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Roles of Phospholipids in Flavor Stability of Soybean Oil

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대두유 향미안정성에 있어서 인지방질의 역할

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Abstract

The effects of phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), phosphatidyl inositol (PI), phosphatidic acid (PA), phosphatidyl glycerol (PG), and cardiolipin (CL) on the flavor stability of purified soybean oil were studied. Purified soybean oil obtained from soybean oil by silicic acid chromatography does not contain measurable iron, tocopherols and phospholipids. Three hundred *ppm* of PC, PE, PI, PA, PG, or CL was added to the purified soybean oil, with and without 1*ppm* ferrous iron added. The flavor stability of sample, which was stored at 60°C for 10 days in dark oven, was determined by a combination of volatile compounds formation and molecular oxygen disappearence in the headspace of air-tightly sealed serum bottle every 48 hrs. Results showed that, in general, phospholipids worked as prooxidant in the pufified soybean oil without ferrous iron added, and worked as antioxidant in the oil, when added 1*ppm* ferrous iron. The results also suggest that phospholipids work as prooxidant by increasing the solubility of oxygen on the surface of oil, and work as antioxidant in the oil containing 1 *ppm* ferrous iron by chelating iron. The results showed that PE and PA are better antioxidants than PC and PG. CL and PI showed the lest antioxidant activities in the oil will 1*ppm* ferrous iron added.

Introduction

Phospholipids in soybean oil have long been recognized as an important factor influencing the keeping quality of the soybean oil.[1] Phospholipids are present in the crude soybean oil at the level of 1.5 to 2.5%, and most of them are removed during oil refining processes. Hydratable phospholipids such as phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), and phosphatidyl inositol (PI), are mostly removed by water-degumming, and nonhydratable phospholipids such as calcium and magnesium salts of phospholipids or lysophospholipids can be removed from soybean oil by alkali refining process.[2]

Phospholipids in crude soybean oil are reported to be PC, PE, PI, phosphatidyl serine (PS), and phosphtidic acid (PA), and PC and PE are also detected in refined, bleached and deodorized (RBD) soybean oil.[3]

Phospholipids have been known as antioxidant synergists, chelating agents, prooxidants and off-flavor precursors in oil. Phospholipids show synergistic effects with antioxidants such as tocopherols on the falvor stability of oil.[4-6] Stasinopoulous[7] reported that PC did not improve the oxidation stability of soybean oil containing no iron, but it increased the oxidation stability of oil containing 0.5 or 1.0 *ppm* iron. These results suggested that PC acted as a chelating agent for iron, thus improving the oxidation stability of soybean oil containing metals such as iron.

Several papers reported that PC and PE also enhanced the statibility of lipids in fishes since the nitrogen moiety of phospholipids such as choline and ethanolamine decomposed the lipid hydroperoxide.[8,9]

Phospholipids, however, can cause darkening of oils and produce off-flavors in finished oils because they are easily oxidized or discolored during heating or prolonged storage.[10,11] Evans et al.[10] reported that the presence of substantial amount of phospholipids can lead to dark-colored oil and to serve as precursor of off-flavors.

Phospholipids can also act as a prooxidant in oils due to higher content of unsaturated fatty acids which are susceptible to oxidation.[12]

The conflicting results as to the effects of phospholipids on the stability of soybean oil, as mentioned above, are considered to be due to the heterogenecity of substrate used, and variety of phospholipids studied, as well as the variety of analytical methods employed.

In this paper, attempts were made to identify 1) the actual roles of phospholipids and 2) the possible mechanism of phospholipids functions in the flavor stability of soybean oil under the defined conditions.

Materials and Methods

Materials

RBD soybean oil was obtained from a local refinery. PC, PE, PI, PA, phosphatidyl glycerol (PG) and cardiolipin (CL) were purchased from Supelco (Bellefonte, PA, U.S.A.) and their purities were higher than 90%. All reagents used were of analytical grade, unless otherwise specified.

Purification of RBD Soybean oil

RBD soybean oil (500*g*) was passed through 4.4×55*cm* column packed with 300*g* activated silicic acid. Silicic acid used in column chromatography was 100 mesh (Mallinckrodt 2847) and was activated according to Sahasrubudhe and Chapman.[13] The portion that passed through the column was considered to be the purified soybean oil.

Test of Phospholipids on the Falvor Stability

To study the effect of phospholipids on the flavor stability of soybean oil, the phospholipids were added to 1) purified soybean oil containign 1*ppm* ferrous stearate added as ferrous iron, and 2) purified soybean oil without iron added to obtain 300*ppm* (*w/w*) of phospholipids in both oils.

Five grams of experimental samples were transferred into a 30*ml* serum bottle and sealed air-tight with Teflon-coated rubber septum and aluminum cap. The samples were stored at 60°C in a dark forced-drift air-oven.

Flavor Stability Measurement

The flavor stability of oils were determined by a combination of volatile compound formation and molecular oxygen disappearence in the headspace of oils in air-tightly sealed bottles.[14] Gas chromatoraphy (GC) used for volatile compounds and oxygen content measurements was Hewlett-Packard GC HP-5880. For determination of volatile compounds in the headspace, 1*ml* of each headspace gas was injected directly into gas chromatograph. Column used for volatile compounds was stainless steel column (10′ × 1/8″ 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 nitrogen carrier gas was 20 *ml/min*.

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

The oxygen content in the headspace was determined using stainless steel column (6′ × 1/8″ OD) packed with Molecular Sieve 13X (Supelco Co.) and column temperature was 35°C. The flame ionization detector and thermal conductivity detector were calibrated for volatile compounds and molecular oxygen measurements using 1% ethyl hexaenoic acid in ethyl ether and air as references. GC peaks were quantified by electronic integrator and expressed in electronic counts.

Statistical Analysis

The qualitative and quantitative effects of phospholipids on flavor stability were analyzed by Duncan's Multiple Range Test programmed by Statistical Analysis System (SAS).[15]

Results and Discussion

Characteristics of RBD and Purified Soybean Oils

Activated silicic acid chromatography effectively removed yellowish color in RBD oil. The eluate from the silicic acid column, which was designated as purified soybean oil , was almost colorless. The efficiency of oil purification by silicic acid seems to be greatly affected by activation condition and kinds of silicic acid used. About 400*g* of purified soybean oil was obtained from 500*g* of RBD soybean oil by silicic acid column chromatography within 10 to 15 hrs under vacuum made by ordinary vacuum pump. Some activated silicic acid did not adsorb coloring material in RBD oil.

It was shown that the purified soybean oil has zero values in acid value, phosphorus content, iron content, and polar compounds, whereas original RBD soybean oil has acid value of 0.1, phosphorus content of 1 *ppm*, iron content of 0.27 *ppm* and polar compounds of 1.1%.[16] Silicic acid chromatography removed free acids, phospholipids and polar compound from RBD oil. Silicic acid chromatography also removes hydroperoxides and tocopherol compounds from RBD soybean oil according to our previous experiments[7,14,16] and from shrimp lipids.[17]

The purified soybean oil does not have any absorbance at 233 and 268*nm*, indicating that conjugated dienes and trienes were not present in the purified soybean oil.

Effects of Iron Added and Phosphatidyl Choline on Flavor Stability of RBD and Purified Soybean Oil.

The qualitative effects of 1 *ppm* ferrous iron added, and 300 *ppm* PC on the flavor stability of RBD and purified soybean oils are shown in Fig. 1 and 2. Figure 1 showed volatile compounds formation, and Fig. 2 showed molecular oxygen contents in the headspace of oil sample during storage.

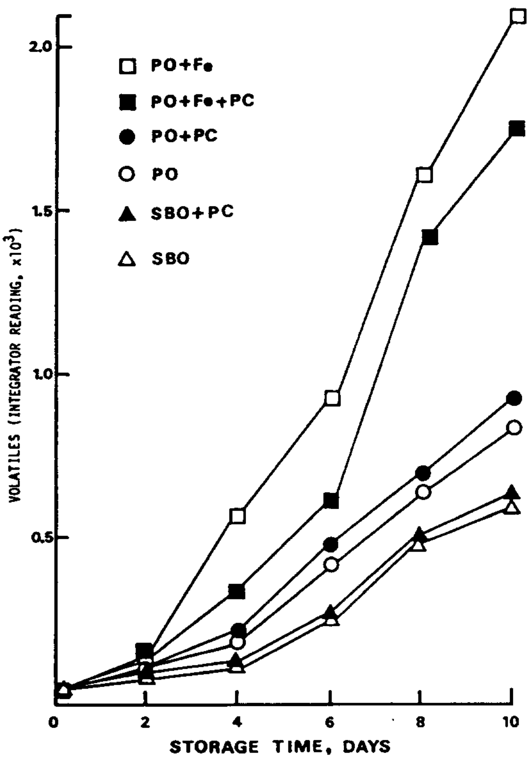

Fig. 1. Effects of Iron (Fe) Added and Phosphatidyl Choline (PC) on Volatile Compounds Formation (Integratior Reading) in RBD Soybean Oil (SBO) and Purified Soybean Oil (PO)

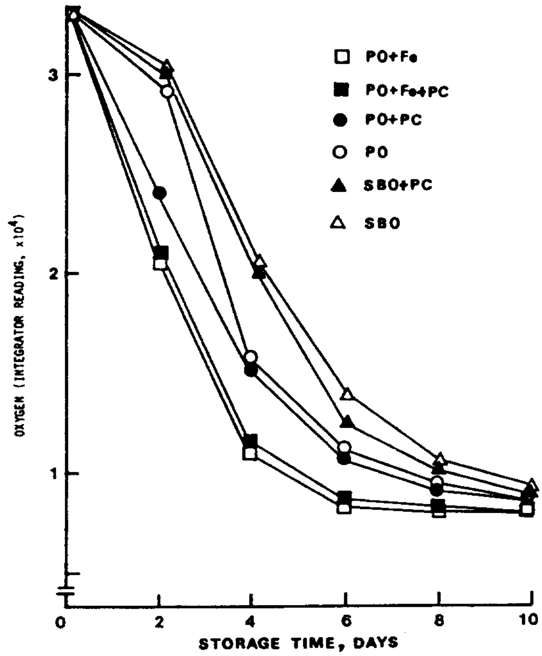

Fig. 2. Effects of Iron Added and Phosphatidyl Choline on Oxygen Disappearence (Integrator Reading) in Headspace of RBD and Purified Soybean Oil.

Legends are the same as in Fig. 1.

The total volatile compounds in the headspace of RBD oil increased from 40 to 600 after 10 days storage at 60°C. It is well known that as the volatile compounds in the soybean oil increases, the flavor quality decreases.[18-21] The volatile compounds were more readily produced in purified soybean oil, than in RBD soybean oil (840 vs. 40). This was considered to be due to the possible elimination of natural antioxidants such as tocopherols from RBD oil during silicic acid chromatography to obtain purified oil.

The formation of volatile compounds in purified oil was greatly accelerated by addition of 1*ppm* ferrous iron. The volatile compounds increased so fast, and reached 2,085 after 10 days storage. This result showed that 1*ppm* ferrous iron possessed the strong prooxidant activity in purified oil, as was expected.

When 300*ppm* of PC was added to purified oil containing 1*ppm* iron, the volatile compounds formation was largely retarded, and the volatile compounds was developed at 60% level of that from purified oil containing iron only. The retardation of volatile compounds formation by PC can be explained by the inactivation or reduction of prooxidant activity of metal irons by phospholipids.

PC(300*ppm*) in purified oil without iron added, however, showed prooxidant activity as shown in Fig. 1. The volatile compounds were formed at a level of 930 after 10 days storage. In RBD soybean oil, the addition of 300*ppm* PC did not significantly affect the volatile compounds formation.

Fig. 2 clearly showed that the effects of added ferrous iron and PC on molecular oxygen disappearence in the headspace. The oxygen content decreased as the storage time increased from 0 to 10 days at 60°C. The oxygen content decreased faster in the headspace of purified oil containing added iron than in purified oil without added iron.

PC was shown to retard the oxygen consumption in purified oil containing added iron, and to accelerate the oxygen consumption in purified oil without added iron.

These results showed that 300*ppm* PC possesses the antioxidant acitivity in the presence of 1*ppm* ferrous iron, and possesses the prooxidant activity in the absence of ferrous iron.

Comparision of Phospholipids Activities in Purified Soybean Oil

Several phospholipids present in soybean oil such as PC, PE, PI and PA, and PG and CL which are not present in soybean oil, were added individually to purified soybean oil at 300 *ppm* level to compare their activities in flavor stability of purified soybean oil. Purified soybean oil containing 1*ppm* ferrous iron added was used as control sample for the determination of antioxidant activity of phospholipids, and purified oil without iron was used for prooxidant activity of phospholipids.

The addition of PA and PE to purified soybean oil containing iron reduced significantly the formation of volatile compounds as shown in Table 1. In other words, PA and PE had considerable antioxidant effect. PC, PG, CL and PI appeared to have less antioxidant activity in decreasing order, and they had no statistically significant effects compared to control sample.

The effects of phospholipids on the voaltile compounds formation in purified soybean oil without added iron are shown in Table 2. Purified oil added with PE produced nearly the same amount of volatile compounds of control sample, which indicates the little prooxidant activity of PE. CL and PG showed the higher prooxidant activity in terms of volatile compounds formation than PA, PI and PC in decreasing order of prooxidant activity.

Amphi-functionality of Phospholipids in Soybean Oil

Hildebrand et al.[4] suggested that the nitrogen moieties of PE and PC, and the reducing sugar of PI donate hydrogen or electron to tocopherols, thus delaying the oxidation of tocopherols to tocopheryl quinone, which indicated the synergistic effect of phospholipids for primary antioxidants. Phospholipids have also been known as chelating agents in oil.[7] It was reported that the addition of 0.1% crude phosphatides to soybean oils prior to deodorization improved the oxidation stability, but produced the oil having dark color and undesirable melony-cucumber flavors.[4]

Table 1. Duncan's Multiple Range Test for Effects of Phospholipids on Volatile Compounds Formation (Integrator Reading) in Purified Soybean Oil (PO) Containing 1ppm Ferrous Iron (Fe)*

Sample	Storage days					Mean**	Duncan's group**
	2	4	6	8	10		
PO	99	198	425	636	840	440	B
PO+Fe+PA	99	276	482	734	1101	538	B
PO+Fe+PE	100	247	502	800	1133	556	B
PO+Fe+PC	162	344	603	1433	1771	863	A
PO+Fe+PG	98	354	638	1479	1800	874	A
PO+Fe+CL	91	385	922	1290	1700	878	A
PO+Fe+PI	113	448	742	1608	1880	958	A
PO+Fe	151	588	930	1609	2085	1073	A

* Abbreviations are the same as described in the text.

** Means with the same letter are not significantly different.

Table 2. Duncan's Multiple Range Test for Effects of Phospholipids on Volatile Compounds Formation (Integrator Reading) in Purified Soybean Oil (PO)*

Sample	Storage days				Mean**	Duncan's group**
	2	4	6	8		
PO	99	198	425	636	340	C
PO+PE	97	200	425	638	340	C
PO+PC	108	215	482	698	376	BC
PO+PI	126	230	462	729	387	ABC
PO+PA	103	268	529	710	403	ABC
PO+PG	95	245	596	892	457	AB
PO+CL	102	280	585	907	469	A

* Abbreviations are the same as described in the text.
** Means with the same letter are not significantly different.

It was shown that phospholipids worked as prooxidants in the oil containing no metals because the hydrophilic groups (polar groups) of the phospholipids will not be dissolved in the oil. Therefore, the polar groups of phospholipids will be situated on the surface of oil and increase the solubility of oxygen in the oil which accelerates the oil oxidation.

It is recommendable that phospholipids should be removed from the oil if the oil does not contain metals, and the phospholipids content in the oil should be just enough to chelate metals. The excessive phospholipids above the mount needed to chelate metals in oil will act as prooxidants to decrease the oxidation stability. In other words, the oil should have optimium phospholipids content to prevent the dark color and off-flavor, and to increase oxidation stability, accordingly flavor stability of oil

요 약

Silicic acid chromatography로 순수정제한 대두유의 향미안정성에 미치는 인지방질의 영향을 조사하였다. 1*ppm* ferrous iron을 첨가한 순수정제 대두유에 300*ppm*의 phosphatidyl choline(PC), phosphatidyl ethanolamine(PE), phosphatidyl inositol(PI), phosphatidic acid(PA), phosphatidyl glycerol(PG), cardiolipin(CL)을 개별적으로 첨가하여 60℃의 오븐에서 10일간 저장한후, 시료병 윗 공간내의 총휘발성물질양 및 산소의 잔유량으로써 향미안정성을 측정하였다. 1*ppm*의 철분을 함유한 시료의 향미안정성은 인지방질을 첨가함으로써 개선되었는데, 이는 인지방질의 금속이온에 대한 chelating 효과에 기인한 것으로써, PA, PE, PC, PG, CL, PI의 순으로 효과적이었다. 그러나 철분을 함유하지 않은 순수정제 대두유에 인지방질을 첨가했을 경우, 그 향미안정성은 감소하였다. 즉 금속이온이 없을 경우 인지방질은 산화촉진효과를 나타내었는데, 이는 인지방질의 극성부분으로 인하여, 유지의 계면에 용존산소의 농도가 증가하는 데에서 기인하는 것으로 생각한다. 그러므로, 유지의 향미안정성을 개선시키기 위하여는 유지내 인지방질의 농도를 금속이온을 chelating 시킬 적당량만을 유지하는 것이 바람직한 것으로 생각된다.

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