Type I caramel products of maltose and sucrose with water and their antioxidant activities

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Abstract

To study the caramel reactions of disaccharides with water, the type I maltose caramel products (MCPs) and sucrose caramel products (SCPs) at three reaction stages were yielded and their reaction rates, UV absorptions, flavor compounds, and antioxidant activities were also detected. It was found that the caramel reactions of sucrose were faster than those of maltose and their reaction rates quickly increased to a high level in the middle stage. UV absorption of SCP in the late stage at 305 nm was much stronger than those of MCPs. Both the MCP and SCP obtained at 180 °C and 4 min had the highest concentrations of 5-hydroxymethylfurfural and total flavor compounds. Moreover, the total flavor concentrations of SCPs were much larger than those of MCPs and the antioxidant effects of SCPs were also stronger. Thus, a preferred condition for both two disaccharide caramel reactions was suggested at 180 °C for 4 min and the sucrose caramels could give better flavors than maltose caramels.

Keywords: caramel reactions; maltose; sucrose; flavor compounds; antioxidant activities.

Practical Application: Caramel manufacture, quality control, and caramel flavors.

1 Introduction

Caramel colors, one of the most widely used dietary additives, have been classified as four distinct types by their processing (Chappel & Howell, 1992; Golon & Kuhnert, 2012). With the participation of ammonia and ammonium salts, type III and type IV caramels can be manufactured through both Maillard and caramel reactions. However, some toxic and cancerogenic substances such as 4(5)-tetrahydroxybutylimidazole (THI) and 2-acetyl-4-methylimidazole (4-MEI) could also be yielded, which led to the limitation of their food use levels (Cunha et al., 2011; Klejdus et al., 2006; Moon & Shibamoto, 2011). Type II caramels are only allowed for several special medicines and foods (Chappel & Howell, 1992). In contrast, the plain caramels (type I) are more safe and promising.

The plain caramels could be yielded using sugars with water or sugars alone. Although the colors of these caramels are slightly weaker than other types, their flavors are more comfortable and natural. Several perfumed compounds like pyranones, furans, and furanones have been reported through heating waterless glucose and fructose (Geng et al., 2019; Golon & Kuhnert, 2013; Pons et al., 1991). Under sugar-water system, the reactions could be controlled easily and accurately. It has been found that disaccharide caramels could give more rich flavors but their characteristic aromatic compounds under sugar-water system are not known clearly enough (Chappel & Howell, 1992; Cunha et al., 2011). Moreover, the qualities and applications of caramels can be associated with their flavor compounds and biological activities. In this study, two kinds of disaccharides (maltose and sucrose) were chosen. Their caramel

reactions with water were performed and the preferred processing conditions were established. The reaction rates, UV absorptions, and characteristic flavors of maltose caramel products (MCPs) and sucrose caramel products (SCPs) were confirmed, as well as the antioxidant effects.

2 Materials and methods

2.1 General experimental procedures

Maltose (99%), sucrose (99%), and trolox were obtained from Aladdin Reagent Co., Ltd (Shanghai, China). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), and 5-hydroxymethylfurfural were purchased from Macklin Reagent (Shanghai, China). A DF-101S magnetic stirrer (Yuhua Co., Ltd, Zhengzhou, China) was used for the caramel reactions. UV and optical density (OD) data were acquired on UV-2450 equipment (Shimadzu, Tokyo, Japan) and iMark microplate reader (BIO-RAD, Contra Costa, USA), respectively. HPLC-ELSD analysis was carried on an Agilent 1290 HPLC equipped with a 385 ELSD detector (Agilent, Santa Clara, USA). GC-MS determination was carried out on an Agilent 8890GC/5977MSD (Agilent, Santa Clara, USA).

2.2 Heating procedure

In two 1000 mL of round-bottomed flasks, maltose (200 g) and sucrose (200 g) were transferred respectively. Then each of the flask was added with purified water (100 g) and heated in an oil bath of 187 °C with a stirring speed of 1000 rpm (Pons et al.,

1991). When the reacting temperature reached 140 (**a**), 170 (**b**), and 180 °C (**c**), each of the MCPs and SCPs (25 g) were transferred at once. Caramel products **d** were obtained that the remaining mixtures were kept at 180 °C for 4 min. After being added with purified water (50% weight), the residual caramels (**d**) were heated at 180 °C for another 2 min to give products **e**. All the caramel products were dissolved in 10% alcohol as the stock solutions (1.0 g/mL).

2.3 Reaction rate analysis

Maltose and sucrose solutions (10% alcohol) were prepared in concentrations of 5.0, 2.0, 1.0, 0.5, and 0.1 mg/mL, which were determined by HPLC-ELSD analysis. Then the standard curves were demonstrated. The MCP and SCP solutions were diluted with 10% alcohol to the concentration of 5.0 mg/mL and determined as above. Then the concentrations of maltose and sucrose in the MCP and SCP solutions and the reaction rates were calculated according to the standard curves and peak areas.

HPLC-ELSD analysis was carried out with an Agilent Pursuit 5 PFP column (250 mm × 4.6 mm). The mobile phase was 5% MeOH with 0.1% HCOOH with a flow rate of 1 mL/min and the injection volume was 5.0 μL. For the ELSD detector, the gas carrier was N_2 with a flow rate of 1.6 L/min, and the temperature of evaporator was set at 45 °C.

2.4 UV and GC-MS analysis

The UV data from 200 to 550 nm were collected using the water dilute solution of MCPs and SCPs (2.0 mg/mL).

In ten round-bottom flasks (100 mL), each of MCP and SCP solutions (12 mL, 1.0 g/mL) was transferred and extracted using ultrasonic treatment with CH_2Cl_2 (10 mL) for 2 min. After being heated at 60 °C for another 20 min under reflux, the CH_2Cl_2 layers were pipetted out to another bottle. The remaining solutions were extracted with CH_2Cl_2 in the same manner as aforementioned. Finally, the two CH_2Cl_2 layers were concentrated to 0.9 mL in

the fume hood and the internal standard 2-phenethyl propionate (0.1 mL, 1.78 mg/mL) was added.

GC-MS analysis was performed on an Agilent 8890GC/5977MSD with a HP-5 MS column (60 m × 0.25 mm). The injection volume was 1.0 μL. The gas carrier was helium with flow rate of 1.0 mL/min and column temperature was set as below: 50 °C, hold for 2 min; 50 °C to 120 °C, 6 °C/min; 120 °C to 240 °C, 12 °C/min, hold for 5 min; 240 °C to 280 °C, 15 °C/min, hold for 3 min.

2.5 Antioxidant assays

Through ABTS and DPPH assays, the radical scavenging effects of MCPs and SCPs were tested (Li et al., 2020; Thomas and Bielski, 1989). Briefly, the ABTS solution and potassium persulfate solution were mixed equally and put in the dark for 18 h. Using 50% ethyl alcohol, the mixture was diluted to 0.70 ± 0.05 ($A_{734 nm}$) and caramel product solutions (50.0-0.5 mg/mL) were also prepared. Then in the 96-well plates, each caramel product solution (20 μL) was pipetted into 180 μL of ABTS solutions. Finally, the OD values at 734 nm were measured after reacting avoiding light for 6 minutes. For the DPPH assays, 100 μL of DPPH solutions (0.25 mM) and equal volume of caramel product solutions were added. After reacting for 30 minutes, the OD values ($A_{517 nm}$) were recorded. All the measurements were carried out in triplicate. The scavenging rate was calculated as following: scavenging rate (%) = $(1 - A_{test}/A_{control}) \times 100$.

3 Results and discussion

3.1 The reaction rates of MCPs and SCPs

Maltose and sucrose solutions were prepared with 10% alcohol to concentrations of 5.0, 2.0, 1.0, 0.5, and 0.1 mg/mL, which were further analyzed by HPLC-ELSD (Table 1). The standard curves were established by the peak areas and the concentrations (Figure 1). Then the MCP and SCP solutions were diluted with 10% alcohol to the concentration of 5.0 mg/mL and analyzed in the same manner (Figure 2). The reaction rates were calculated through the peak areas and the standard curves (Table 2).

Based on the reaction phenomena and the reaction rates, three reaction stages could be revealed, namely the early stage, the middle stage, and the late stage. From the reaction beginning to the arriving of 170 °C (**b**) could be supposed as the early stage. It is interesting to note that the caramel reaction of sucrose was faster than those of maltose since the reaction rate of SCP **b** (61.3%) was larger than those of maltose (22.8%). The middle stage was defined as from arriving of 170 °C (**b**) to 180 °C 4 min (**d**) and the reaction rates quickly increased to 87.0% and 92.3%

Table 1. The HPLC-ELSD peak areas of different maltose and sucrose solutions.

concentrations (mg/mL)	maltose	sucrose
0.1	112.6	123.5
0.5	515.4	679.6
1.0	1387.5	1501.2
2.0	2281.2	2754.8
5.0	5683.0	6504.3

Table 2. The reaction rates of different MCPs and SCPs.

products	maltose			sucrose		
	peak area	concentration	reaction rate (%)	peak area	concentration	reaction rate (%)
a	5249.0	4.60	8.0	5328.4	4.05	18.9
b	4411.6	3.86	22.8	2588.8	1.93	61.3
c	2117.4	1.83	63.4	1281.9	0.92	81.5
d	789.8	0.65	87.0	585.6	0.38	92.3
e	225.2	0.15	97.0	117.6	0.02	99.6

for maltose and sucrose, respectively. In this stage, the sugars were reacting drastically to become dilated foams, which were finally broken and cooled down at around 4 min (180 °C). The following **d** to **e** were regarded as the late stage and the reaction rates were in the stable levels.

3.2 UV absorptions of MCPs and SCPs

Along with the reaction, it was found that the UV absorptions from 200 to 550 nm were significantly enhanced and two peaks (305 and 290 nm) were discovered (Figure 3) (Uríčková & Sádecká,

2015). In the early stage, the UV absorptions at 290 nm and 305 nm were slightly increased. In the middle stage, $UV_{290\,nm}$ was quickly enhanced to the stable level and $UV_{305\,nm}$ also obviously increased. It has been known that the sugars were first polymerized before decomposition into flavor compounds in the caramel reactions and the UV absorptions at 290 nm could be associated with the polymerized sugars (Chappel & Howell, 1992; Golon & Kuhnert, 2013). As mentioned above, the sucrose could react faster than maltose since the UV absorptions at 290 nm of SCP **b** was larger than those of MCP **b** (Figure 3). In the late stage, it was found that sucrose caramel exhibited a much darker color
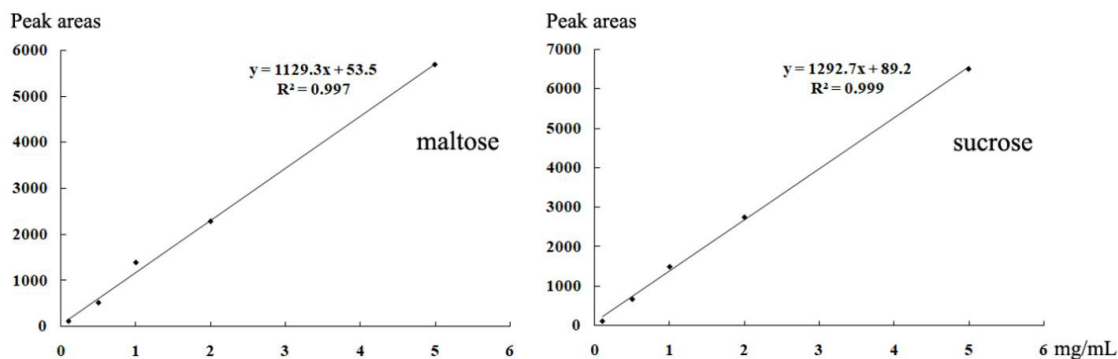

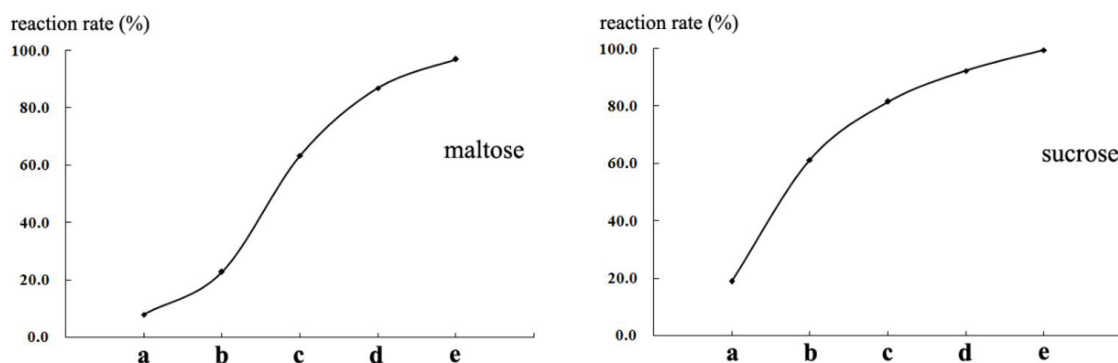**Figure 1**. HPLC-ELSD standard curves of maltose and sucrose.

Figure 2. The reaction rate curves of MCPs and SCPs. (**a**) 140 °C, 0 min; (**b**) 170 °C, 0 min; (**c**) 180 °C, 0 min; (**d**) 180 °C, 4 min; (**e**) 180 °C, 4 min, 50% water supplement, reacted at 180 °C for another 2 min. The same below.
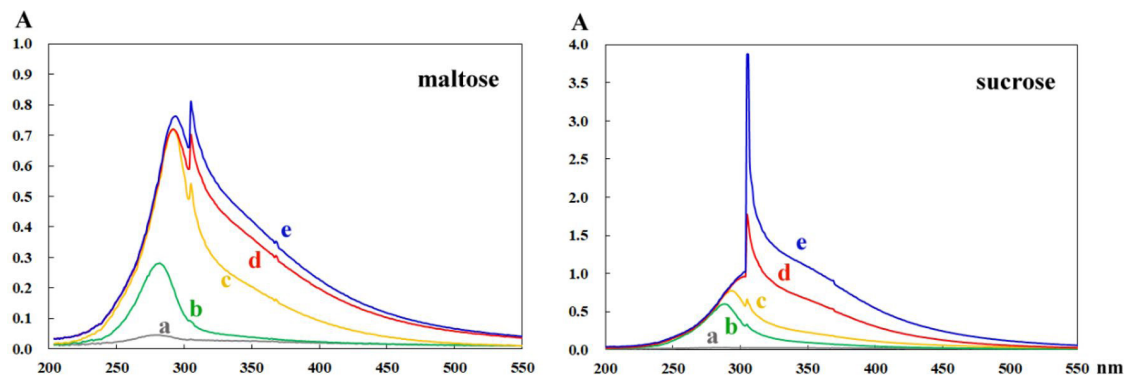

Figure 3. UV absorptions of MCPs and SCPs in water (A, absorbance).

than maltose caramel since the UV absorption at 305 nm of SCP **e** was much larger than those of MCP **e** (Figure 4).

3.3 Flavor compounds of MCPs and SCPs

Careful analysis of the GC-MS data of MCPs and SCPs revealed 18 flavor compounds that can be distinguished as three classes (Table 3 and Figure 5) (Golon & Kuhnert, 2013; Pons et al., 1991). 4-MEI and THI were not detected. Class I was the flavor compounds that found in both MCPs and SCPs, namely the compounds **1**-**7**. Among them, 5-hydroxymethylfurfural (5-HMF, **1**), methyl-2-furoate (**2**), furfural (**3**), 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one (DDMP, **4**), and 1-(2-furanyl)-ethanone (**5**) could be recognized as the main flavor compounds since they were found in both middle and late stage products. In addition, compounds **8**-**11** (class II) were the flavors that only yielded in MCPs and the remaining **12**-**18** (class III) were only observed in SCPs.

Among MCPs, it is interestingly to note that product **d** was disclosed to have highest concentration values of 5-HMF and total flavor compounds (Table 3 and Figure 6). For SCPs, the same result could also be observed although the number of flavor compounds in **e** was larger than those of **d**. Thus, a proposed condition for both maltose and sucrose caramel reactions was established at 180 °C for 4 min. In this condition, the total flavor concentration of SCP **d** was eight fold more than those of MCP (Table 3), which suggested that sucrose caramels could give better flavors than maltose caramels.

3.4 Antioxidant effects

Along with the caramel reactions, the antioxidant effects of MCPs and SCPs were fast enhanced, which reached the stable levels at the late stages with IC_{50} values decreased to 0.93 and 0.44 mg/mL for MCP and 0.45 and 0.24 mg/mL for SCP, respectively (Table 4 and Figure 7). It has been reported that DDMP exhibited ABTS and DPPH radical scavenging effects due
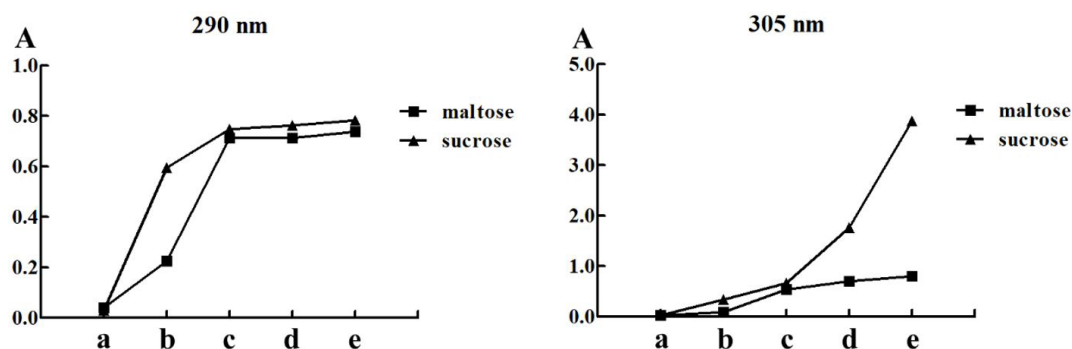

Figure 4. UV absorptions of MCPs and SCPs at 290 and 305 nm (A, absorbance).

Table 3. Concentrations of the flavor compounds in MCPs and SCPs (μg/mL).

No.	flavor compounds	maltose a	maltose b	maltose c	maltose d	maltose e	sucrose a	sucrose b	sucrose c	sucrose d	sucrose e
1	5-hydroxymethylfurfural	–*	48.21	67.47	86.78	41.11	–	142.46	398.74	673.45	512.60
2	methyl-2-furoate	–	0.54	0.71	1.22	1.14	–	2.33	10.57	27.96	33.61
3	furfural	–	–	1.62	0.93	0.71	–	7.97	35.19	41.33	50.01
4	2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one	–	0.54	1.58	1.82	0.74	–	2.90	4.83	12.36	14.66
5	1-(2-furanyl)-ethanone	–	0.28	0.56	1.27	1.79	–	–	0.69	0.72	0.75
6	5-methyl-2-furancarboxaldehyde	–	–	–	0.03	–	–	–	–	0.77	2.18
7	5-methyl-2(3*H*)-furanone	–	–	–	0.05	–	–	–	–	0.39	0.79
8	2-furanmethanol	–	–	0.09	0.44	0.59	–	–	–	–	–
9	3-methyl-2-furoic acid	–	–	0.11	0.04	–	–	–	–	–	–
10	2-acetyl-5-methylfuran	–	–	0.04	–	–	–	–	–	–	–
11	3,4-furandimethanol	–	–	–	0.03	–	–	–	–	–	–
12	5-methyl-2(5*H*)-furanone	–	–	–	–	–	–	–	–	1.28	2.59
13	2,4-dihydroxy-2,5-dimethyl-3(2*H*)-furan-3-one	–	–	–	–	–	–	–	–	1.96	1.42
14	2,5-furandicarboxaldehyde	–	–	–	–	–	–	–	–	1.54	1.97
15	2-furaldehyde diethyl acetal	–	–	–	–	–	–	–	–	0.72	2.92
16	5-hydroxy-2-methyl-4*H*-pyran-4-one	–	–	–	–	–	–	–	–	–	0.86
17	5-(2-furanylmethyl)-2-furancarboxaldehyde	–	–	–	–	–	–	–	–	–	3.55
18	5-[(5-methyl-2-furanyl)methyl]-2-furancarboxaldehyde	–	–	–	–	–	–	–	–	–	3.34
	total	–	49.57	72.18	92.61	46.08	–	155.66	450.02	762.48	631.25

*–, not detected.

to the olefinic alcohol group in its structure (Yoshiki & Okubo, 1995; Yu et al., 2013). Thus, the stronger antioxidant effects of SCPs were associated with their higher concentrations of DDMP than those of MCPs (Figure 6). Also, since the concentration of DDMP in MCP **e** was decreased, its radical scavenging effects were reduced (Figures 6 and 7).

Table 4. IC_{50} values of MCPs and SCPs in the antioxidant assays (mg/mL).

products	maltose		sucrose	
	DPPH	ABTS	DPPH	ABTS
a	16.95 ± 1.88	11.74 ± 0.93	13.62 ± 1.73	9.58 ± 0.92
b	6.28 ± 0.93	3.50 ± 0.23	4.43 ± 0.72	0.86 ± 0.14
c	2.20 ± 0.36	0.66 ± 0.10	2.26 ± 0.19	0.50 ± 0.08
d	0.84 ± 0.07	0.41 ± 0.07	0.47 ± 0.06	0.25 ± 0.08
e	0.93 ± 0.06	0.44 ± 0.09	0.45 ± 0.04	0.24 ± 0.07
5-HMF	> 2.00	> 2.00	> 2.00	> 2.00
Trolox*	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01

*Positive control. Maltose and sucrose were considered as inactive since its $IC_{50} > 50.0$ mg/mL. 5-HMF was inactive since its $IC_{50} > 2.0$ mg/mL. Data are expressed as mean of three measurements of triplicate tests ± standard deviation.
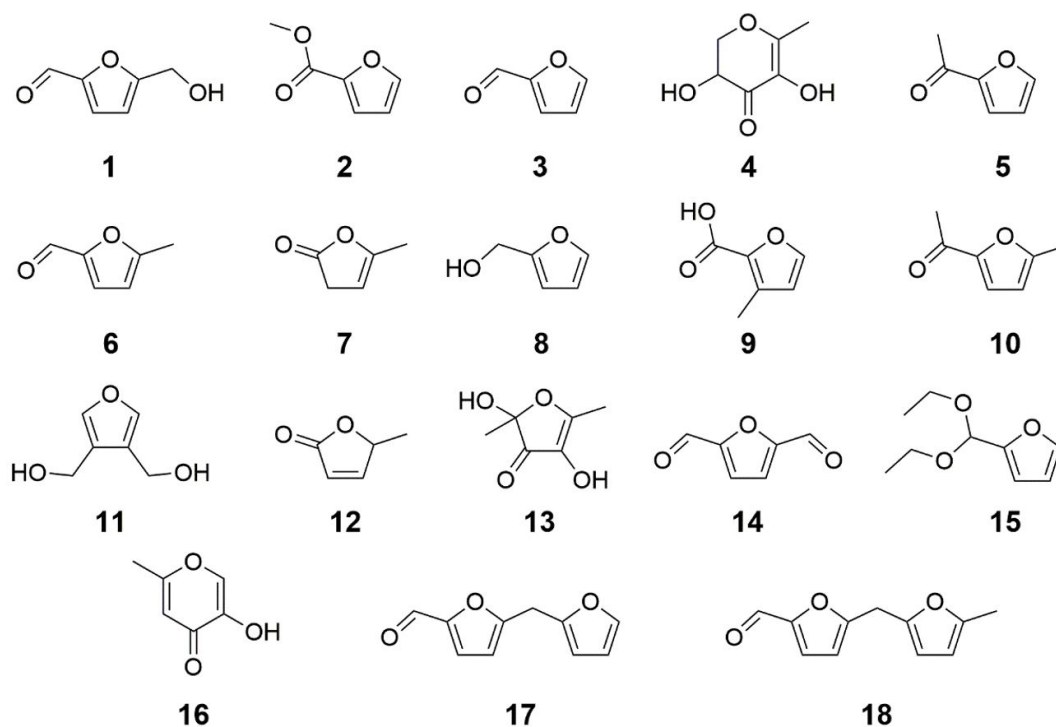

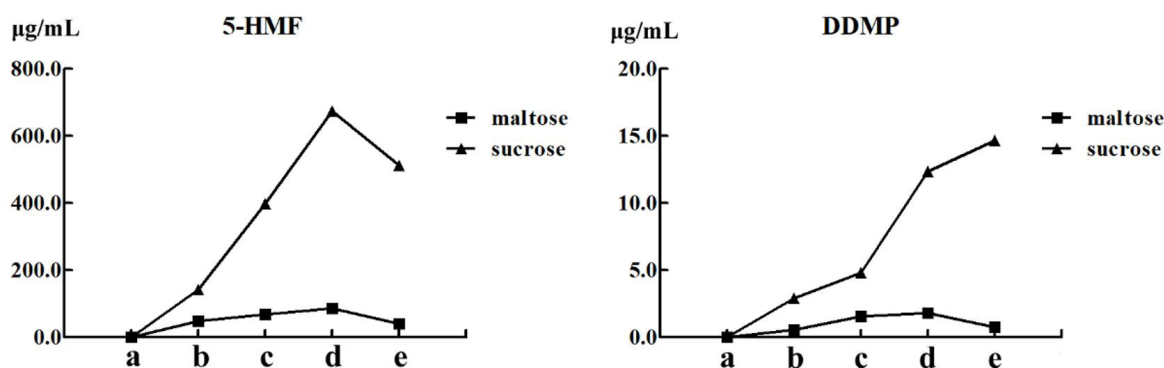**Figure 5**. Structures of flavor compounds identified by GC-MS.

Figure 6. Concentrations of 5-HMF and DDMP in MCPs and SCPs.
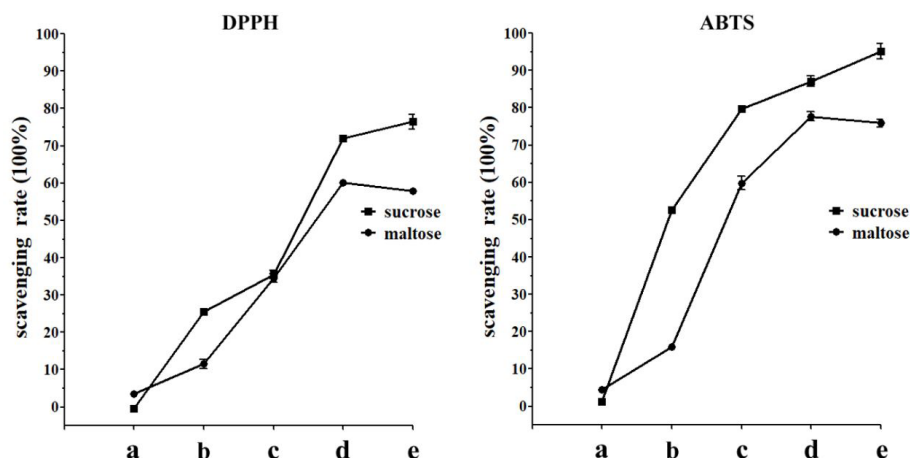
Figure 7. Radical scavenging effects of MCPs and SCPs at 1.0 mg/mL.

4 Conclusion

The results showed that a suggested condition for both maltose and sucrose caramel reactions was proposed at 180 °C for 4 min. And sucrose caramels exhibited better reaction rates, colors, flavors, and antioxidant effects than those of maltose caramels. Based on their characteristic flavors, antioxidant activities, and safeties, these caramels are promising in the application of food industry.

Acknowledgements

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