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Glucose-Ammonia 및 Glucose-Glycine 褐色化 反應液에서 얻어진 Ethanol 抽出物의 抗酸化效果의 比較

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Comparison of Antioxidant Activity of Ethanol-Extracts obtained from a Glucose-Ammonia and a Glucose-Glycine Browning Mixtures

by
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Abstract

An attempt was made to compare the antioxidant activity of ethanol extracts of a glucose-ammonia (0.2 M+0.2 M) browning mixture with that of the corresponding glucose-glycine mixture, in soybean oil substrates, on the basis of peroxide value (POV), thiobarbituric acid value (TBA-value) and acid value (AV) development.

Absorbances, at 470 nm, of the former mixture after 2 and 5 hour browning were 1.88 and 3.42 while those of the latter mixture were 0.02 and 0.07. The POVs of the supstrates containing the extracts taken after 2, 15, and 40 hrs from the former mixture were 15.8, 14.2, and 12.6 after 30 day storage at 42.3 ± 2.6 °C. Those of the latter mixture were 17.4, 16.1, and that of the control was 82.1. TBA and acid value developnent followed similar trends.

These results indicated that the antioxidant activity of the extracts of the glucose-ammonia mixture was slightly stronger than that of the glucose-glycine mixture. They also suggested that effective antioxidants had already been formed in the earlier stages of the glucose-anmonia mixture, and that brown-pigments formed did not contribute significantly to the activity of the mixture.

I. Introduction

Maillard-type non-enzymatic browning reactions were considered to be one of the most important deteriorative changes during processing and upon storage of processed foods. However, an increasing number of many researchers have recently reported the stabilizing effect of browning reaction products on the fat ingredients of these foods.

For example, Griffith et al[1] found that the addition of 5% dextrose to sugar cookies exhibited greater stability to oxidative rancidity than did the cookies in which browning occurred. Evans et al[2] investigated the antioxidant effects of amino-reductones in soybean, cottonseed, and corn oils. They demonstrated that the reductones treated oils had longer induction periods, slower oxygen absorption, and lower rates of peroxide

formation, It has been reported that ethanol or acetone extracts of Maillard-tvpe[3,4] or caramelization-type[5] browning reaction mixtures demonstrated strong antioxidant activity. The antioxygenic properties of brown-colored pigments in aqueous systems have also been reported by several researchers such as Kirigaya et al[6], and Yamaguchi and Fujimaki[7]. Pokorny et al[8] reported that brown pigments produced by condensation of alpha, beta-carbonyl compounds with amino derivatives protected lipids against autoxidation even in the presence of ionic copper. El-Zeany et al[9] reported that the brown pigments produced in a Maillard-type browning mixture were slightly active when suspended in an anhydrous system, i.e., lard, and that the activity of the pigments depended very much on the mode of application because of their low solubility in fat phase. With reference to this, it was reported by Kim[10] that acetone extracts of a Maillard-type browning mixture demonstrated considerable antioxidant activity in a water impregnated gelatine-soybean oil mixture.

The nature of effective antioxidant compounds in browning reaction mixtures has not been satisfactorily explained. This is partly due to the fact that reaction mechanisms of a Maillard-type browning reaction are very complicated,[11,12] and that so many compounds are produced that sorting and identifying effective antioxidant compounds would be extremely difficult. For the elucidation of the nature of the effective antioxidants, it would be necessary to use a model Maillard-type browning system which is as simple as possible. In this connection, Kawashima et al[13] reported the activity of browning products prepared from low molecular carbonyl compounds, such as trioses, and amino acids. However, it appears that little work has been done on the antioxidant actvity of the products of a Maillard-type browning system which utilizes the simplest form of amino compounds, namely, ammonia.

The purpose of the present study was firstly to determine whether or not ethanol extracts from a glucose-ammonia browning mixture would exhibit antioxidant activity, and secondly to compare the activity, if any, with that of ethanol extracts of the corresponding glucose-glycine mixture.

II. Experimental

Substrate

A commercial edible soybean oil was used as a substrate. The peroxide, iodine, acid, TBA, and carbonyl values were respectively 1.0±0.1 meq/kg, 97.7 ±4.6, 0.10±0.01, 0.081±0.011, and 7.38±0.03 micromoles/g, The peroxide and iodine values were determined respectively by Wheeler's method[14] and the A.O.A.C.-Wijs method.[15] The acid values were determined by the method established by Unilever laboratory.[16] The TBA and carbonyl values were determined by the methods described by Sidwell et al[17] and Henick et al.[18]

Browning reaction mixtures

A 0.2 M glucose and 0.2 M ammonia aqueous mixture was introduced into a 1,000 ml round bottom flask fitted with a reflux condensor, and heated at 100±1°C. for 40 hours. Fifty ml aliquots of the reaction mixture were withdrawn at intervals of 2, 15, 40 hrs after the heating had started. A 0.2 M glucose and 0.2 M glycine aqueous mixture was heated in the same manner. Fifty ml aliquots of the mixture were taken at the same intervals.

Measurement of the color of browning mixture

Ten ml of each aliquot was filtered and absorbance, at 470 nm, of the filtrate was measured with a Beckmann Model 25 spectrophotmeter.

Preparation of Ethanol extracts

Absolute ethanol extracts were prepared by the method previously reported[3,4]; 10 ml of each aliquot of the mixtures was concentrated by means of a rotary vacuum evaporator at 40±2.5 °C. for 15 min. Each residue resulted was extracted with 30 ml of ethanol, and the extract was dehydrated with anhydrous Na_2SO_4 and filtered.

Preparaton of substrates and determination of antioxidant activity

Each extract was added to a 150 g soybean oil. After mixing the oil thoroughly with the extract, the solvent was removed from the oil on a water bath. The supstrates, which had contained the extracts of the glucose-ammonia mixture taken at the intervals of 2, 15, and 40 hrs, were termed A-2, A-15, and A-40

respectively. The substrates, which had contained the extracts of the glucose-glycine mixture taken at the same intervals, were termed G-2, G-15, and G-40. The soybean oil, which contained only the same amount of ethanol, was used as a control,.

Each substrate was equally divided into three Petri dishes and were stored in an incubator which was kept at 42.3±2.6°C. for a period of 39 days. The peroxide, TBA, and acid values of each substrate were determined regularly during the storage period by the methods described earlier. The antioxidant activity of the ethanol extracts was determined on the basis of TBA, acid, and especially peroxide value development of the substrates relative to that of the control.

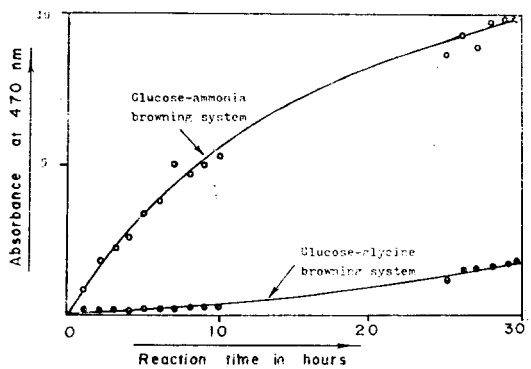

Fig. 1. Variation of absorbance, at 470nm, of glucose-ammonia and glucose-glycine browning mixtures with reaction time.

III. Results and Discussion

The results of the color measurement are presented in Fig. 1. The absorbance of the glucose-glycine mixture increased approximately in proportion to the length of reaction time, as had been previously reported.[3,4,10] The browning of the glucose-ammonia mixture, however, proceeded much faster than that of the glucose-glycine mixture. For example, the former mixture remained almost colorless after 3 hour heating, whereas the latter mixture became already reddish brown at this stage. The browning rate of the glucose-ammonia mixture fell off rapidly as the reaction continued. Neverthless, the absorbance of the glucose-ammonia mixture was 5.5 times greater than that of the glucose-glycine mixture at the end of 30 hr browning.

The results of the peroxide, TBA, and acid value determination are presented in Table 1, Figures 2 & 3, and Table 2 respectively. A-2, A-15, and A-40 showed far lower peroxide values than the control, indicating that the extracts of the glucose-ammonia mixture possessed strong antioxidant activity. G-2, G-15, and G-40 demonstrated comparable activity.

The peroxide values of A-series were, in a decreasing order, A-2> A-15> A-40, and those of G-series

Table 1. Variation of peroxide values[1] of soybean oil substrates[2], containing an equal amount of ethanol-extracts obtained at intervals of 2, 15, & 40 hrs from glucose-ammonia and glucose-glycine browning systems, with time in days

Time in Days / Sample	0	3	7	16	25	30
Control	1.0±0.1	3.4±0.4	5.8±0.5	21.2±1.6	55.5[3]	82.1±5.9
G-2	1.0±0.1	2.9±0.5	4.3±0.4	6.2±1.0	14.0±0.6	17.4±3.4
G-15	1.0±0.1	2.7±0.0	3.6[3]	5.8±0.4	12.1±1.3	16.1±2.6
G-40	1.0±0.1	2.3±0.2	4.0±0.2	5.6±0.2	10.7±1.0	15.8±0.6
A-2	1.0±0.1	2.3±0.2	4.6±1.2	6.8±0.4	9.6±2.4	15.8±1.9
A-5	1.0±0.1	2.3±0.4	3.9±0.3	6.4±0.4	9.5±1.1	14.2±2.0
A-40	1.0±0.1	1.9±0.1	3.7±0.5	7.1±0.5	8.0±1.1	12.6[3]

1. Peroxide values were expressed as milli-equivalent peroxides per kg oil.
2. All substrates were stored in an incubator kept at 42.3±2.6°C.
3. Figures without SDs are mean values.

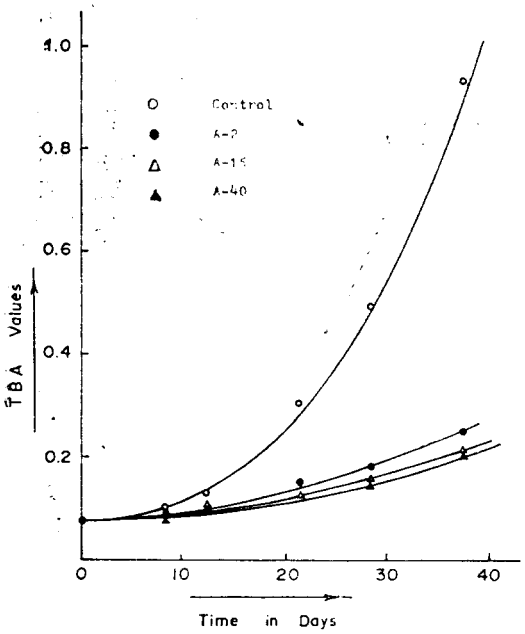

Fig. 2. Variation of TBA-values of soybean oil substrates, containing an equal amount of ethanol-extracts obtained at intervals of 2, 15, & 40 hrs from glucose-ammonia browning system, with time

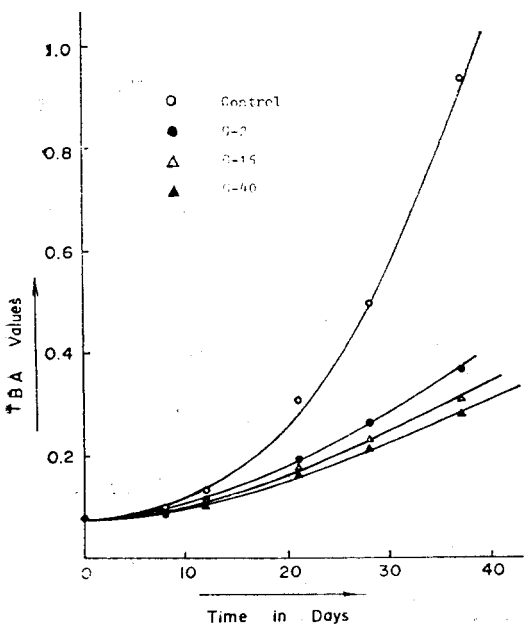

Fig. 3. Variation of TBA-values of soybean oil substrates, containing an equal amount of ethanol-extracts obtained at intervals of 2, 15, & 40 hrs from glucose-glycine browning system, with time

were also, in a decreasing order, G-2> G-15> G-40 throughout the storage period. The peroxide values of the control, A-2, A-15, and A-40 after 30 day storage were respectively 82.1±5.9, 15.8±1.9, 14.2 ±2.0, and 12.6. Those of G-2, G-15, and G-40 after 30 days were respectively 17.4±3.4, 16,1±2.6, and 15.8±0.6.

The TBA values of the A-series were, in a decreasing order, A-2> A-15> A-40, and those of the G-series were also, in a decreasing order, G-2> G-15> G-40 throughout the storage period. The TBA values of the conrtol, A-2, A-15, and A-40 after 37 days were 0.936±0.201, 0.252±0.004, 0.208±0.024, and 0.204±0.023. Those of G-2, G-15, and G-40 were 0.252±0.121, 0.310, and 0.276±0.014.

The acid values of the A-series were, in a decreasing order, A-2> A-15> A-40, and those of the G-G-2> series were likewise, in a decreasing order, G-15> G-40 throughout the storage period. The acid values of the control, A-2, A-15, and A-40 after 35 days were 1.46±0.22, 1.14±0.16, 1.12, and 1.11, while those of G-2, G-15, and G-40 were 1.25±0.19, 1.23±0.19, and 1.13±0.10.

In all cases, the acid values increased very rapidly in the initial stages of the storage period, but the rate of increase fell off quickly as time passed.

Table 2. Variation of acid values[1] of soybean oil substrates,[2] containing an equal amount of ethanol-extracts obtained at intervals of 2, 15, & 40 hrs from glucose-ammonia and glucose-glycine browning systems, with time in days

Time in Days / Sample	0	8	15	35
Control	0.10±0.00	0.97±0.05	1.29±0.04	1.46±0.22
G-2	0.10±0.00	0.84[3]	1.04±0.03	1.25±0.19
G-15	0.10±0.00	0.77±0.02	1.01±0.04	1.23±0.19
G-40	0.10±0.00	0.74±0.08	0.95±0.00	1.13±0.10
A-2	0.10±0.00	0.79±0.10	1.01±0.05	1.14±0.16
A-15	0.10±0.00	0.80[3]	0.99±0.04	1.12[3]
A-40	0.10±0.00	0.79±0.10	0.95[3]	1.11[3]

1. Acid values were determined by the method established by Unilever laboratory.
2. All substartes were stored in an incubator kept at 42.3±2.6°C.
3. Figures without SDs are mean values.

The fact that the extract of the glucose-ammonia browning mixture taken after 2 hrs demonstrated antioxidant activity nearly as strong as that of the mixture taken after 40 hrs once more indicated that some of effective antioxidants had already been formed in the earlier stages of browning of the mixture. This was in agreement with the findings previously reported by several workers.[3,4,10]

Strong antioxidant activity of the glucose-ammonia mixture was expected, as not only a sugar and amino acid mixture such as a glucose-glycine, but also a browning solution of glucose had been reported to possess considerable activity.[5] However, the fact that the extracts of the glucose-ammonia mixture demonstrated activity slightly stronger than that of the corresponding glucose-glycine mixture is very noteworthy for two reasons.

Firstly, it appears that it is not an amino acid per se, but the amino group that is important for the formation of effective antioxidants in these mixtures, just as it is the carbonyl group and not a reducing sugar per se that is important for the formation of effective antioxidants in Maillard-type browning mixtxres.

Secondly, it seems that brown-pigments may not be very important as a source of antioxidants, at least, in the glucose-ammonia browning mixture. Since the absorbances of both glucose-ammonia and glucose-glycine mixtures after 2 hour browning were respectively 1.88 and 0.02, it is obvious that the former contained far greater amount of brown-colored compounds than the latter, if not 94 times as much.

요 약

Glucose-ammonia(각 0.2M)와 glucose-glycine(각 0.2M) 褐色化 反應液의 색깔을 측정하고, 또한 이들의 ethanol 抽出物들이 함유된 大豆油의 POV, TBA價 및 酸價를 정기적으로 측정하여 그抗酸化作用을 비교하였다.

Glucose-ammonia 反應液의 2, 5시간후의 absorbance (470 nm)는 각 1.88 및 3.42였으며, 後者의 경우 0.02와 0.07였다. 反應後 2,15 및 40시간이 경과한 glucose-ammonia 및 glucose-glycine 反應液에서 얻은 抽出物들이 들은 基質(42.3±2.6°C)의 30일간 저장후의 過酸化物價는 15.8, 14.2, 12.6과 17.4, 16.1. 15.8였으며, control의 경우 82.1였다. TBA價및 酸價도 비슷한 추세를 보였다.

Glucose-ammonia反應液의 ethanol抽出物의 抗酸化作用은 glucose-glycine 反應液의 경우보다 다소 컸으며, 다같이 反應初期에 이미 抗酸化物質들이 形成된듯 했다. 한편, 前者의 경우, 形成된 褐色物質들은 그抗酸化作用에는 크게 寄與하지 않는듯 했다.

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