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Antioxidant Activity of Maltol, Kojic Acid, Levulinic Acid, Furfural, 5-Hydroxymethyl Furfural, and Pyrazine

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Maltol, Kojic Acid, Levulinic Acid, Furfural, 5-Hydroxymethyl Furfural과 Pyrazine의 항산화작용

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Abstract

An attempt was made to investigate the antioxidant activity of maltol, kojic acid, levulinic acid, furfural, 5-hydroxymethyl furfural (5-HMF), and pyrazine which had been known to be important intermediates of Maillard browning reactions. The activity of these compounds was determined by comparing induction periods of soybean oil substrates containing each compound at a 0.01M level with that of a control. The induction period was arbitrarily taken as the time in hours for a substrate to reach a peroxide value of 60 meq/*kg* oil. The substrates and control were stored at 45.0±1.0°C for 30 days. The induction periods of the control, kojic acid, furfural, 5-HMF, maltol, levulinic acid, and pyrazine were respectively 468, 592, 510, 498, 486, 450, and 402 hours. Kojic acid demonstrated considerable antioxidant activity, whereas furfural, 5-HMF, and maltol showed weak activivity. Pyrazine and levulinic acid showed prooxidant activity. Although the prooxidant activity of pyrazine seemed definite, that of levulinic acid appeared very weak.

Introduction

The antioxidant activity of non-enzymatic browning reaction products for fats and oils and food products containing fats and oils has been reported by many research workers. For example, Lips[1] reported the stabilizing effect of heat treatment on

the lard which had contained a small amount of carbohydrates and nitrogenous compounds as impurities. It was suggested that antioxidants were formed by heat possibly through the interaction of the carbohydrates and nitrcgecus ccmpounds. Baker and Hellmann[2] reported that antioxidants were also formed during the heat treatment of malt and malt sprouts through the interaction of proteins and sugars in the products. Griffith *et al.*[3] demonstrated the antioxidant property of the lyophilized water extracts of browned cookies and a synthetic dextrose-glycine browning mixture. Kirigaya *et al.*[4,5] reported that high molecular weight brown-colored compounds, i.e., melanoidins which had been formed in browning systems demonstrated effective antioxidant activity.

The antioxidant activity does not seem to be limited to the products of Maillard-type browning mixtures. Rhee and Kim[6] reported that acetone extracts from a caramelization-type browning mixture exhibited definite antioxidant activity in a soybean oil substrate.

The nature of antioxidants formed in Maillard-type browning reactions, however, has not been fully investigated. The type of solvent used for the extraction of browning reaction products seems to affect the nature of the antioxidants extracted and hence the antioxidant effectiveness of the extracts. For example, Won and Kim[7] reported that ethanol extracts of a Maillard-type browning reaction mixture showed the strongest antioxidant activity among the eight different solvent-extracts tested.

As the initial step towards the elucidation of the nature of antioxidants formed in non-enzymatic browning mixtures, some workers have tested the antioxidant activity of those compounds which had been known to be intermediates of Mallard browning reactions. Paik and Kim[8] tested the antioxidant activity of methylene chloride extracts of a glucose-ammonia(1 M+8 M) browning mixture and furfural, and reported that the activity of furfural was very weak while the methylene chloride extracts exhibited considerable activity. Kim *et al.*[9] tested the antioxidant actvity of furfural, furyl alcohol, lactic acid, imidazole, furan, formaldehyde, glycoaldehyde, formic acid, acetic acid, propionic acid, and pyridine, all of which had been regarded as major intermediates of Maillard browning reactions. They reported furfural, furfuryl alcohol, lactic aicd, furan, and especially imidazole showed antioxidant activity. Anderson and Huntly[10] investigated the antioxidant activity of kojic acid, maltol, isomaltol, and cyclotene and reported only kojic acid exhibited antioxidant activity. Abe and Takasaki[11] tested the activity of o-acyl and alkyl derivatives of kojic acid, and reported they exhibited some antioxidant activity.

In the present study, an attempt was made to determine the antioxidative property of maltol, kojic acid, levulinic acid, furfural, 5-hydroxymethyl furfural, and pyrazine which had been known to be important intermediates of Maillard browning reactions and of which antioxidant activity had not been well-established.

Materials and Methods

Soybean oil used

A commercial soybean oil was used as substrate in the present study. The peroxide, acid, and iodine values of the edible oil were as follows:

Peroxide	value	1.0 ± 0.1 meq/*kg* oil
Acid	value	0.060 ± 0.003 *mg* KOH/*g* oil
Iodine	value	110.7 ± 1.5

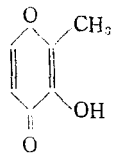
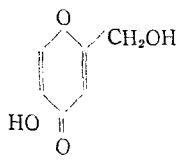
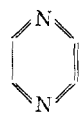
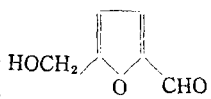
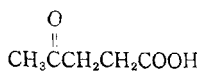
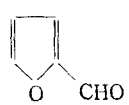
The peroxide value of the oil was determined by the AOCS method[12]. The acid value was determined by the Unilever method[13], whereas the idoine value was measured by the AOAC-Wijs method[14].

Compounds known to be intermediates of maillard browning reactions

As stated earlier, the compounds selected for use in the present study were those which had been regarded as important intermediates of Maillard browning reactions and of which antioxidant activity had not been well established. They were maltol, kojic acid, levulinic acid, furfural, 5-hydroxymethyl furfural(5-HMF), and pyrazine.

The molecular structures, melting and boiling points, solubilities in water, alcohol, and ether, and Merck index of the compounds are tabulated and shown in Table 1. These compounds were purchased

Table 1. Molecular structures, melting and boiling points, solubilities in water, alcohol, and ether, and Merck index numbers of the compounds used

Compound	Molecular structure	M.P.(C)	B.P.(C)	Solubility			Merck index number
				Water	Alcohol	Ether	
Maltol $C_6H_6O_3$		161~162	Sublime at 93°C.	1*g*/85*ml* Freely in hot	Soluble	Sparingly soluble	5540
Kojic acid $C_5H_6O_4$		153~154		Freely soluble	Freely soluble	Sparingly soluble	5165
Pyrazine $C_4H_4N_2$		53.0	115-118	Freely soluble	Freely soluble	Soluble	7741
5-HMF $C_6H_6O_3$		31.5	110	Freely soluble	Freely soluble	Soluble	4738
Levulinic acid $C_6H_5O_3$		33~35	245~246	Soluble in hot	Freely soluble	Freely soluble	5316
Furfural $C_5H_4O_2$		36.5	161.8	11 parts	Very soluble	Very soluble	4155

from Aldrich Chemical Company, Inc., WI, USA. These compounds were dissolved in the soybean oil at a 0.01 M concentration. The molecular weights and 0.01 Molar weights expressed in *g*/210 *g* oil are shown in Table 2.

Determination of antioxidant activity of the compounds

Each 30 *ml* 100% ethanol solution of the weighed compounds was added to a 210 *g* of the soybean oil, and the ethanol was evaporated from the mixture at 45°C. These oils containing each compound at

Table 2. Molecular weight and 0.01 molar weight of each compound

Compound	Molecular weight(*g*)	0.01 Molar weight[1]
Moltol	126.11	0.2863
Kojic acid	142.11	0.3266
Pyrazine	80.09	0.1818
5-Hydroxymethyl furfural	126.11	0.2863
Levulinic acid	116.11	0.2636
Furfural	96.08	0.2181

[1] Molar weight expressed in *g*/210 *g* oil.

a 0.01 M concentration were used as substrates. A control was made by adding a 30 *ml* of 100 % ethanol to a 210 *g* of the oil, and then by evaporating the solvent.

Each substrate and the control were poured eqally into three Petri dishes, and the Petri dishes were stored at 45.0±1.0°C for 30 days. The peroxide values of the substrates and control were determined regularly by the AOCS method[12] described earlier during the storage period.

The antioxidant activity of the compounds was estimated by comparing the induction period of each substrate with that of the control. The induction period of a substrate was arbitarily taken as the time in hours for the substrate to reach a peroxide value of 60 meq/*kg* oil(30 *m*mol/*kg* oil)[7]. The induction periods of each substrate and the control were estimated graphically from Fig. 1 and 2.

Results and Discussion

The results of the peroxide value determination are shown in Fig. 1 and 2. The peroxide values of the substrates containing kojic acid, furfural, and 5-HMF at a 0.01 M level were significantly lower than that of the control throughout the storage period. The peroxide value of the substrate containing maltol at a 0.01 M level was slightly lower than that of the control throughout the storage period.

The induction periods of the control and the substrates containing kojic acid, furfural, 5-HMF, and maltol were respectively 468, 592, 510, 498, and 486 hours(Table 3).

These results indicate that kojic acid possessed considerable antioxidant activity, while furfural, 5-HMF, and especially maltol had weak activity. The relative antioxidant activity of these compounds was in decreasing order, as follows:

Kojic acid ⟫ Furfural > 5-HMF > Maltol

The substrates containing pyrazine and levulnic acid exhibited higher peroxide values than the control. The peroxide value of the substrates containing levulinic acid was especially higher than that of the control throughout the storage period. The induction periods of the substrates containing levulinic acid and pyrazine were respectively 450 and

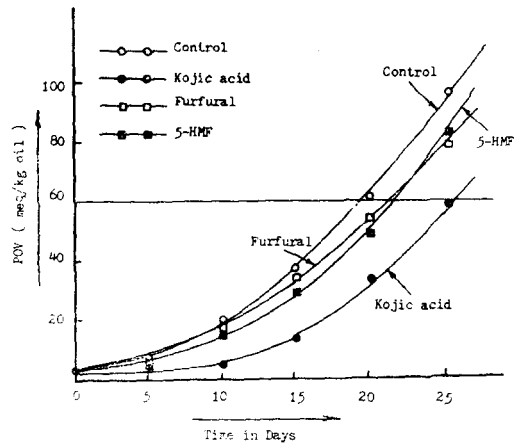


Fig. 1. Variations of peroxide values of the control and substrates containing each compound at a 0.01M level with time

402 hours (Table 3).

These results show that while pyrazine possessed distinct prooxidant activity, levulinic acid had very weak activity.

In the present study, kojic acid demonstrated significant antioxidant activity in the soybean oil. It was in good agreement with the result of Anderson and Huntly[10], although they had used different concentration(100 ppm instead of 0.01 M), substrates (corn and safflower oil), and autoxidation temperature(57°C).

While maltol exhibited weak activity in the soybean oil in the present study, Anderson and Huntly[10] had reported prooxidant activity of maltol.

It is noteworthy that both furfural and 5-HMF possessed some antioxidant activity. The result of the present study concerning the antioxidant activity of furfural seems to confirm the experimental result of Paik and Kim[8] and Kim *et al.*[9] The antioxidant activity of 5-HMF seems very significant because 5-HMF has been considered one of the most important intermediates of non-enzymatic browning reactions[15]. Both Wahhab[16] and Stadtman[17] reported 5-HMF and furfurals had been produced during the browining of dried apricots. Stadtman reported further that no browning of apricot concentrate occurred as long as extraction of 5-HMF and furfurals with ethyl acetate continued.

Paik and Kim[8] reported the methylene chloride

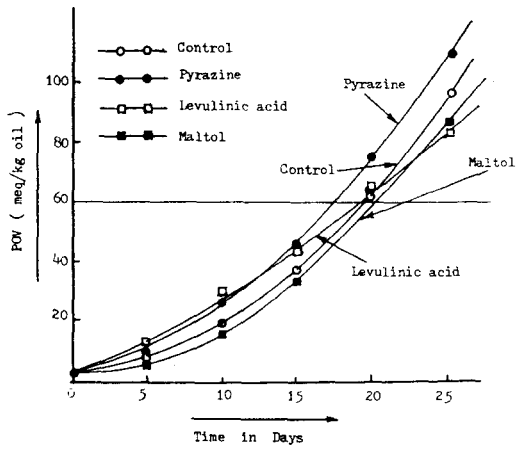


Fig. 2. Variations of peroxide values of the control and substrates containing each compound at a 0.01 M level with time

extracts of a glucose-ammonia (1 M+8 M) browning mixture showed considerable antioxidant activity. As stated earlier, pyrazine exhibited distinct prooxidant activity in the present study. It is probable that the methylene chloride extracts contained not only pyrazine and its derivatives, but also many other intermediates which were structurally unrelated to pyrazine, and yet possessed considerable antioxidant activity.

Variations of the acid values of the control and substrates containing each compound with time were also investigated in the present study. The acid values of the substrates containing levulinic acid,

Table 3. Induction periods of the control and soybean oil substrates containing each compound at a 0.01M level[1]

Sample	Induction period in hours
Control	468
Kojic acid	592
Furfural	510
5-HMF	498
Maltol	486
Levulinic acid	450
Pyrazine	402

[1] The induction periods are time required for a substrate to reach a peroxide value of 60 meq/*kg* oil.

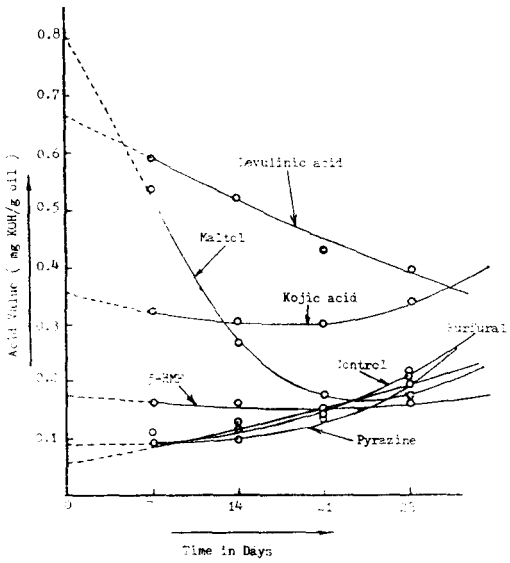


Fig. 3. Variations of acid values of the control and substrates containing each compound at a 0.01 M level with time (dotted lines are extrapolated ones)

kojic acid, and maltol were much higher than that of the control even at the initial stages of the storage period (Fig.3). These higher values at the initial stages seemed to be due to the fact that levulinic acid, kojic acid, and the enol form of maltol are acid themselves.

The rapid decrease of the acid values of the substrates containing leuvlinic acid and maltol during the storage period seemed to indicate that these compounds were very unstable at the experimental conditions of the present study. The substrate containing 5-HMF showed consistently higher acid values up to three weeks of storage time than that of the control. A possible interpretation for this is the acid formation through rapid oxidation of 5-HMF at the initial stages.

The low acid value of the substrate containing pyrazine was in agreement with the result reported by Paik and Kim[15]. A probable explanation for this lower acid value is that basic pyrazine neutralized the free fatty acid formed in the substrate.

요 약

마이알 갈색화반응의 중요한 중간생성체로 알려진 maltol, kojic acid, levulinic acid, furfural, 5-hydroxymethyl furfural(5-HMF)와 pyrazine의 항산화작용을 조사하였다. 각 화합물의 작용은 이들 화합물이 0.01 M의 농도로 들어있는 콩기름 기질들과 실험대조기질의 유도기간의 길이를 비교함으로써 추정하였다. 기질들은 45.0±1.0°C.에서 30일간 저장되었으며, 이들 기질의 과산화물값이 60 meq/*kg* oil가 되는데 소요된 시간으로써 그 기질의 유도기간으로 삼았다.

실험대조기질, kojic acid, 5-HMF, furfural, maltol, levulinic acid와 pyrazine의 유도기간은 각각 468, 592, 510, 498, 486, 450와 402시간 였었다.

Kojic acid는 뚜렷한 산화방지작용을 갖고 있는 반면에 furfural와 5-HMF는 약한 작용을, maltol은 아주 약한 작용을 갖고 있었다.

한편, pyrazine과 levulinic acid는 산화촉진작용을 보였었다. Pyrazine은 뚜렷한 촉진작용을 보였으나, levulinic acid의 산화촉진작용은 매우 미약했었다.

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