Effect of Extraction Solvents on Oxidative Stability of Crude Soybean Oil

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Oxidative stabilities of crude soybean oils obtained by different extraction solvents such as hexane, water and Folch's solvent (mixture of two volumes of chloroform and one volume of methanol) were determined by gas chromatographic analyses of headspace and peroxide value of oil samples. For the determination of oxidative stability of oil samples, total volatile compounds formation, molecular oxygen disappearance in the headspace and peroxide value of oil samples were measured. Iodine value (133-136), saponification value (195-198), unsaponifiable matters (0.3-0.4%), iron (0.6 ppm), sterols content (2,400-2,590 ppm), tocopherols content (1,250-1,520 ppm) and fatty acid composition of crude oils obtained by different solvent extraction were not significantly different. Acid value of Folch-extracted oil was the highest as 1.3, whereas those of hexane- and aqueous-extracted oils were 0.5 and 0.4, respectively. Crude soybean oil extracted by Folch's method was found to contain the most phosphorus, while hexane- and aqueous-extracted oils contained similar amounts of phosphorous. Crude soybean oil obtained by Folch extraction was most stable in oil oxidation, and oxidative stabilities of oils obtained by hexane and aqueous extraction, which were significantly much less stable than Folch-extracted oil, were not significantly different during ten weeks storage.

Most vegetable oils conventionally have been obtained from oilseeds by either pressing or organic solvent extraction methods. Since the conventional organic solvent extraction method is a high energy-consuming process, alternative extraction methods have been extensively studied (1,2). One of the alternative extraction methods is aqueous extraction. The aqueous extraction method has been successfully applied to soybean (3), sunflower seed (4), peanut (5) and coconut oils (6).

Oxidative stability, which is one of the most important keeping qualities of oil, is known to be greatly influenced by minor components present in oils such as phospholipids, tocopherols, fatty acids and trace metals (7,8). It is reported that the type and content of minor components in crude oils are primarily dependent upon extraction solvents, extraction temperature, pretreatment of oilseeds, etc. (8,9).

The objectives of this study were (i) to determine the contents of minor components, and (ii) to measure the oxidative stability of oils obtained by different extraction solvents such as hexane, water and a mixture of chloroform and methanol.

MATERIALS AND METHODS

Materials. Full-fat soybean flour (100-120 mesh) was purchased from a local market in Korea. Protease of *Aspergillus oryzae* (3.7 unit/mg solid) was obtained from Sigma Chemical Co. (St. Louis, MO). All reagents used were of analytical grade unless otherwise specified.

Preparation of oil samples. To prepare aqueous-extracted crude soybean oil, one part soybean flour was dispersed in six parts distilled water at 40°C by continuous stirring with a magnetic stirrer, and then 0.2% (wt enzyme/wt soybean) of protease was added to enhance extraction of oil. The pH of the dispersion was adjusted to 8.0 with 1 N NaOH solution. After 1 hr stirring, the dispersion was centrifuged (Beckman J2-21M) at 10,000 *g* for 15 min, and the dispersion was separated into four layers of solid, water, emulsion and oil. To separate the oil from the emulsion layer, acetone was added and the miscella recovered was evaporated at 50°C under vacuum. Crude soybean oil was also obtained by conventional hexane extraction and Folch's extraction method using solvent mixture of two volumes of chloroform and one volume of methanol (10). Crude soybean oils obtained were steam distilled under vacuum to remove residual extraction solvents with 1% (wt water/wt oil) steam for 10 min. Pressure and temperature of the steam distillation process were 1 torr and 150°C, respectively.

Analytical methods. Acid value, iodine value, saponification value, unsaponifiable matter, phosphorous, peroxide value and fatty acid composition were determined by AOCS methods Cd 3a-63, Cd 1-25, Cd 3-25, Ca 6a-40, Ca 12-55, Cd 8-53 and Ce 1-62, respectively (11). Phospholipids content in oil was calculated by multiplying phosphorus content by a conversion factor of 30 [(11), Ca 12-55]. Iron was measured using a Perkin-Elmer 360 atomic absorption spectrophotometer (Perkin-Elmer, Norwalk, CT) according to AOAC methods 2.109-2.112 (12). Sterols and tocopherols were determined by gas chromatographic analyses, respectively (13,14).

Oxidative stability measurement. To determine oxidative stability of oils, oil samples obtained were stored at 60°C in a dark, forced-draft air-oven for ten weeks.

The oxidative stability of oils was determined by a combination of volatile compounds formation and molecular oxygen disappearance in the headspace of oils in air-tight, sealed bottles and peroxide value of oil samples. Fifteen grams of experimental samples were transferred into a 50 ml serum bottle and sealed air-tight with a Teflon-lined rubber septum and an aluminum cap. The gas chromatograph used for volatile compounds and oxygen content measurements was a Hewlett-Packard 5890. For determination of volatile compounds in the headspace, 1 ml of headspace gas was directly injected into the gas chromatograph. Column used for volatile compounds measurement was a stainless steel column (10′ × ⅛″ OD) packed with 80/100 mesh Tenax GC coated with 10% polymetaphenoxylene. Temperatures of column, injector and flame ionization detector were 120°C, 200°C, and 250°C, respectively. Flow rate of helium carrier gas was 20 ml/min.

For the determination of oxygen content in headspace, a thermal conductivity detector was used. The oxygen

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content in the headspace was determined using a stainless steel column (10′ × ¼″ OD) packed with Molecular Sieve 5A, and column temperature was 60°C. Temperature of thermal conductivity detector was 210°C, and flow rate of argon carrier gas was 20 ml/min. GC peaks were quantified by electronic counter and expressed in electronic counts. The methods are essentially the same as described in Yoon *et al.* (15).

Statistical analysis. The qualitative and quanitative effects of extraction solvents and storage time on oxidative stability of oils were analyzed by Duncan's Multiple Range Test (16).

RESULTS AND DISCUSSION

Characteristics of oil samples. Acid value, iodine value, saponification value, unsaponifiable matter, iron content, phosphorus and phospholipids contents, sterols, tocopherols and fatty acid composition of crude soybean oils obtained by different extraction solvents are shown in Table 1. Iodine value, saponification value and unsaponifiable matter content of crude soybean oils were found in their typical ranges, such as 133-136, 195-198 and 0.3-0.4%, respectively. The extraction solvent did not significantly affect iodine value, saponification value and unsaponifiable matter content in crude soybean oils.

Acid value of Folch-extracted crude soybean oil was 1.3, whereas those of hexane- and aqueous-extracted oils were 0.5 and 0.4, respectively. Free fatty acid content in hexane-extracted oil, 0.25%, which was assumed as half of an acid value, implied that the full-fat soybean flour used as a raw material was not substantially deteriorated before the experiment.

Iron contents in oils were measured as 0.6 ppm, which indicated the oils were not heavily contaminated.

Phospholipids contents (which are calculated from phosphorus contents) in hexane-, aqueous- and Folch-extracted crude soybean oils were 0.7%, 0.6% and 2.4%, respectively. In plant scale extraction, which uses hexane as extraction solvent, phospholipids content in crude soybean oil generally ranges from 1.5-2.5% (17). In this study, however, phospholipids contents in bench scale hexane- and water-extracted soybean oils were shown to be less than that from plant scale extraction. Phospholipids content in Folch-extracted soybean oil (2.4%) was measured as more than three times higher than that in hexane-extracted oil (0.7%). Folch's solvent, being more polar than hexane, was shown to be a better solvent for extracting phosphorus containing compounds from soybean (18).

Sterols and tocopherols contents in oils ranged from 2,400-2,590 ppm, and from 1,250-1,520 ppm, respectively. It was found that sterols in all three oil samples were mainly composed of campesterol, stigmasterol and β-sitosterol in the approximate ratio of 1:1:2.

Fatty acid compositions of oil samples were not notably different, depending on the extraction solvents.

Effect of extraction solvent on oxidative stability of soybean oil. To test the effect of extraction solvent on the oxidative stability of soybean oil, crude soybean oils obtained by hexane-, aqueous- and Folch-extraction followed by steam distillation were stored at 60°C for ten weeks.

The major components of volatile compounds in the headspace of soybean oil were butane, pentane, propanol and hexanal as determined by mass spectra, and these compounds are formed by lipid oxidation (19). Total volatile compounds in the headspace of aqueous- and hexane-extracted soybean oils increased from an initial value of 65,000 to 6,075,000 and 4,951,000, respectively, after ten weeks storage, whereas that of Folch-extracted oil increased from 65,000 to 650,000 during the same period (Fig. 1).

It is well-known that as the volatile compounds in the soybean oil increase, the oxidative and flavor quality decrease (20,21).

The headspace oxygen of aqueous- and hexane-extracted soybean oils decreased from an initial oxygen content of 21% in headspace air to 6.9% after six weeks storage, whereas the headspace oxygen of Folch-

TABLE 1

Characteristics of Soybean Oils Obtained by Hexane-, Aqueous- and Folch-Extraction

Item	Hexane	Aqueous	Folch
Acid value	0.5	0.4	1.3
Iodine value	136	133	135
Saponification value	195	195	198
Unsaponifiable matters, %	0.3	0.4	0.3
Iron, ppm	0.6	0.6	0.6
Phosphorus, ppm	240	210	790
Phospholipids, %	0.7	0.6	2.4
Sterols, ppm	2,490	2,400	2,590
Tocopherols, ppm	1,250	1,300	1,520
Fatty acid composition			
Palmitic acid	11.7	11.3	11.7
Stearic acid	4.1	4.0	4.1
Oleic acid	22.0	22.1	21.7
Linoleic acid	54.0	54.4	54.1
Linolenic acid	7.6	7.6	7.8
Others	0.6	0.6	0.6

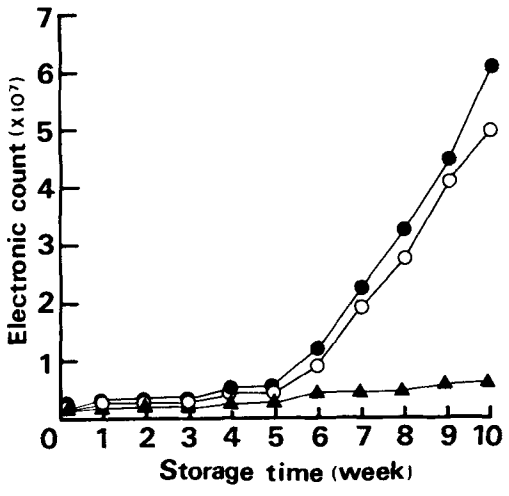

FIG. 1. Effect of storage time on the total volatile compounds formation in headspace of crude soybean oil obtained by hexane-(○), aqueous-(●) and Folch-extraction (▲).

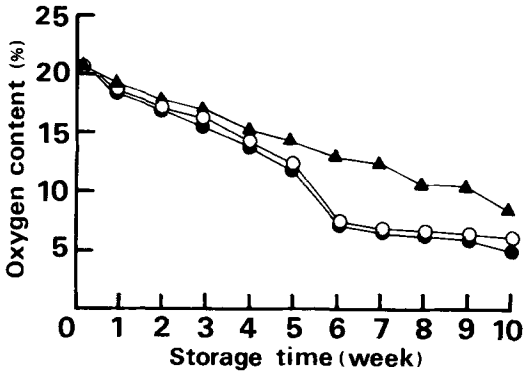

FIG. 2. Effect of storage time on the oxygen content in headspace of crude soybean oil. Legends are the same as in Figure 1.

extracted oil decreased to 8.6% after ten weeks storage (Fig. 2), which showed that oxygen reacted more rapidly with aqueous- and hexane-extracted oil than Folch- extracted oil.

Duncan's Multiple Range Tests indicated that the amounts of volatile compounds and oxygen in the headspaces of aqueous- and hexane-extracted oils were not significantly different depending on the extraction solvent throughout storage ($P < 0.05$). However, volatile compounds and oxygen contents of Folch-extracted oil were shown to be significantly different from those of aqueous- and hexane-extracted oils ($P < 0.05$) (Fig. 3). Peroxide values of aqueous- and hexane-extracted soybean oils increased rapidly and reached a maximum of 22 after six weeks storage and decreased thereafter, whereas peroxide values of Folch-extracted oil increased gradually up to maximum of 5.3 during ten weeks storage.

The combined results of contents of volatile compounds and oxygen in the headspace and peroxide values of oil samples, indicated that the oxidative stability of soybean oil obtained by Folch-extraction was significantly better than that of soybean oils obtained by conventional hexane extraction and aqueous extraction ($P < 0.05$).

It is reported that the oxidative stability of oil is affected by such pro- and antioxidant materials such as free fatty acids, iron, tocopherols and phospholipids (22-24). Among the analytical results in this study, the contents of iron and tocopherols were shown to be similar, whereas acid value and phospholipids content were shown to be significantly different, depending on the extraction solvents. Phospholipids are known to possess prooxidant activity in the absence of iron, and to possess antioxidant activity in the presence of iron at 1 ppm level in purified soybean oil (23). The better oxidative stability of Folch-extracted soybean oil than that of aqueous- and hexane-extracted soybean oils can be explained partly by a combination of (i) increased antioxidant effect, the phospholipid acts as a synergist for the primary antioxidant present in crude soybean oil tocopherols; and (ii) increased metal chelating effect caused by the higher phospholipids content (23). These are observed in spite of the higher amount of free fatty acid (0.65%) [which showed prooxidant activity (22)] present in Folch- extracted oil than in hexane- and aqueous-extracted oils, 0.25% and 0.,2%, respectively.

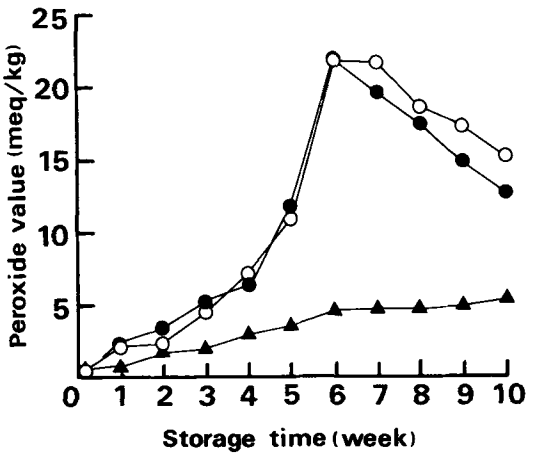

FIG. 3. Effect of storage time on peroxide value of crude soybean oil. Legends are the same as in Figure 1.

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