

Soybean lecithin: acetone insoluble residue fractionation and their volatile components

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RESUMEN

Lecitina de soja: fraccionamiento del residuo insoluble en acetona y sus componentes volátiles.

El residuo insoluble en acetona fue aislado de la lecitina de soja. Este residuo fue fraccionado por solventes en cuatro fracciones: soluble en ácido acético, insoluble en ácido acético, fase benceno y fase insoluble en benceno.

Concerniente a la constitución de los fosfolípidos de estas cuatro fracciones, se encontró que la primera fracción contiene PC, PE y PI en porcentajes del 56.0, 21.6 y 19.0 respectivamente. La segunda fracción tuvo 39% PC y 60% CER, junto a algunas trazas de PE y PI. La fase benceno está constituida principalmente por PC con algunas trazas de PE. La última fracción tuvo 80.6% CER y 20% PC. La composición en ácidos grasos de estas cuatro fracciones junto a los solubles en acetona, aceite de soja crudo y desgomado y fosfolípidos totales fueron registradas. Por lo general, se encontró que los ácidos grasos mayoritarios saturados e insaturados fueron el palmítico y linoléico. Los componentes volátiles de estas muestras excepto la insoluble en ácido acético fueron determinadas. Cuarenta y nueve compuestos fueron separados. Treinta y dos componentes incluyendo aldehídos alifáticos, cetonas, alcoholes, ésteres y ácidos fueron identificados. Los aldehídos y cetonas mostraron un cambio en las siete muestras. Estos aumentaron por el desgomado.

El 4,5-dimetilelisoxazol tuvo un fuerte flavor a lecitina, así puede usarse como indicador para el proceso de desgomado. El 2-pentilfurano mostró una disminución significativa por el desgomado. Otros compuestos, tales como ésteres y alcoholes no tuvieron efectos distintivos sobre los productos volátiles a lo largo del proceso.

PALABRAS-CLAVE: *Aceite desgomado – Acetona – Acido acético – Componentes volátiles – Fracción bencénica – Fosfolípido – Lecitina de soja.*

SUMMARY

Soybean lecithin: acetone insoluble residue fractionation and their volatile components.

The acetone insoluble residue was isolated from soybean lecithin. This residue was solvent fractionated resulted in four fractions, namely, acetic acid soluble, acetic acid insoluble, benzene phase and benzene insoluble phase.

Concerning phospholipid constitution of these four fractions, it was found that the first fraction contains PC, PE and PI in percentages of 56.0, 21.6 and 19.0 respectively. The 2nd fraction makes 39% PC and 60% CER, besides some traces of PE and PI. The benzene phase is mainly all PC with some traces of PE. The last fraction is 80.6% CER and 20% PC. The fatty acid composition of these four fractions besides soluble in acetone, crude and degummed soybean oil and total phospholipids was recorded. Generally, it was found that the major saturated and

unsaturated fatty acids were palmitic and linoleic. Volatile components of these samples except acetic acid insoluble were reported. Forty nine compounds were separated. Thirty two components including aliphatic aldehydes, ketones, alcohols, esters and acids were identified. Aldehydes and ketones showed a changed through the seven samples. They increased by degumming.

4,5-Dimethylelisoxazole had a strong lecithin like flavour, so it can be used as an indicator for the degumming process. 2-Pentylfuran showed a significant decrease by degumming. Other compounds, such as esters and alcohols had no distinguish effect on the volatile products through process.

KEYWORDS: *Acetic acid - Acetone - Benzene fractions - Degummed oil – Phospholipid - Soybean lecithin - Volatile components.*

1. INTRODUCTION

The soybean is a commercial source of lecithin (1). Worldwide consumption of this by-product of soybean processing is estimated at 10,000 tons / year (2). Industrially, lecithin is removed by treating the crude oil with water (2), inorganic and organic acids (3) or acetic anhydride (4) at elevated temperatures; the gums precipitate from the oil and are removed by centrifugation or sedimentation.

The term "lecithin" as used to day refers to the material obtained by degumming crude vegetable oils and drying the hydrated gums. Lecithin, then, consists not only of a mixture of phospholipid components removed from the degumming process.

Brain (1) gives the proximate composition of commercial crude soybean lecithin (soy lecithin) as phosphatidylcholine, 20%; phosphatidylethanolamine, 20%; soybean oil, 35%; and sugars, sterols and moisture, 5%.

2. MATERIALS AND METHODS

2.1. Preparation of soy lecithin

Soybean oil (2.000 g) was charged into a 3 liter round bottom flask with a stirring shaft and a paddle shaped Teflon impeller 7.5 cm long driven by a variable speed motor. The oil was purged with nitrogen through a sinter glass stick for minute and brought to the desired temperature under a nitrogen

blanket; then the motor was started, and the desired amount of distilled water was added. When degumming was completed, the mixture was cooled to 40°C and the soy lecithin was separated by centrifugation at 1.900 rpm for 15 minute. The degummed oil was removed by decantation. Soy lecithin was purified by extraction of the rest of soybean oil with n-hexane and was separated by filtration and washed several times with the same solvent and dried under vacuum at 40°C while bubbling nitrogen then kept under nitrogen in refrigerator.

2.2. Isolation of the acetone - insoluble residue

Fifty grams of soy lecithin were dissolved in the least amount of ether and the solution then filtered through Whatman No. 1 filter paper on a Buchner funnel. Twenty volumes of cold acetone were added to the filtrate, small portion at a time while continuous stirring. The solution was then allowed to settle over night in the refrigerator. The acetone - insoluble residue was centrifuged and then successively washed with voluminous amounts of cold acetone. The acetone insoluble residue was redissolved in a small volume of diethyl ether and reprecipitated with acetone in the same manner.

2.3. Solvent fraction of the acetone - insoluble residue

The acetone insoluble residue was slurried with glacial acetic acid (1: 3, v/v) in a warring blender. The suspension was poured into 350 ml glacial acetic acid in a 2 liter beaker while continuous stirring. The suspension was allowed to stand at room temperature for 2 hour and then filtered through Whatman No. 1 filter paper on a Buchner funnel. The residue was re-extracted with 200 ml of glacial acetic acid in the same manner. The acetic acid - soluble (fraction I) was evaporated under vacuum at 45°C while bubbling nitrogen in the solution and the residue was dissolved in 20 ml of hot distilled water and re-evaporated in the same manner, and the process repeated to get ride of all acetic acid vapours. The acetic acid - insoluble (fraction II) was suspended in 100ml of benzene and 50 ml distilled water in a centrifuge bottle. The bottle was shaken vigorously for 15 minutes and then centrifuged. The benzene phase was re- extracted twice with 75 ml portions of benzene. The combined benzene extracts, after being dried over anhydrous Na₂ SO₄ (fraction III) and the benzene - insoluble phase, aqueous phase, (fraction IV) were evaporated as described previously in acetic acid - insoluble fraction I.

2.3. Fatty acid composition of Crude soybean oil, Degummed oil, Acetone soluble, Acetic acid soluble and total phospholipid, besides Benzene insoluble phase and Benzene phase

The component fatty acids of these seven samples were converted to their methyl esters by esterification (5), the mixed methyl esters were subjected to gas liquid chromatographic (GLC) analysis (6).

2.4. Volatile components of Crude soybean oil, Degummed soybean oil, Acetone soluble and Acetic acid soluble besides Total phospholipid, Benzene insoluble phase and Benzene phase

The volatile components of soluble in acetone, soluble in acetic acid, benzene phase and benzene insoluble phase fractions were prepared. Volatile components of crude soybean oil, Degumming soybean oil and total phospholipid were also collected for comparison.

The identification of the components of each flavour was done using coupling gas liquid chromatography-mass spectrometry (Varian 1400-Mat 112). Temperature of ion source of mass spectrometry is 200°C under reduced pressure 10⁻⁶ torr and electron volt 70 ev.

2.5. Gas liquid chromatography

It was done using Varian 3700 dual flame ionization detector under the following conditions:

Column package 20% Diethylene glycol succinate (DEGS) on chromosorb W(60-80 mesh), column length 6 feet with internal diameter ¼ inch (stainless steel).

Column temperature for flavour 70-190°C with programming rate 4°C / minute.

Injection temperature 220°C.

Detector temperature 300°C.

Carrier gas (He) flow rate 30ml / minute.

Hydrogen flow rate 30ml / minute.

Air flow rate 300 ml / minute.

2.6. Preparation of the Aroma concentrate

The aroma concentrate of each sample was as follow :

Suitable amount of the sample was heated on silicon oil bath at 190°C in 500 ml round bottom flask under reduced pressure (about 30 mm Hg). The aroma was collected in traps cooled to -20°C using crushed ice-salt- acetone mixture. The collected distillate was thrice extracted with peroxide free ether, and the combined extracts were dried over

anhydrous sodium sulphate. The extract was filtered and ether was then removed by distillation at 40°C to obtain the aroma concentrate.

3. RESULTS AND DISCUSSION

3.1. Phospholipid Constitution of Acetic Acid Soluble (A.S.), Benzene Phase (B.P.), and Benzene Insoluble Phase (B.I.)

It can be seen from table I that:

1. The acetic acid soluble contain PC, PE and PI in percentages of 56.0, 21.6 and 19.0 respectively.
2. The benzene phase mainly all PC with some traces of PE.
3. The major part of the benzene insoluble phase was CER which makes 80.6 % and PC makes 20 %.

Table I

Phospholipid Constitution of Fractions Acetic Acid Soluble, Benzene Phase and Benzene Insoluble Phase

Samples	A.S.	B.P.	B.I.
PC (LE)	57.9	99.9	19.86
PE	22.3	trace	trace
PI	19.6	trace	trace
PA	trace	trace	trace
CER	trace	trace	80.04

Where : AS = Acetic Acid Soluble .
BP = Benzene Phase Fraction.
BI = Benzene Insoluble Phase.
CER = cerebroside (glycolipid).

Table II

Fatty Acid Composition of Fractions: Crude Soybean Oil, Degummed Oil, Acetone Soluble, Acetic Acid Soluble Besides Total Phospholipid, Benzene Insoluble Phase, and Benzene Phase

Fatty Acid	C.O.	D.O.	S.A.	A.S.	P.L.	B.I.	B.P.
16:0	14.4	10.2	13.7	33.8	15.7	32.4	20.2
18:0	2.4	4.4	2.6	6.0	7.5	8.0	5.9
18:1	25.9	25.3	25.4	12.0	12.8	11.7	10.9
18:2	49.9	50.2	49.7	45.1	60.1	45.8	58.5
18:3	7.4	9.9	8.5	3.0	3.8	2.0	4.5

Where : C.S.O. =Crude Soybean Oil Fraction.
D.S.O. = Degummed Soybean Oil Fraction.
S.A.= Acetone Soluble Fraction.
P.L.= Total Phospholipid Fraction (Acetone Insoluble).

3.2. Fatty Acid Composition of Crude soybean oil (C.O), Degummed oil (D.O), Acetone soluble (S.A), Acetic acid soluble (A.S) Besides Total phospholipid (PL), Benzene insoluble phase (B.I) and Benzene phase (B.P)

Table II shows the fatty acid composition of these compounds.

The major fatty acids are linoleic acid and palmitic acid. The ratio of saturated-unsaturated fatty acids ranges from 1:1.5 to 1:5.85.

3.3. Volatile Components of Crude soybean oil (C.O), Degummed oil (D.O), Acetone soluble (S.A), Acetic acid soluble (A.S) Besides Total phospholipid (PL), Benzene insoluble phase (B.I) and Benzene phase (B.P)

The gas chromatograms of the volatile components of crude soybean oil, degummed oil, acetone soluble, acetic acid soluble, total phospholipids, benzene insoluble phase and benzene phase together with their concentrations are illustrated in table III.

Fourty nine compounds were separated. Thirty two compounds including aliphatic aldehydes, ketones, alcohols, esters and acids were identified.

Among these compounds, aldehydes are formed to be present in high concentration reaching more than 55 % in crude oil 2,4 undecadienal and 2,4,6 dodecatrienal has the highest concentration 11.94 % and 15.64 % respectively among them.

The short chain aliphatic aldehydes showed a remarkably decrease for the total phospholipid.

The lack of these aldehydes (C₃ - C₁₂) in the volatile decomposition products of phospholipid may result from the immediate reaction of the aldehydes produced from the fatty acid moieties towards the amino group of phospholipids, especially phosphatidylethanolamine (7,8). 2, Pentylfuran and 4,5 Dimethylisoxazole were identified in the volatile components of total phospholipid. 2,4-Dimethylisoxazole may derive from the reaction of 1,3-dicarbonyl compound with the decomposition products phosphatidylethanolamine (9).

The mechanism that forms ketones, aldehydes, alcohols and esters through the decomposition of hydroperoxides of the unsaturated fatty esters has been reviewed (10). The aliphatic aldehydes (saturated and unsaturated) showed a remarkable increase and in particularly 2,4 undecadienal (21.25 %) and 2,4,6 dodecatrienal (23.01 %) among the volatile compounds of degummed soybean oil. This was in agreement with Kim *et al.* (11), who found that oil free

Table III
 Volatile Components of Crude Soybean Oil (C.O.S), Degummed Oil (D.O.), Acetone Soluble (S.A.), Acetic Acid Soluble (A.S.), Total Phospholipid (P.L.), Benzene Insoluble Phase, and Benzene Phase

Peak No.	tR	C.O.	D.O.	S.A.	A.S.	PL	B.I.	B.P.	Compounds
1	1.2	1.4	trace	0.21	0.16	0.33	trace	trace	Ethanol
2	2	trace	0.35	0.68	1.74	0.09	trace	trace	Propanol
3	2.3	trace	trace	trace	0.39	0.41	trace	trace	Unidentified
4	3	trace	0.41	1.42	1.29	0.25	trace	trace	Butanoic acid
5	5	0.8	trace	0.44	0.15	0.38	trace	trace	2-Hexenol
6	5.4	trace	0.44	0.44	0.22	0.88	trace	trace	Unidentified
7	6.5	trace	trace	0.21	0.29	trace	trace	trace	Hexanal
8	7.7	trace	trace	0.28	1.52	0.82	2.02	trace	Methyl Valerate
9	9	trace	0.38	trace	1.54	0.44	1.43	trace	Hexanoic acid
10	10	trace	0.76	1.68	0.42	0.35	2.99	trace	Methylhexanoate
11	11	2.69	0.35	2.25	0.22	0.84	trace	trace	Heptanal
12	12	trace	0.81	0.33	0.89	0.51	trace	trace	Octanal
13	13	1.2	trace	trace	0.09	0.27	3.49	trace	Nonanal
14	15	0.33	trace	0.94	0.14	trace	1.78	trace	Decanal
15	16	trace	trace	0.37	0.26	0.41	trace	trace	Unidentified
16	17.5	1.42	trace	1.04	0.56	1.02	trace	4.26	1-Octaan-3-ol
17	18.7	1.35	0.29	0.19	0.27	0.17	4.65	trace	Hendecanal
18	20.3	0.85	0.76	3.21	0.1	trace	3.71	4.26	Octanol
19	21.6	trace	0.85	0.27	0.94	trace	5.56	3.39	Dodecanal
20	22.3	trace	trace	1.01	0.89	0.49	0.44	7.3	Unidentified
21	23.2	0.47	8.06	0.7	3.92	0.53	9.77	13.88	Decanoic Acid
22	23.4	trace	trace	trace	trace	0.82	trace	trace	2-Pentyl furan
23	23.7	trace	trace	trace	0.43	0.65	1.5	trace	4,5Dimethylisoxazol
24	23.9	6.4	11.38	6.23	5.39	1.09	9.27	7.62	2-Nonenal
25	24.1	1.89	trace	0.51	0.56	trace	2.28	3.85	2,5 Hexadione
26	25.3	2.26	0.58	4.24	1.62	0.82	5.35	trace	2,4 Nonadienal
27	27.9	3.51	trace	10.45	2.11	1.46	4.2	3.35	3 Ethyl hexanone
28	28.5	2.62	trace	0.33	trace	3.33	3.16	2.29	3 Ethyl heptanone
29	30.2	4.39	trace	1.18	2.22	1.76	trace	trace	3 Ethyl octanone
30	31.3	2.43	15.9	4.51	2.52	1.63	8.86	1.83	Ethyl nonanone
31	32.7	5.9	1.35	2.16	3.63	1.45	4.29	trace	2,4Decadienal
32	34.8	0.47	trace	1.84	1.89	1.82	3.2	trace	Dodecanol
33	35.1	trace	trace	0.34	0.37	0.89	3.7	trace	Unidentified
34	36.9	10.9	0.86	0.68	1.69	1.38	2.37	4.59	Undecanol
35	37.2	trace	trace	1.14	0.86	0.46	0.44	0.47	Unidentified
36	39.2	1.22	trace	trace	trace	0.19	trace	trace	2-Nonenal
37	41.2	10.77	18.04	trace	2.69	7.69	trace	trace	2,4Undecadienal
38	42.1	0.33	0.81	0.46	1.62	0.54	0.44	0.47	Unidentified
39	43.0	trace	trace	trace	1.39	1.47	0.88	0.94	Unidentified
40	43.5	trace	trace	trace	0.35	1.33	trace	0.94	Unidentified
41	44.0	trace	1.76	2.48	1.22	3.97	0.88	1.88	Unidentified
42	45.1	trace	trace	1.66	2.04	0.37	0.26	0.47	Unidentified
43	46.8	trace	trace	0.51	1.38	0.19	0.17	0.47	Unidentified
44	47.5	14.11	19.53	39.13	22.58	21.46	trace	32.74	2,4,6Dodecatrienal
45	51.7	5.27	trace	trace	17.69	3.57	0.44	0.47	Unidentified
46	55.7	7.09	trace	trace	3.10	1.18	trace	trace	Unidentified
47	56.0	trace	trace	trace	1.47	20.53	trace	trace	Unidentified
48	58.0	trace	trace	trace	trace	4.74	trace	trace	Unidentified
49	59.0	trace	trace	trace	trace	trace	trace	trace	Unidentified

soybean lecithin enhance the formation of aliphatic aldehydes.

The volatile components of acetone soluble have high concentration of aldehydes, in particularly 2,4,6 dodecatrienal (41.29 %). This fraction is neutral lipid, in which by heating gives rise to aliphatic aldehydes,

ketones, esters, and alcohols. The large number of aldehydes with these high concentrations have been reported in the volatile decomposition products of neutral lipids (12,13). Ketones are forming the secondary remarkable concentrations after aldehydes, in the volatile components of acetone

soluble. They contained the highest percent of aldehydes, followed by ketones then alcohols and esters. No significant variations were observed in their three samples.

Concerning the volatile components of the benzene insoluble phase, it contained high percent of short chain aldehydes (C₉, C₁₀, C₁₁, C₁₂) and unsaturated aldehydes 2- nonenal, 2,4 nonadienal (3.98 %, 2.03 %, 5.3 %, 6.34 %, 10.57 % and 6.1 %) respectively. Decanoic acid was present in high concentration 11.14 % compared to all the other mentioned samples.

Benzene phase showed a quite different constitution. Many component disappeared, in particularly short chain aldehydes. Decanoic acid and 2,4,6 dodecatrienal were present in very high concentrations (14.8 %, and 34.9 % respectively).

4. CONCLUSION

From the above forgoing results, it is concluded that:

1. Aldehydes showed a remarkable change through the seven samples.

2. Also ketones showed the same trend as aldehydes, as they form through the decomposition of hydroperoxides of unsaturated fatty acids.

3. Aldehydes and ketones have the largest amount among all the components, as they considered the responsible for the odour of heating sample.

4. 4,5 Dimethylisoxazole had a strong lecithin like flavour, so it can be used as an indicator for the degumming process.

5. 2-Pentylfuran showed a significant decrease by degumming, it is formed from the autoxidation decomposition of linoleate.

6. Other compounds such as esters and alcohols were seen to have no distinguish effect on the volatile products through degumming process.

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