KOREAN J. FOOD SCI. TECHNOL.
Vol. 12, *No.* 4 (1980)

Antioxidant Activity of Various Solvent Extracts Obtained from A Maillard-type Browning Reaction Mixture

Jong-Tai Won and Dong-Hoon Kim
Department of Food Technology, Korea University, Seoul 132
(Received June 30, 1980)

各種溶媒로 부터 抽出한 Maillard型 褐色化反應 生成物의 酸化抑制作用

元 鍾太·金 東勳
高麗大學校 農科大學 食品工學科
(1980년 6월 30일 수리)

Abstract

Equal portions of a Maillard-type browning mixture (0.2 *M* glucose+0.2 *M* glycine), heated at 100°C for 12 hr, were extracted with the same amounts of eight solvents, respectively. The extracts were then dissolved in equal amounts of an edible soybean oil, and the resulting substrates and a portion of the soybean oil (Control) were stored in an incubator kept at 45.0±1.0°C for three weeks. Peroxide values and TBA values of Control and the substrates were determined regularly during the storage period.

The POVs of Control and the substrates containing acetone, benzene, chloroform, ethanol, diethyl ether, methanol, methylene chloride, and petroleum ether extracts after 12 days of storage were respectively 60.0±3.6, 31.9±0.9, 37.6±2.2, 48.1±1.1, 11.9±1.3, 4.85±0.4, 11.5±1.0, 45.3±0.3, and 43.3±4.2 *m. mole/kg oil*. The TBA values after 16 days of storage were respectively 0.28±0.02, 0.20±0.01, 0.21±0.01, 0.26±0.03, 0.16±0.02, 0.28±0.02, 0.17±0.01, 0.33±0.05, and 0.31±0.02.

The induction periods (arbitrarily taken as the time in hours for a substrate to reach a peroxide value of 30 *m. mole/kg oil*) of Control and the substrates were respectively 193, 280, 252, 220, 478, 229, 455, 217, and 214 hr.

The antioxidant activity of each extract estimated on the basis of the length of the induction periods was, in decreasing order, as follows; ethanol>methanol>acetone>benzene>diethyl ether>chloroform, pertroleum ether, methylene chloride.

Introduction

Non-enzymatic and especially Maillard-type browning reactions occurring in heat-processed food products affect not only the color and flavor, but also the nutritional values of the products. It has also been frequently reported that heat-processed products demonstrated increased stability against the rancidity development of the products[1~4].

The active antioxidants in heat-processed products or in Maillard-type browning mixtures have

been assumed to be reductones[1,2,5~9]. Evans *et al*[10], Cooney *et al*[11], and Yamaguchi[12] reported that aminohexose-reductones or amino-reductones showed considerable antioxidant activity. On the other hand, Kirigaya *et al*[13~15], Yamaguchi *et al*[16,17], and Pokorny *et al*[18] reported that high molecular weight brown pigments, i.e., melanoidins, which had been formed at the later stages of browning reactions, showed strong antioxidant activity. The nature of the antioxidants produced in Maillard-type browning reactions has not been well established. Antioxidant activity of browning products of hydroxyacetone, xylose, and glucose and 13 amino acids was investigated[19]. The antioxidant activity of 11 compounds known to be intermediates of Maillard-type browning reactions has been recently studied[20].

Some researchers used a portion of a Maillard-type browning mixture directly for the investigation of the antioxidant activity of the mixture[13,17]. Others[7,9,12,20,21] used acetone or ethanol extracts of Maillard-type browning mixtures. Lee *et al*[22] reported that both absolute ethanol and 90% ethanol extracts of a Maillard-type browning mixture possessed comparable activity. Methylene chloride extracts of a Maillard-type browning mixture was lately shown to have antioxidant activity [23]. Burton *et al*[24] separated ether, cyclohexanone, petroleum ether, and methanol soluble extracts from a glucose-glycine browning mixture, but they did not test the antioxidant activity of the extracts. Yamaguchi *et al*[6] reported that ethanol and acetone extracts of biscuits and cookies showed the strongest antioxidant activity among the solvents tested (benzene, ether, chloroform, acetone, ethanol, and ethyl acetate). However, no attempt seemed to have been made to compare the effects of various solvents on the antioxidant activity of extracts of Maillard-type browning mixtures.

In the present study, an attempt was made to determine the effects of various solvents on the antioxidant activity of the extracts obtained from a Maillard-type browning mixture. Eight typical polar and non-polar solvents were selected and used for the study.

Materials and Methods

Substrate used for measurement of antioxidant activity

A refined edible soybean oil (a commerical product) was used as substrate in this study. Some of the chemical properties of the soybean oil were as follows:

Peroxide value(POV)	:	1.9±0.2
Thiobarbituric acid value	:	0.12±0.01
Acid value (AV)	:	0.42±0.08
Saponification value (SV)	:	192.1
Iodine value (IV)	:	116.1±1.3

The peroxide and thiobarbituric acid values of the oil were determined respectively by Wheeler's method[25] and the method described by Sidwell *et al*[26]. The determination of the acid value was carried out by the method described by Triebold and Aurand[27], while the saponification and iodine values were measured by the method reported by Pearson[28] and by the A.O.A.C.-Wijs method[29].

Preparation of extracts of a Maillard-type browning mixture

A 0.2 *M* equimolar mixture of glucose and glycine was introduced into a 1 *l* flask with a reflux conde nser, and heated at 100°C for exactly 12 hr. A 10 *ml* portion of the browning mixture was extracted five times with 20 *ml* portions of a solvent used in this study and the extracts were combined. The combined extracts was first dehydrated with anhydrous Na_2SO_4 for 12 hr, and then concentrated with a rotary vacuum evaporator held at 45.0± 1.0°C. A residue thus obtained was again extracted with a 20 *ml* portion of the solvent and the extract was made exactly to 30 *ml*. The solvents used were acetone, benzene, chloroform, diethyl ether, ethanol (anhydrous), methanol, methylene chloride, and petroleum ether.

Determination of antioxidant activity of the extracts

Ten *ml* of each extract was added to 210 *g* of

the soybean oil and the mixture was thoroughly mixed. After the removal of the solvent on water bath, the mixture was divided equally into three Petri dishes (diam. $=9.4\pm0.1$ *cm*, height $=1.7\pm0.1$ *cm*), and was taken as substrate. A substrate, in 3 Petri dishes, containing the acetone extract was designated as Acetone, and a substrate containing the benzene extract as Benzene, and so on (cf. Table 1). Similarly, 210 *g* of the soybean oil without any extract was divided into 3 Petri dishes, and was taken as Control. Control and the substrates were stored in an incubator kept at 45.0 ± 1.0°C for three weeks, and peroxide[25] and TBA values[26] of Control and the substrates were determined regularly.

Induction periods of Control and the substrates were determined graphically from peroxide value -time curves. An induction period was taken as the time expressed in hours for a substrate to reach a peroxide value of 30 *m. mole/kg* oil. The antioxidant activity of each extract was estimated chiefly on the basis of the length of the induction periods of Control and the substrates. POV and TBAvalue development of Control and the substrates were also taken into consideration.

Results and Discussion

Variations of peroxide values during the storage period

Variations of peroxide values (POVs) of Control and the substrates during the storage period were shown in Fig. 1 and 2. The POVs of Control increased very rapidly at the earlier stages of the storage period. The rate of peroxide formation reached a maximum value ca. 8 days after the storage experiment had begun, and then decreased. It appears that the rate of hydroperoxide destruction. i.e., the rate of further oxidation of accumulated hydroperoxides became sufficiently greater after 8 days to reduce the net increase in the rate of peroxide formation. Privett *et al*[30] reported in their study of autoxidation of methyl linoleate that at constant temperature the rate of oxidation of the accumulated hydroperoxides depended on their concentration. On the other hand, the POVs

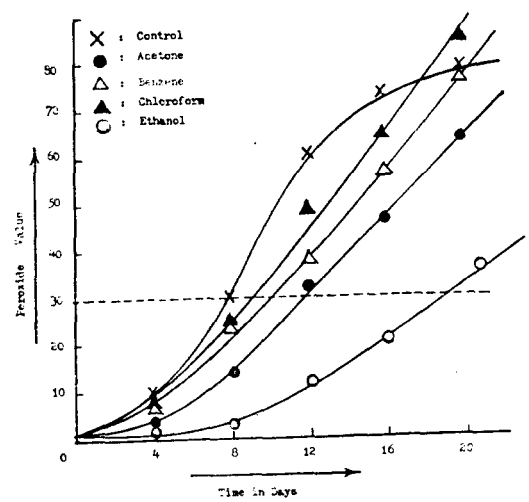

Fig. 1. Variations of peroxide values[1] of soybean oil substrates[2] containing equal amounts of various solvent-extracts obtained from a Maillard-type browning mixture

1) Peroxide values were expressed as *m. mole/kg* oil.
2) All substrates were stored in an incubator kept at 45.0 ± 1.0°C.

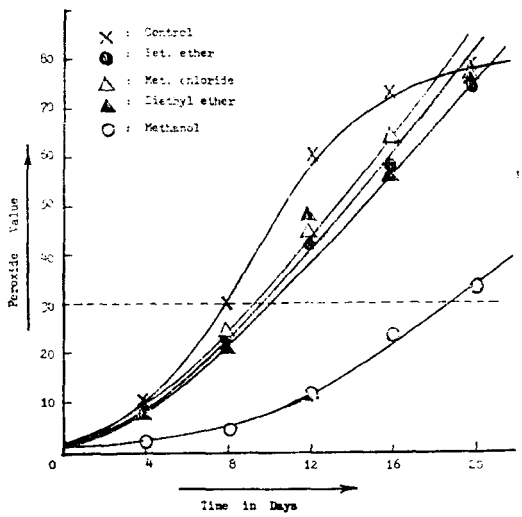

Fig. 2. Variations of peroxide values[1] of soybean oil substrates[2] containing equal amounts of various solvent-extracts obtained from a Maillard-type browning mixture

1) Peroxide values were expressed as *m. mole/kg* oil.
2) All substrates were stored in an incubator kept at 45.0 ± 1.0°C.

of the substrates increased more slowly and steadily throughout the experimental period. The substrates containing the ethanol and methanol extracts showed the slowest increase in peroxide value among the substrates.

The POVs of Control and the substrates containing acetone, benzene, chloroform, ethanol, diethyl ether, methanol, methylene chloride, and petroleum ether after 12 days of storage were 60.3±3.6, 31.9 ±0.6, 37.6±2.2, 48.1±1.1, 11.9±1.3, 48.5±0.4, 11.5±1.0, 45.3±0.3, and 43.3±4.2, respectively. The POVs after 16 days of storage were 72.2±5.2, 46.5±0.1, 55.7±0.7, 63.7±2.4, 20.4±0.7, 56.8± 2.9, 22.8±3.0, 64.2±1.3, and 54.2±7.7. On the basis of the comparison of POV development of the substrates with that of Control, all the extracts seemed to possess antioxidant activity.

Induction periods of control and the substrates

An induction period of a substrate in this study was taken arbitrarily as the time in hours for the substrate to reach a peroxide value of 30 *m. mole/kg* oil, and obtained graphically from Fig. 1 and 2. Koo *et al*[31] used, in their study on the effects of various light on a soybean oil substrate, an induction period for a peroxide value of 15 *m. mole/kg* oil. The induction periods of Control and the substrates containing acetone, benezene, chloroform, ethanol, diethyl ether, methanol, methylene chloride, and petroleum ether extracts were 193, 280, 252, 220, 478, 229, 455, 217, and 214, respectively (Table 1). It was noteworthy that while the substrates containing acetone, benzene, chloroform, diethyl ether, methylene chloride, and petroleum ether possessed the induction periods ranging from 280 to 214 hr, the substrates containing ethanol and methanol extracts demonstrated far greater induction periods of 478 and 455 hr.

Variations of thiobarbituric acid values during the storage period

Variations of the TBA values of Control and the substrates during the storage period were shown in Fig. 3 and 4. Except the substrates containing methylene chloride, chloroform, and petroleum ether extracts, other substrates showed lower TBAvalues than Control throughout the period. As in the case of the POV variations, the substrates containing ethanol and methanol extracts demonstrated by far the lowest TBA values throughout the period, The substrate containing the acetone extract also exhibited considerably lower TBA values than Control. The TBA values of Control and the substrates containing acetone, benzene, chloroform, ethanol, diethyl ether, methanol, methylene chloride, and petroleum ether extracts after 8 days of storage were 0.21±0.02, 0.14±0.03, 0.19±0.03, 0.24±0.01, 0.08±0.01, 0.20±0.04, 0.10±0.01, 0.28±0.04, and 0.26±0.01, respectively. The TBA values of Control and the substrates after 16 days of storage were 0.28±0.02, 0.20± 0.01, 0.21±0.01, 0.26±0.03, 0.16±0.02, 0.28± 0.02, 0.17±0.01, 0.33±0.05, and 0.31±0.02, respectively.

The TBA value development of the substrates was generally in agreement with the POV development except a few anomalous cases. The substrate containing the petroleum ether extract showed slightly higher TBA values than Control throughout the storage period. It appears that the petroleum ether extract did not contain those compounds which were effective in retarding the formation of malonaldehyde described by Sinnhuber *et al*[32] and other

Table 1. Induction periods[1] of Control and soybean oil substrates containing equal amounts of various extracts obtained from a Maillard-type browing mixture[2]

Sample	Induction period in hours
Control	193
Acetone	280
Benzene	252
Chloroform	220
Ethanol	478
Diethyl ether	229
Methanol	455
Methylene chloride	217
Petroleum ether	214

1) The time required for a substrate to reach a peroxide value of 30 *m. mole/kg* oil.
2) Equal portions of the browning mixture were extracted with eight solvents respectively.

compounds[33,34] responsible for color development. The higher TBA values of the substrates containing chloroform and especially methylene chloride are hard to explain. They might have been caused by the interference of these chlorine containing solvents with colored complex formation in the TBA determination. The chlorine containing solvents might have caused increased formation of colored complex counteracting the action of antioxidant compounds extracted with these solvents. Dahle *et al*[35] reported that TBA color and diene conjugation were essentially linear in relationship throughout the whole course of oxidation of polyunsaturated fatty acid esters, and that β-γ-unsaturated hydroperoxides could be precursors of cyclic peroxides and of malonaldehyde. It is also possible that these solvents might have facilitated the production of malonaldehyde and other precursors of the TBA reactions. Paik *et al*[36] reported that methylene chloride extracts of a glucose-ammonia browning mixture demonstrated lower TBA values than the control in a rapeseed oil substrate. However, the comparison of the results with each other seems not so easy, since the control used in the above report had been treated with a small amount of "blank" methylene chloride, while the control in the present study was not treated with any solvent. In other respects, the pattern of the TBA value development of Control and the substrates was very similar to that of the POV development.

Comparison of the antioxidant activity of the extracts

Although there were a few exceptions, the antioxidant activity of each extract estimated on the basis of POV and TBA value development of Control and the substrates was generally in agreement with that based on the length of the induction periods of Control and the corresponding substrates. The antioxidant activity of each extract based on the length of the induction period was, in decreasing order, ethanol>methanol>>>acetone>benzene>diethyl ehter>chloroform, methylene chloride, and petroleum ether.

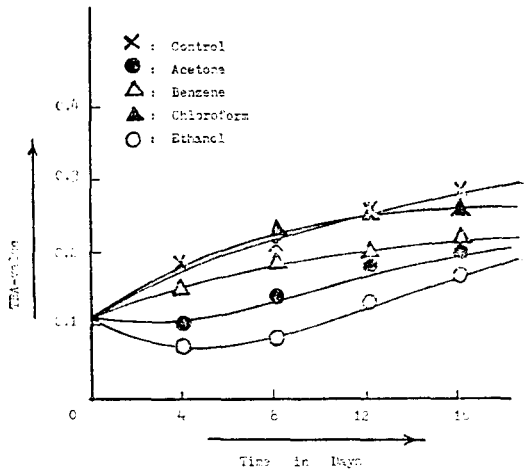

Fig. 3. Variations of TBA-values[1] of soybean oil substrates[2] containing equal amounts of various solvent-extracts obtained from a Maillard-type browning mixture

1) TBA values were determined by the method described by Sidwell *et al.*
2) All the substrates were stored in an incubator kept at 45.0±1.0°C.

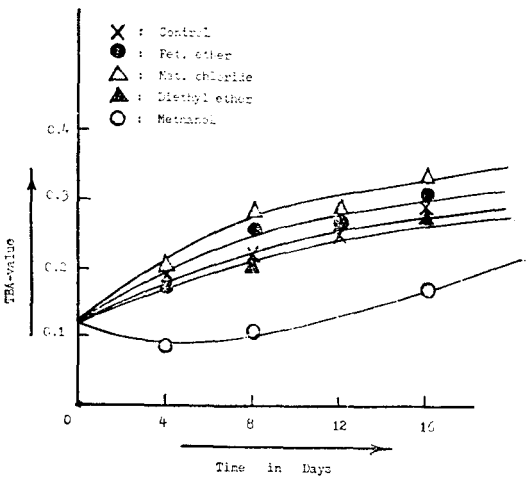

Fig. 4. Variations of TBAvalues[1] of soybean oil substrates[2] containing equal amounts of various solvent-extracts obtained from a Maillard-type browning mixture

1) TBAvalues were determined by the method described by Sidwell *et al.*
2) All the substrates were stored in an incubator kept at 45.0±1.0°C.

Yamaguchi *et al*[6] reported that ethanol and acetone extracts of biscuits and cookies demonstrated the strongest antioxidant activity among the

six different solvent-extracts in a lard substrate. Although direct comparison of the above result with those of the present study may not be possible, there is no doubt that ethanol and methanol and, to a lesser extent, acetone were the most effective solvents among the eight solvents for the extraction of the antioxidant compounds produced in the Maillard-type browning reaction.

要 約

100°C에서 12시간 가열한 Maillard型 褐色化反應液을 8개의 溶媒로 추출하여 각 抽出物의 酸化抑制 作用을 大豆油를 基質로 하여 비교하고저 하였다.

實驗對照와 아세톤, 벤젠, 클로로포름, 에탄올, 에테르, 메탄올, 메틸렌 클로라이드, 석유에테르 抽出物이 들은 基質의 저장 12일 후의 過酸化物값, 16일 후의 TBA값은 각각 60.0, 31.9, 37.6, 48.1, 11.9, 48.5, 11.5, 45.3, 43.3 (*m. mole/kg* oil)과 0.28, 0.20, 0.21, 0.26, 0.16, 0.28, 0.17, 0.33, 0.31이었다. 過酸化物값이 30이 되는데 소요한 시간을 誘導期間으로 할 때 實驗對照와 各溶媒抽出物이 들은 基質의 誘導期間은 각각 193, 280, 252, 220, 478, 229, 455, 217과 214시간이었다.

References

1. Lips, H. J.: *J. Am. Oil Chem.* Soc., **28**, 58 (1951)
2. Griffith, T. and Johnson, J. A.: *Cereal Chem.*, **34**, 159 (1957)
3. Zipser, M. W. and Watts, B. M.: *Food Technol.*, **15**, 445 (1961)
4. Anderson, R. H., Moran, D. H., Huntley, T. E. and Holahan, J. L.: *Food Technol.*, **17**, 1587 (1963)
5. Yamaguchi, N., Yokoo, Y. and Koyama, Y.: *J. Food Sci. Technol. (Japan)*, **11**, 184 (1964)
6. Yamaguchi, N. and Koyama, Y.: *J. Food Sci. Technol. (Japan)*, **14**, 106 (1967)
7. Hwang, C. I. and Kim, D. H.: *Korean J. Food Sci. Technol.*, **5**, 84 (1973)
8. Obata, H., Satou, E. and Tokuyama, T.: *J. Agr. Chem. Soc. (Japan)*, **45**, 489 (1971)
9. Rhee, C. and Kim, D. H.: *J. Food Sci.*, **40**, 460 (1975)
10. Evans, C. D., Moser, H. A., Cooney, P. M. and Hodge, J. E.: *J. Am. Oil Chem. Soc.*, **35**, 84 (1958)
11. Cooney, P. M., Hodge, J. E. and Evans, C. D.: *J. Am. Oil Chem. Soc.*, **35**, 167 (1958)
12. Yamaguchi, N.: *J. Food Sci. Technol. (Japan)*, **16**, 94 (1969)
13. Kirigaya, N., Kato, H. and Fujimaki, M.: *Agr. Biol. Chem. (Japan)*, **32**, 287 (1968)
14. Kirigaya, N., Kato, H. and Fujimaki, M.: *J. Agr. Chem. Soc. (Japan)*, **43**, 484 (1969)
15. Kirigaya, N., Kato, H. and Fujimaki, M.: *J. Agr. Chem. Soc. (Japan)*, **45**, 292 (1971)
16. Yamaguchi, N. and Fujimaki, M.: *J. Food Sci. Technol. (Japan)*, **21**, 13 (1974)
17. Yamaguchi, N. and Fujimaki, M.: *J. Food Sci. Technol. (Japan)*, **21**, 280 (1974)
18. Pokorny, J., El-Zrany, B. A., Velisek, J. and Davidek, J.: *Proc. Symposium Metal Catalyzed Lipid Oxidation*, 3rd, Paris, p.71, (1974)
19. Itoh, H., Kawashima, K. and Chibata, I.: *Agr. Biol. Chem. (Japan)*, **39**, 283 (1975)
20. Kim, D. H., Yoon, J. W. and Paik, H. D.: *Theses Collection of Agriculture and Forestry*, **19**, 141, College of Agriculture, Korea University (1979)
21. Kim, D. H.: *Theses Collection of Agriculture and Forestry*, **17**, 223, College of Agriculture, Korea University (1977)
22. Lee, S. S., Rhee, C. and Kim, D. H.: *Korean J. Food Sci. Technol.*, **7**, 37 (1975)
23. Paik, H. D. and Kim, D. H.: *Korean J. Food Sci. and Technol.*, **11**, 93 (1979)
24. Burton, H. S., McWeeny, D. J. and Biltcliff, D. O.: *Food Sci.*, **28.**, 631 (1963)
25. Wheeler, D. H.: *Oil and Soap*, **9**, 89 (1932)
26. Sidwell, C. G., Salwin, H., Benca, M. and Mitchell, J. H. Jr.: *J. Am. Oil Chem. Soc.*, **31**, 603 (1954)
27. Triebold, H. O. and Aurand, L. W.: "*Food Composition and Analysis.*" D. Van Nostrand Inc., New York, p.164 (1963)
28. Pearson, D.: "*The Chemical Analysis of Foods,*" 7 th ed. Churchill Livingston, Lond-

on, p.491 (1976)

29. Assoc. Official, Anal. Chemists: *Method of Analysis of A.O.A.C.*," 12 th ed. Assoc. Official Anal. Chemists, Washington, D. C., p. 489 (1975)
30. Privett, O. S. and Nickell, C.: *J. Am. Oil Chem. Soc.*, **33**, 156 (1956)
31. Koo, J. H. and Kim, D. H.: *Korean J. Food Sci. Technol.*, **3**, 178 (1971)
32. Sinnhuber, R. O. and Yu, T. C.: *Food Technol.*, **12**, 9 (1958)
33. Tarladgis, B. G. and Watts, B. M.: *J. Am. Oil Chem. Soc.*, **37**, 403 (1960)
34. Asakawa, T., Nomura, Y. and Matsushita, S.: *Oil Chemistry (Japan)*, **24**, 88 (1975)
35. Dahle, L. K., Hill, E. G. and Holman, R. T.: *Arch. Biochem. Biophys.*, **98**, 253 (1962)