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Antioxidant Activity of Ethanol-Extracts of Defatted Soybean, Sesame, and Perilla Flours in a Soybean Oil-Water Emulsion System

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脫脂 콩, 참깨 및 들깨粕의 에탄올 抽出物의 콩기름-물 基質에서의 酸化抑制效果

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Abstract

The antioxidant activity of ethanol-extracts of defatted soybean, sesame, and perilla flours was compared with that of 0.02% BHT in a soybean oil-water emulsion system. The emulsion substrates and control were stored at 46.0±0.5°C for 25 days. The peroxide and TBA values of the substrates and control were determined regularly. The activity of the oilseed flour extracts and BHT was estimated by comparing the POV development of the substrates with that of the control.

The POVs of the substrates containing the soybean, sesame, and perilla flour extracts and BHT and that of the control after 25 day storage were respectively 43.3±0.1, 22.6±0.7, 21.5±0.2, 38.6±0.4, and 80.1±0.8. The TBA values after 20 day storage were 0.91±0.05, 0.67±0.02, 0.68±0.01, 0.38±0.01, and 0.62±0.01.

The soybean, sesame, and perilla flour extracts exhibited considerable antioxidant activity in the oil-water emulsion system. The activity of the sesame and perilla flour extracts was far stronger than that of 0.02% BHT in the emulsion system. The abnormally high TBA values of the oilseed flour extracts in the present study might be attributed to the interference of some carbonyl compounds in the extracts in the TBA value determination.

Introduction

Much research effort has recently been directed to the antioxidant activity of soybean or soybean components, particularly to those associated with flavonid compounds and phenolic acids. For example, Hammerschmidt and Pratt[1] reported that phenolic acids in dried soybeans exhibited potent antioxidant property. It has also been reported that many flavonoid compounds[2] and aqueous extracts of several plant tissues[3] demonstrated

antioxidant activity. The antioxidative property of soybean flour and its derivatives was reviewed by Hayes *et al*[4]. Several research workers[5~7] have indicated that the incorporation of soybean protein into meat products might give an added advantage of providing the meat products with further antioxidative protection.

It has been reported[8~10] that the superior stability of sesame oil against oxidation is due to the presence of natural antioxidants, sesamin, sesamol, and sesamolin, in the oil. The antioxidant activity of sesame flour or its constituents such as sesamin and sesamol just mentioned has been well studied[8~11]. There is, at least, one research work[11] which has reported that, aside from propyl gallate, sesamol was more effective than butylated hydroxytoluene (BHT) and several other synthetic antioxidants. Rhee *et al*[12] have recently studied the antioxidant activity of defatted flours, concentrates, and isolate of cottonseed, peanut, and soybean in various substrates including safflower oil. They have repoted[12] that the methanol-extracts of cottonseed protein ingredients showed uniquely and consistently higher activity than those of peanut and soybean protein ingredients.

Although there have been some works on the nutritive value of perilla flour[13~15], research works on the antioxidative activity of the perilla flour seem very rare. Therefore, as an initial step for the investigation of the antioxidant activity of the perilla flour and its constituents, the activity of ethanol-extracts of defatted soybean, sesame, and perilla flours was compared with that of a commercial antioxidant BHT in an oil-water emulsion system.

Materials and Methods

Materials

The oil used in the present study was a commercial edible soybean oil (Dong-Bang Oil Manufacturing Co., Seoul). The saponification and iodine values of the oil were determined by the method roported by Pearson[16] and the A.O.A.C.-Wijs method[17] respectively. The peroxide value (POV) and thiobarbituric acid value (TBA value) were determined by the A.O.C.S. method[18] and the me thod described by Sidwell *et al*[19]. The results of the analyses for the soybean oil are as follows;

Saponification value[15]	189.0
Iodine value[17]	127.1±0.1
Peroxide value[18] (*meq/kg* oil)	0.4±0.1
Thiobarbituric acid value[19]	0.08±0.01

Soybean, sesame, and perilla were purchased at Kyung-Dong Market, Dong-Dae Moon, Seoul in February, 1981. The moisture, ash, crude fat, and crude protein contents were determined by the respective methods described in "Official Methods of Analysis of the A.O.A.C."[20]. The results of the proximate analysis are given in the following table :

	Soybean (%)	Sesame (%)	Perilla (%)
Moisture	8.0	4.4	5.9
Ash	5.1	4.7	4.1
Crude fat	16.6	45.7	41.7
Crude protein	46.1	25.8	26.8

Butylated hydroxytoluene (BHT) used as a reference phenolic antioxidant was obtained from Ueno Chemical Co. (Osaka, Japan). The emulsifiers, Tween 80 and Span 80 used for the preparation of the soybean oil-water emulsion system, were obtained from Wako Pure Chemical Co. (Tokyo, Japan).

Preparation of ethanol-extracts of the defatted oilseeds flours

Ethanol-soluble components of the soybean, sesame, and perilla flours were extracted with 95% ethanol by the method reported by Hammerschmidt and Pratt[1]. Hundred *g* of each crushed oilseed was defatted with *n*-hexane for 42 hr prior to the extraction. The defatted flour was soaked in 95% ethanol for 16 hr, and then homogenized with the ethanol. The homogenate was boiled in the ethanol for 5 min and filtered through Whatman No. 1 paper. The residue was washed with additional 50 *ml* hot ethanol. The filtrates were combined and the combined filtrate was concentrated at ca. 40°C to a final volume of 30 *ml*. The

procedure used for the preparation of the ethanol-extracts is presented schematically in Fig. 1.

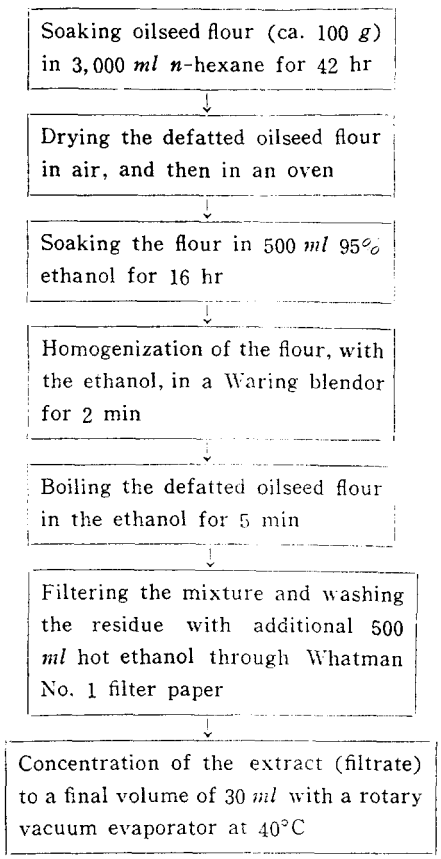

Fig. 1. Procedure used for the preparation of the ethanol-extracts of the defatted soybean, sesame, and prilla flours

Preparation of the soybean oil-water emulsion

Tween 80 and Span 80 (1 : 1, w/w) were added to a mixture of the soybean oil and distilled water (50 : 45, w/w) in such a way as the final concentration of the emulsifiers in the mixture became 5% by weight. Sodium dehydroacetate (0.02% by weight) was also added to the mixture as preservative. The mixture was then homogenized for 4 min at 5,000 rpm.

POV and TBA value determination of the emulsion

The ethanol-extracts of the defatted oilseed flours and 0.02% BHT were added respectively to 260 *ml* of the emulsion. A 260 *ml* portion of the emulsion which did not contain any extract or BHT was used as a control. Each of these emulsion substrates including the control was divided equally into three 100 *ml* Erlenmeyer flasks. They were termed respectively Soybean, Sesame, Perilla, and BHT. The substrates including the control were stored at 46.0 ±0.5°C for 25 days.

Fifty *ml* of each substrate was evenly taken out of the flasks and the oils in the substrates were extracted with small amounts of petroleum ether. The petroleum ether extracts were dehydrated with anhydrous Na_2SO_4 and filtered. The solvent in the filtrates was removed at ca. 40°C and the extracted oils were used for the determination of POV and TBA values. The determination was carried out every 5 days during the storage period.

Determination of antioxidant activity

The POV and TBA values of each substrate and control were determined respectively by the A.O.C.S. official method[18] and the method described by Sidwell *et al*[19]. The antioxidant activity of the ethanol-extracts and BHT in the soybean oil-water emulsion was estimated by comparing the POV development of the corresponding substrates with that of the control. The TBA value development of the corresponding substrates and the control was used as reference.

Results and Discussion

The results of the POV and TBA value determination are presented in Table 1 and 2.

As is shown in Table 1, the POVs of the emulsion substrates containing the ethanol-extracts of the defatted soybean, sesame, and perilla flours were consistently lower than those of the control throughout the storage period. The POVs of the substrate containing 0.02% BHT were also lower than those of the control throughout the storage period.

The POVs of Soybean, Sesame, Perilla, BHT, and control after a10 day storage period were respectively 15.4±0.3, 8.6±0.2, 11.6±0.2, 16.8±0.3, 16.3 ±0.1, and 28.5±0.3. Those after a 25 day storage period were 43.3±0.1, 22.6±0.7, 21.5±0.2, 38.6 ±0.4, 42.0±0.6, and 80.1±0.8. The ethanol-extracts of the defatted oilseed flours seemed to

Table 1. Variations of POVs[1] of soybean oil-water emulsion substrates[2], containing the ethanol-extracts of defatted soybean, sesame, and perilla flours and 0.02% BHT with storage time

Substrate	Storage time in days					
	0	5	10	15	20	25
Soybean	1.5±0.1	6.7±0.1	15.4±0.3	28.3±0.4	40.5±0.1	43.3±0.1
Sesame	1.5±0.1	2.7±0.1	8.6±0.2	13.1±1.0	20.1±0.1	22.6±0.7
Perilla	1.5±0.1	4.2±0.2	11.6±0.6	15.1±0.3	20.4±0.2	21.5±0.2
BHT	1.5±0.1	11.5±0.2	16.8±0.3	32.9±0.4	37.5±1.2	38.6±0.4
Control	1.5±0.1	13.6±0.7	28.5±0.3	52.3±0.3	67.8±0.1	80.1±0.8

1) POVs were determined by the A.O.A.C. method[18] and expressed as *meq* peroxides/*kg* oil.
2) All substrates including Control were stored in an incubator kept at 46.0±0.5°C.

Table 2. Variations of TBA values[1] of soybean oil-water emulsion substrates,[2] containing the ethanol-extracts of defatted soybean, sesame, and perilla flours and 0.02% BHT with storage time

Substrate	Storage time in days				
	0	5	10	15	20
Soybean	0.10±0.01	0.33±0.05	0.45±0.01	0.68±0.04	0.91±0.05
Sesame	0.10±0.01	0.25±0.01	0.34±0.02	0.50±0.01	0.67±0.02
Perilla	0.10±0.01	0.29±0.05	0.40±0.01	0.53±0.01	0.68±0.01
BHT	0.00±0.01	0.21±0.01	0.26±0.01	0.32±0.01	0.38±0.01
Control	0.10±0.01	0.23±0.02	0.31±0.01	0.48±0.01	0.63±0.01

1) TBA values were determined by the method described by Sidwell *et al*[19] and expressed in absorbance at 530 *nm*.
2) All substrates including Control were stored in an incubator kept at 46.0±0.5°C.

possess stronger antioxidant activity than the commercial antioxidant BHT in the oil-water emulsion system, The activity of the extracts and 0.02% BHT based on the POV development of the emulsion substrates was, in decreasing order, as follows :

Perilla, Sesame ≫ BHT > Soybean

The findings that the soybean flour extract showed considerable antioxidant activity in an oil-water emulsion system is in agreement with the previous findings reported by many workers[1~3,5,21]. It should be noted here, however, that the activity was much lower than that of the sesame or perilla flour extracts. The activity of the soybean flour extract was stronger than that of BHT in the earlier stages of the storage period, but the activity became slightly weaker than that of BHT in the later stages. The rapid loss of the activity of the soybean flour extract might be partly due to the rapid oxidation of antioxidative phenolic acids such as chlorogenic acid into *o*-quinone derivatives as reported by Sosulski[22].

The ethanol-extracts of both defatted sesame and perilla flours showed strong activity in the oil-water emulsion as mentioned earlier. The strong activity of the sesame flour extract was in good agreement with the previous findings of Budowski[8,9] and Lyon[11]. The activity of the sesame flour extract was slightly stronger than that of the perilla flour extract in the earlier stages of the storage period, but it became almost indistinguishable with that of the perilla flour extract in the later stages of the storage period. Beroza *et al*[10] and Park[22] have reported that the sesamol con-

tent of sesame oil tends to increase with the rancidity development of the oil. The conversion of sesamine and especially sesamolin, which are known to be very potent antioxidants in sesame flours or oils[23], into sesamol might have caused the partial loss of the activity of the sesame flour extract in the later stages of the storage period.

As is shown in Table 2, the TBA values of the emulsion substrates containing the soybean flour extract were greater than those of the control throughout the storage period. The TBA values of the substrates containing the sesame and perilla flour extracts were similar to those of the control throughout the storage period.

The TBA values of Soybean, Sesame, Perilla, BHT, and Control after a 10 day storage period were respectively 0.45±0.01, 0.34±0.02, 0.40±0.01, 0.26±0.01, and 0.31±0.01. Those after a 20 day storage period were 0.91±0.05, 0.67±0.02, 0.68±0.01, 0.38±0.01, and 0.62±0.01.

It seems probable that the ethanol-extracts of the defatted oilseed flours, especially the extract of the soybean flour, might have contained some ethanol-soluble compounds of aldehydic or ketonic nature.

These carbonyl compounds could certainly interfere with the TBA-malon aldehyde colored complex formation by forming various colored complexes with TBA. Many volatile carbonyl compounds have been detected from soybean oil[24] or soybean[25] at the incipient stages of oxidation.

Tocopherols and their esters are known to be widely distributed in many plants and in seed germ oils[26,27]. The antioxidant activity of tocopherols and their esters has been well studied[27~30]. Since tocopherols and their esters are very soluble in fats and oils and fat solvents[26], no appreciable amounts of tocopherols and their esters could have remained in the defatted oilseed flours or in their ethanol-extracts. There is, therefore, little possibility that the tocopherols and their esters in the oilseed flour extracts had contributed to the antioxidant activity of the extracts. It has been shown that not only the methanolic extracts of soybean flour but also the methanolic extracts of cottonseed and peanut flours possessed antioxidant activity in various substrates[12].

The results of the present study seem to indicate the ethanol-extract of the defatted perilla flour has potent antioxidant activity comparable with that of the defatted sesame flour extract. It seems warranted that the compounds in the perilla flour extract responsible for the potent antioxitant activity should be further investigated, as has been the case for soybean or sesame flour extract.

要 約

콩, 참깨 및 들깨의 脫脂粕의 에탄올抽出物의 酸化防止 效果를 콩기름-물 乳濁液基質에서 0.02% BHT의 效果와 비교하고저 했다. 抽出物 및 BHT가 들은 基質과 control을 46.0±0.5°C에서 저장하면서 그 過酸化物값 (POV)과 TBA값을 定期的으로 측정하였다.

콩, 참깨 및 들깨의 脫脂粕의 抽出物 및 BHT가 들은 基質과 control의 저장 25일후의 過酸化物값(平均値)은 43.3, 22.6, 21.5, 38.6과 80.1였으며, 저장 20일후의 TBA값은 각각 0.91, 0.67, 0.68, 0.38과 0.62였다.

콩, 참깨 및 들깨의 脫脂粕의 抽出物은 乳濁液基質에서 상당한 酸化防止效果를 보였다. 특히 참깨와 들깨의 경우, 그 效果는 0.02% BHT보다 훨씬 컸었다. 抽出物이 들은 基質의 TBA 값은 豫想外로 컸으나, 이는 脫脂粕에서 抽出된 一部 化合物들이 TBA 값 測定에 干涉한 結果로 推測된다.

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