. Determination of chelating activity

The chelating activity was determined according to the method of Tang, Kerry, Sheehan, and Buckley

(2002) with slight modification. CPs (200 μl) were mixed with distilled water (800 μl). Then the mixture was mixed with 0.1 ml of 2 mM $FeCl_2$ and 0.2 ml of 5 mM ferrozine. The mixture was allowed to stand for 20 min and the absorbance was read at 562 using UV-160 spectrophotometer. Distilled water was used as a control. Chelating activity was calculated as follows

$$\text{Chelating activity (\%)} = [1 - A_{562} \text{ of sample}/A_{562} \text{ of control}] \times 100.$$

2.3.6. Preparation of comminuted fish model system

Comminuted fish model system was prepared according to the method of Kamil, Jeon, and Shahidi (2002) with a slight modification. 200–250 g of Pacific saury (*Cololabis saira*) was purchased from the local market in Tokyo. Upon arrival at the laboratory, fish were filleted and finely chopped. The mince was mixed with 20% distilled water (w/w) and used as the control. CPs prepared under conditions rendering the highest reducing power and DPPH radical scavenging activity were used as the antioxidant. The pH of CPs was adjusted to a neutral pH using 6 M HCl prior to adding into the fish mince. Neutralized CPs were added at the level of 10% and 20%. At 10% CPs level, 10% distilled water was added to obtain the total amount of 20% based on fish mince. After addition of neutralized CPs or distilled water, the mixtures (10 g) were placed in a Ziploc® plastic bag (Seisan Nippon, Tokyo, Japan) and kept at 4 °C for 0, 2, 4, 6 and 8 days. After the storage time designated, the samples were taken for thiobarbituric acid-reactive substances (TBARS) analysis.

2.3.7. Determination of thiobarbituric acid-reactive substances (TBARS)

TBARS in the samples were determined as described by Benjakul and Bauer (2001) with some modification. Comminuted fish model systems (1 g) were mixed with 5 ml of a solution containing 0.0375% thiobarbituric acid, 15% trichloroacetic acid and 0.25 N HCl. The mixture was heated in boiling water for 10 min, followed by cooling with the running water. The mixture was centrifuged at 4000*g* using a 05 PR-22 centrifuge (Hitachi, Tokyo, Japan) for 10 min. The supernatant was collected and absorbanace was read at 532 nm using a UV-160 spectrophotometer. TBARS was calculated from the standard curve of molonaldehyde and expressed as mg malonaldehyde/kg sample.

2.3.8. Statistical analysis

Data were subjected to analysis of variance. Mean differences were determined by the least significant difference multiple range test (Steel & Torrie, 1980). Analysis was performed using a SPSS package (SPSS 8.0 for windows, SPSS Inc, Chicago, IL). All of the experiments were performed in duplicate.

3. Results and discussion

3.1. The loss of sugar

The increased degradation of all sugars was observed as the heating time increased ($P < 0.05$) (Fig. 1). The rate of sugar degradation was much greater under alkaline pH conditions, compared with at neutral pH. At pH 7, a slight decrease in sugar was found during the first 60 min of heating. Thereafter, sugars, especially fructose and ribose, underwent more extensive degradation as evidenced by the marked decrease in reducing sugar content (Fig. 1(a)). Fructose and ribose decreased to 73.5% and 75.4% after heating time for 150 min. At pH 10, sharp degradation was observed in all sugars during the first 20 min of heating. Subsequently, sugars underwent degradation gradually up to 150 min. Among all of the sugars tested, glucose was degraded to a smaller extent, compared to the other. Higher levels of degradation of both fructose and glucose occurred at 100 °C under alkaline conditions (Ajandouz & Puigserver, 1999; Ajandouz et al., 2001; Yang & Montgomery, 1996). After 150 min, glucose, fructose, ribose and xylose had degraded to 46.9%, 34.9%, 23.4% and

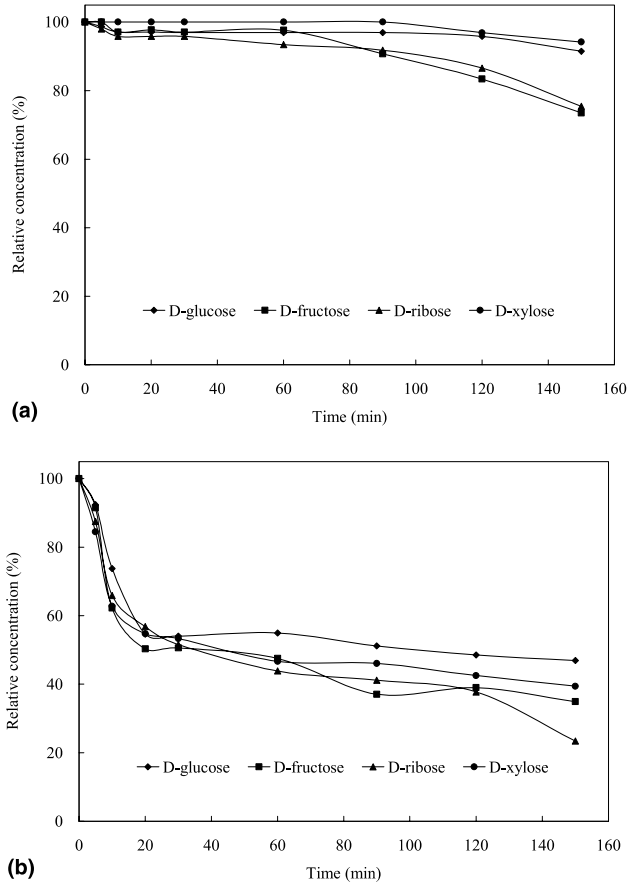

Fig. 1. Changes in reducing sugar content of CPs from different sugars during heating at pH 7 (a) and pH 10 (b) for various times.

39.7%, respectively. From these results, it was determined that the rate of degradation was dependent upon pH and the type of sugar involved.

3.2. UV-absorbance and browning of CPs

UV-absorbance of CPs prepared from different sugars at pH 7 and 10 during heating is depicted in Fig. 2. Regardless of sugar type, UV-absorbance of CPs prepared under alkaline conditions increased to a higher extent, compared to those prepared at neutral pH. UV-absorbance was used to monitor the intermediate degradation products of nonenzymatic browning reactions (Ajandouz et al., 2001; Lerici, Barbanti, Manzano, & Cherubin, 1990). Under neutral conditions, UV-absorbance at both 205 and 285 nm of all sugars increased gradually as the heating time increased, and it was found that ribose showed higher UV-absorbance than the other sugars. The increase in absorbance at 205 nm due to CPs from xylose was higher than those from fructose. Conversely, CPs from xylose showed lower absorbance at 285 nm than those from fructose. The results suggested that different intermediate products with different absorbance maxima were formed for different sugars. CPs from glucose had the lowest increase in absorbance at both 205 and 285 nm. Different patterns of changes in the UV-absorbance of CPs prepared under alkaline conditions were noted, compared to those found under neutral conditions. Sharp increases in UV-absorbance at both 270 and 285 nm were found in all sugars within the first 60 min of heating. Thereafter, no marked changes in UV-absorbance at 270 and 285 nm were observed in ribose and xylose up to 150 min of heating. Continuous increases in absorbance at 270 nm were observed in glucose and fructose heated up to 150 min, although no changes in absorbance at 285 were observed. From these results, the accumulation of intermediate degradation products was generally coincidental with the increase in sugar degradation (Fig. 1). Fructose showed the highest rate of degradation with the concomitant accumulation of intermediate products.

Browning of CPs from different sugars prepared at pHs 7 and 10 as monitored by the increase in absorbance at 420 nm is shown in Fig. 3. The final stage of the browning reaction was monitored by the increase in absorbance at 420 nm (Ajandouz et al., 2001). Increase in browning was generally observed as the heating time increased ($P < 0.05$). Regardless of sugar type, browning reactions occurred to a greater extent at alkaline pH, compared with at neutral pH. Browning at pH 7 increased continuously with increasing heating time, whereas browning occurred sharply within the first 20–

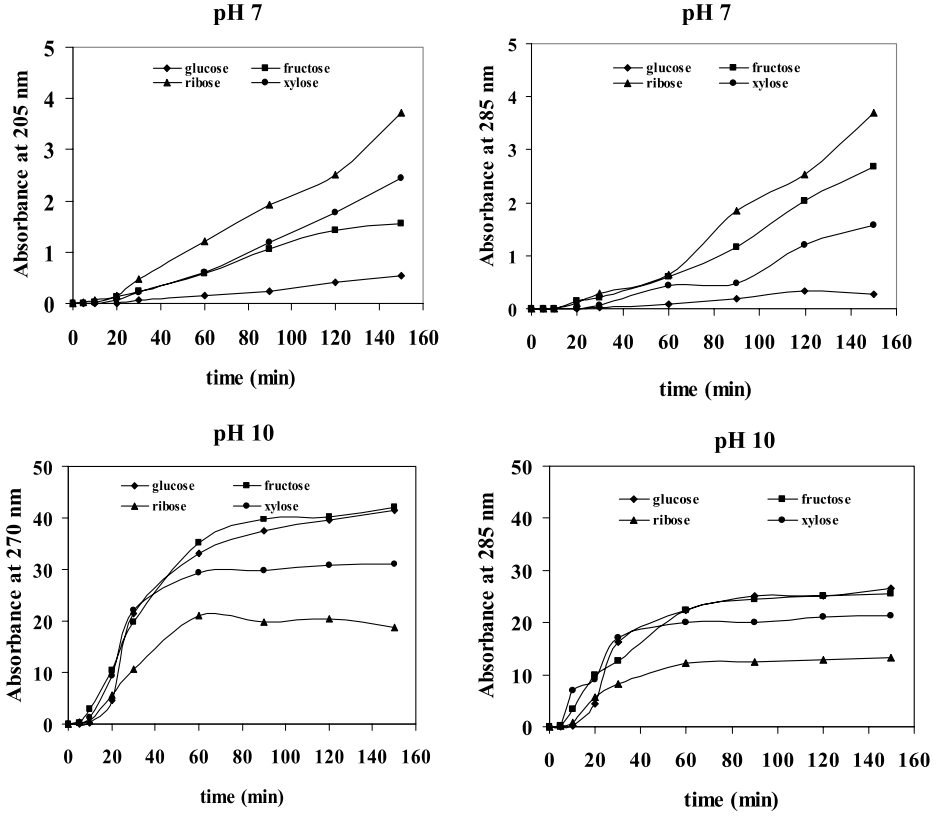

Fig. 2. Changes in UV-absorbing compounds in CPs from different sugars during heating for various times. Development of intermediate products was indicated by the increase in A_{205}, A_{270} and A_{285}.

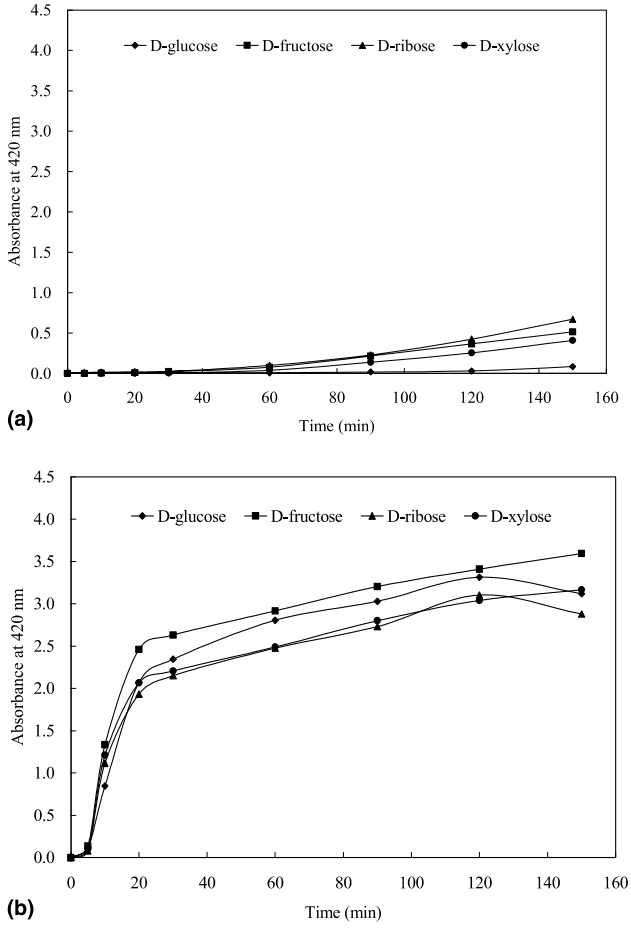

Fig. 3. Changes in browning of CPs from different sugars during heating at pH 7 (a) and pH 10 (b) for various times. Browning development was indicated by the increase in A_{420}.

30 min at pH 10. Subsequently, the browning was increased at a slower rate. The result was in agreement with Ajandouz et al. (2001) who reported that browning development of fructose was mainly observed in alkaline pH ranges. At pH 7, glucose was more reactive to caramelisation than the other sugars as indicated by the development of browning. However, fructose was more likely to undergo browning via caramelisation at pH 10. The differences in browning found among all sugars tested might be related to their different relative structural stability, including mutarotation, opening of the hemiacetal ring and enolization of the sugar (Buera, Chirife, Resnik, & Lozano, 1987b). Browning development is influenced by the type of sugar and pH and the rate of color development decreased as the pH decreased. Buera et al. (1987b) reported that rates of browning development of reducing sugars via caramelisation processes were in the descending order: fructose > xylose > lactose > maltose > glucose. A similar relationship between the increase in UV-absorbance and browning (absorbance at 420 nm) suggested that a large proportion of the intermediate product was converted to a brown polymer (Ajandouz et al., 2001). Thermolysis causes dehydration of sugar molecules with the introduction of double bonds or formation of anhydro rings. Introduction of double bonds leads to unsaturated rings and conjugated double bonds absorb light and produce color. Unsaturated rings will condense to polymers leading to the development of color (BeWiller & Whistler, 1996).

The absorbance ratios of sugar solutions heated for different times at pH 7 and 10 is shown in Tables 1 and 2. Under neutral conditions, pH 7, both absorbance ratios (A_{205}/A_{420} and A_{285}/A_{420}) for ribose, xylose and fructose solutions increased within the first 10 min of heating with the development of browning (Table 1). Conversely, no browning was formed with glucose solutions until 30 min of heating. The ratios for fructose reached a maximum after 20 min of heating, whereas glucose and ribose solutions gave maximum ratios after 60 and 30 min of heating, respectively. For xylose, the highest A_{205}/A_{420} and A_{285}/A_{420} ratios were observed after 30 and 60 min, respectively. The increased absorbance ratios suggested that the intermediates were generated to a larger extent with lower transformation to brown polymers. The subsequent decrease in absorbance ratios suggested the formation of brown polymers from the intermediate. From the result, it was suggested that the formation of brown polymers from different intermediates varied with the type of sugar.

Table 1
Changes in the absorbance ratio of CPs from different sugars during heating at pH 7 for various times

Time (min)	D-glucose		D-fructose		D-ribose		D-xylose	
	A_{205}/A_{420}	A_{285}/A_{420}	A_{205}/A_{420}	A_{285}/A_{420}	A_{205}/A_{420}	A_{285}/A_{420}	A_{205}/A_{420}	A_{285}/A_{420}
5	NC	NC	NC	NC	0.89	NC	1.20	1.00
10	NC	NC	6.58	0.20	4.26	0.62	2.47	0.82
20	NC	NC	12.1	14.9	6.93	5.37	11.6	1.25
30	16.2	5.12	11.0	8.97	20.3	12.4	24.5	7.27
60	17.9	10.6	7.82	8.03	12.0	6.38	15.6	11.4
90	12.1	9.76	4.88	5.38	8.36	8.03	8.54	3.48
120	13.0	10.1	3.92	5.57	5.92	5.95	6.96	4.73
150	6.21	3.04	3.03	5.20	5.56	5.50	5.97	3.89

NC: Not calculated due to no browning detected.

Table 2
Changes in the absorbance ratio of CPs from different sugars during heating at pH 10 for various times

Time (min)	D-glucose		D-fructose		D-ribose		D-xylose	
	A_{270}/A_{420}	A_{285}/A_{420}	A_{270}/A_{420}	A_{285}/A_{420}	A_{270}/A_{420}	A_{285}/A_{420}	A_{270}/A_{420}	A_{285}/A_{420}
5	0.37	0.27	1.57	1.67	4.43	3.95	2.39	1.92
10	0.32	0.30	2.22	2.52	0.64	0.83	1.02	5.72
20	2.18	2.11	4.26	4.07	2.89	2.93	4.56	4.40
30	9.14	6.97	7.54	4.82	4.98	3.81	10.0	7.74
60	11.8	7.96	12.1	7.69	8.47	4.91	11.8	8.07
90	12.4	8.27	12.4	7.62	7.24	4.59	10.6	7.13
120	11.9	7.56	11.8	7.35	6.55	4.12	10.1	6.93
150	13.3	8.54	11.7	7.12	6.52	4.63	9.80	6.76

NC: Not calculated due to no browning detected.

At pH 10, fructose, ribose and xylose solutions showed a marked increase in the A_{270}/A_{420} and A_{285}/A_{420} ratios within the first 5 min of heating (Table 2). However, slight increase in both ratios were observed for glucose. Further heating resulted in a continuous increase in the ratios, suggesting more extensive formation of intermediates, compared to the development of brown polymers. The highest ratios of glucose and fructose solutions were observed with the heating time of 90 min, whereas ribose and xylose had the maximum ratios when heated for 60 min. The subsequent decrease in ratio might indicate the transformation of intermediates into polymers. The slightly higher ratio for CPs prepared under neutral pH condition indicated that intermediates were generated to a larger extent, compared to final product formation. This was coincidental with the greater browning intensity of CPs prepared under alkaline condition, compared with CPs prepared under neutral condition (Fig. 3). It is suggested that the caramelisation occurred to a larger extent under alkaline conditions and that it depended upon type of sugar. Generally, hexose sugars, both glucose and fructose, were more prone to caramelisation than pentose, ribose and xylose as evidenced by the greater browning as well as intermediate formation.

3.3. Reducing power of CPs

Reducing power of CPs from different sugars prepared by heating at pH 7 and 10 for different times is depicted in Fig. 4. Under neutral conditions, the reducing power of CPs, as indicated by the increase in absorbance at 700 nm, increased linearly as the heating time increased ($P < 0.05$). Fructose CPs showed the highest reducing power, compared with CPs from other sugars. For CPs prepared under alkaline conditions, reducing power increased exponentially with increasing heating time. A sharp increase in reducing power was observed when heating was conducted for up to 20 min. Heating for a longer time did not result in increased reducing power. Generally, CPs from fructose exhibited the highest reducing power and CPs from glucose

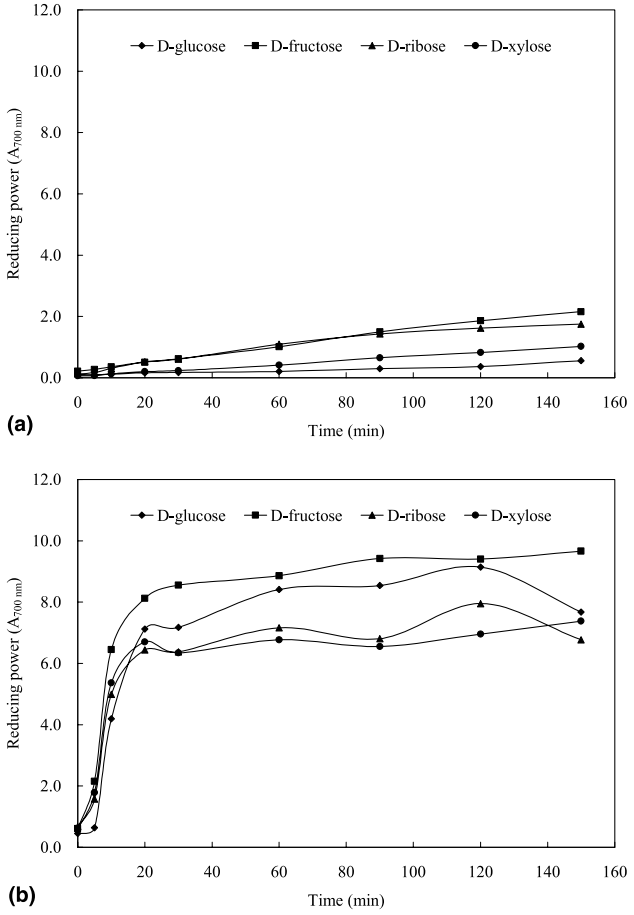

Fig. 4. Changes in reducing power of CPs from different sugars during heating at pH 7 (a) and pH 10 (b) for various times. Reducing power was indicated by the increase in A_{700}.

showed higher reducing power than those from ribose and xylose. Thus, it can be concluded that CPs from hexose sugars rendered the greater reducing power, compared with CPs from pentose. During heating of sugar solutions, especially under alkaline conditions, reducing compounds might be formed and these could exhibit antioxidative activity. Antioxidative activity of Maillard reaction products was associated with reducing

power (Yen & Hsieh, 1995). The reducing power of CPs might be due to hydrogen-donating ability (Shimada, Fujikawa, Yahaa, & Nakamura, 1992).

3.4. DPPH radical scavenging activity

DPPH radical scavenging activity of CPs from sugars prepared by heating under neutral and alkaline condition is shown in Fig. 5. DPPH radical scavenging activity of CPs prepared under neutral conditions increased linearly as the heating time increased. Among CPs from all sugars tested, those from ribose showed the highest activity (Fig. 5(a)). CPs from glucose were found to exhibit to lowest activity, compared to CPs from other sugars. For CPs prepared under alkaline conditions, an exponential increase in DPPH radical scavenging activity was observed with increasing heating time (Fig. 5(b)). CPs from fructose exerted greater DPPH radical scavenging activity compared to CPs from other sugars. DPPH radical scavenging activity was in the descending order: fructose > glucose > ribose > xylose. From the result, it was noted that DPPH radical scavenging activity of CPs prepared under alkaline conditions was approximately five-fold greater than that of CPs prepared under neutral conditions. The higher radical scavenging activity of CPs prepared at pH 10 was coincidental with the higher reducing power, browning and intermediate formation. DPPH is one of compounds that possesses a proton free radical with a characteristic absorption, which decreases significantly on the exposure to proton radical scavengers (Yamaguchi, Takamura, Matoba, & Terao, 1998). It was found that CPs were able to reduce the DPPH radical to the yellow-coloured diphenylpicrylhydrazine. The reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant is due to the formation of the non-radical form, DPPH-H (Shon, Kim, & Sung, 2003). Thus it was suggested that either intermediates or the final brown polymer could function as hydrogen donors. Kirigaya, Kato, and Fujimaki (1968) found that antioxidant activity increased with increasing color intensity. However, Rhee and Kim (1975) reported that effective antioxidant compounds were formed at an earlier stage of browning reactions. Therefore, CPs, especially those from caramelisation under alkaline conditions, exhibited antioxidant activity. Furthermore, the possible involvement of CPs in concert with MRPs as the antioxidant in high-temperature cooked foods, such as grilled or roasted products, can be proposed.

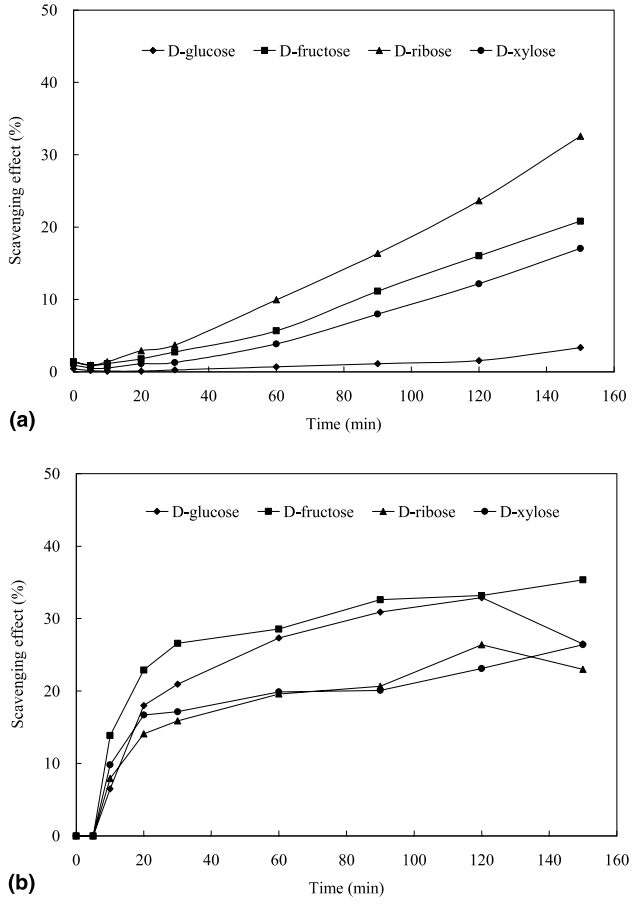

Fig. 5. Changes in DPPH radical scavenging activity of CPs from different sugars during heating at pH 7 (a) and pH 10 (b) for various times. Four-fold and twenty-fold dilution was made for CPs prepared at pH 7 and 10, respectively, prior to analysis.

3.5. Ferrous ion chelating activity

Ferrous ion chelating activity of CPs from fructose is shown in Table 3. The chelating activity increased as the amount of CPs increased. The Fe^{2+} ion is the most powerful prooxidant among various species of metal ions (Yomauchi, Tatsumi, Asano, Kato, & Ueno, 1988). Ferrous iron can interact with hydrogen peroxide in a Fenton reaction to produce the hydroxyl free radical, which can initiate lipid oxidation (Hultin, 1994). Therefore, CPs might retard lipid oxidation by chelating ferrous ions.

3.6. Effect of CPs addition on lipid oxidation of saury mince model system

Lipid oxidation of comminuted saury during iced storage was monitored as shown in Fig. 6. TBARS

Table 3
Chelating activity of fructose CPs for ferrous ions

Amount of CPs (μl)	Chelating activity (%)[a]
5	5.80 ± 0.76
10	19.7 ± 2.00
15	36.1 ± 1.65
20	47.2 ± 1.71
25	56.3 ± 0.77

[a] Mean ± SD from triplicate determinations.

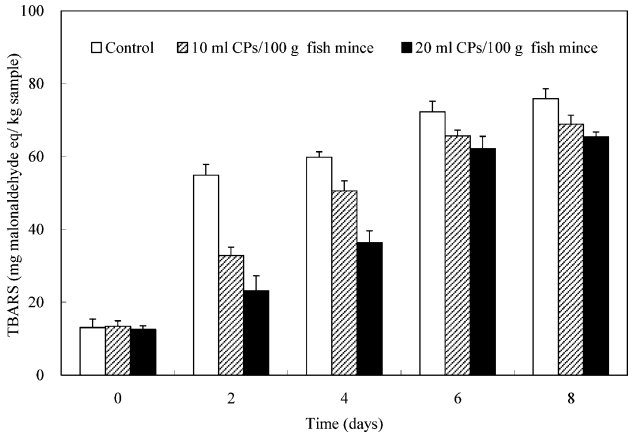

Fig. 6. Changes in TBARS values of comminuted saury model system without and with 10% or 20% fructose CPs addition during iced storage. Bars indicate the standard deviation from six determinations.

values of the control increased sharply after 2 days of storage. TBARS values of the samples that included 10% and 20% CPs were 40.1% and 58.0% lower than that of control, respectively. A slower rate of increase in TBARS values was found with increasing CPs. After 8 days of storage, TBARS values of samples with 20% CPs were slightly lower than both the control and that of sample with 10% CPs. Lipid oxidation in comminuted fish meat can be initiated and promoted by a number of mechanisms including autoxidation, photosensitized oxidation, lipoxygenase, peroxidase and microsomal enzymes (Decker & Hultin, 1990; German & Kinsella, 1985). Pacific saury used in this study is a fatty dark-fleshed fish, which is prone to lipid oxidation. Lipid deterioration in fatty fish is due to the progressive oxidation and enzymatic hydrolysis of unsaturated fatty acids in fish (Srikar & Hiremath, 1972). Several sources of protein-bound iron including myoglobin, haemoglobin, ferritin and transferrin exist in fish tissue, particularly dark-fleshed fish. This iron can be released from the complex during storage and processing, leading to the acceleration of lipid oxidation (St. Angelo, 1996). Also, the damage of tissue membranes during the mincing process can induce lipid oxidation (Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998). Membrane phospholipids, which are high in polyunsaturated fatty acid, are responsible for the initial development of oxidation in meat during storage (Gray & Pearson, 1987). CPs might retard lipid oxidation by scavenging free radicals as evidenced by the DPPH radical scavenging activity (Fig. 5). Also, CPs were good hydrogen or electron donors as indicated by their high reducing power (Fig. 4). In lipid peroxidation, free radicals abstract a hydrogen from a fatty acid double bond to produce fatty acid free radicals, which further react with oxygen to produce fatty acid hydroperoxide. The hydroperoxide is unstable and decomposes readily to shorter chain hydrocarbons such as aldehydes, etc. Those final products can be determined as TBARS. In the presence of CPs, the propagation step might be inhibited, leading to lower oxidation in the samples with CPs. Owing to chelating property of the ferrous ions, CPs might retard the lipid oxidation by eliminating their prooxidant activity. It was likely that higher concentration of CPs prevented oxidation in saury mince more effectively via greater iron chelating potential.

4. Conclusion

CPs from sugar subjected to heating at 100 °C showed antioxidative activities that depended upon type of sugar and pH. CPs from fructose prepared under alkaline conditions exhibited the highest radical scavenging activity and reducing power. Fructose CPs also exhibited ferrous ion chelating properties. CPs from fructose could inhibit lipid oxidation in fish mince effectively during extended iced storage.

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