

Studies on Processing and Analysis of Red Pepper Seed Oil

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고추씨 기름의 정제와 분석에 관한 연구

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Abstract

An attempt was made to remove red color and capsaicin which were contaminated in crude oil extracted from red pepper seeds with *n*-hexane. Both clay bleaching and deodorization were responsible for the removal of red color. Clay bleaching was also the most effective for the removal of capsaicin, which was removed completely with 4 % clay.

Capsaicin in oil was adsorbed onto the clay particles exponentially and it followed Freundlich equation: $\frac{x}{m} = 1.1 c^{0.31}$, where x is amount of capsaicin adsorbed (*mg/ml*), m is amount of clay used in bleaching (%) and c is amount of residual capsaicin (*mg/ml*).

Neutral lipid of the seed oil consisted of 95.8 % triglyceride, 1.55 % sterol ester, 1.45 % monoglyceride, 0.78 % free fatty acids, 0.2 % diglycerides and 0.15 % sterol. Major components of fatty acids in the red pepper seed oil were linoleic acid (78.79 %), palmitic acid (15.27 %) and linolenic acid content was only 0.63 % by high performance liquid chromatography.

Introduction

Annual local production of red pepper in 1978 reached 93,500 %⁽¹⁾. The amount of seeds were approximately 10,000 %, equivalent to 10 to 12 % of the whole production⁽²⁾. The consumption pattern of red pepper seed is diverse. Park and Chun⁽³⁾ reported that only 45.5 % of the seeds were utilized as resource of edible oil. The rest of the seeds are either consumed with red pepper or discarded.

Even though part of the seeds have been locally processed for edible oil production, the characteristics and processing methods of the oil have not been well documented. Furthermore, results on fatty acid composition and neutral lipid composition of the oil published in several research papers are in conflict⁽⁴⁻⁶⁾. Therefore, we decided to study the processing of the seed oil and to characterize the oil in the hope that the red pepper seeds can be utilized efficiently as an edible oil resource in Korea. Specific objectives are to determine the neutral lipid composition and fatty acid composition of the seed oil

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and to develop the process for removal of capsaicin and color pigments.

Materials and Methods

Materials

Red pepper seeds were obtained from local markets at Seoul and at Suwon, Kyungki-do. Activated clay was the product of Wako Pure Chemicals, Osaka, Japan. Monopalmitin, dipalmitin, tripalmitin, cholesterol, cholesterol palmitate, phosphatidyl ethanolamine, linoleate and capsaicin were purchased from Sigma Chemicals, St. Louis, U.S.A. Acetonitrile, tetrahydrofuran and silica gel were the products of Merck, Darmstadt, W. Germany. All other reagents were analytical grade. Waters Associates ALC/GPC 244 was used for high performance liquid chromatography (HPLC) and Varian Aerograph 204 for gas liquid chromatography (GLC).

Oil processing

1. Pretreatment and solvent extraction

To remove red pepper particles and other undesirable material, the seeds were passed through No. 5 mesh sieve and then through No. 7 mesh sieve. The seeds were crushed with a Wiley mill and then the crushed seeds were extracted with *n*-hexane at 55°C in a specially-made extractor. After extraction, *n*-hexane were evaporated from the crude oil.

2. Degumming

In order to reduce refining loss, the crude oil was degummed by adding approximately 2 % water and 500 ppm of phosphoric acid, followed by agitation at 70°C for 30 min. As gum was hydrated, the mixtures were centrifuged at 500 r.p.m. for 15 min in a RC-5 superspeed refrigerated centrifuge to remove the gummy material.

3. Caustic refining

Caustic refining was done according to the dry method⁽¹⁹⁾.

4. Clay bleaching

In order to test the possibility for the removal of color pigments as well as capsaicin, seven levels of activated clay (between 0.5 % and 4.0 % with an increment of 0.5 %) were added to the oil. The slurry was heated to 115°C and bleached under nitrogen gas blanketing with vigorous agitation for

20 min at that temperature. After 20 min, the slurry was filtered through a Büchner filter.

5. Deodorization

Deodorization was done according to Hunt-wesson test method⁽²⁰⁾.

Analysis

1. General characterization

Red pepper seeds were analyzed for crude fat^(7a), crude protein⁽⁸⁾, crude fiber⁽⁹⁾, moisture^(7b) and ash^(7c). Characteristic values of the seed oil such as specific gravity^(7d), refractive index^(7e), acid value^(7f), iodine value^(7g), unsaponifiables^(7h), phosphatides⁽⁷ⁱ⁾, color^(7j) were also measured.

2. Neutral lipid composition

Amenta's method⁽¹⁰⁾ was used for the determination of neutral lipid composition of the crude oil.

3. Fatty acid composition

Fatty acid composition of the crude oil was determined with high performance liquid chromatography and gas liquid chromatography. The types and ope-

Table 1. Operating conditions of high performance liquid chromatography and gas liquid chromatography

	HPLC	GLC
Instrument	Waters, ALC/GPC 244	Varian Aerograph 204
Detector	RI, R 401	FID
Column	Fatty acid	5 % FFAP on Chromosorb-W, 20' × 1/8" stainless steel
Solvent	THF* : AN** : H ₂ O = 25:45:35	Diethyl ether
Flow rate	1.0 ml/min	—
Attenuation	8x	—
Injector	U6K	—
Detector temperature	—	250°C
Injector temperature	—	200°C
Column temperature	—	Initial 50°C Final 225°C
Programmed rate	—	8°C/min
Carrier gas	—	N ₂ , 30 ml/min

* Tetrahydrofuran

** Acetonitrile

rating conditions of HPLC and GLC are shown in Table 1. Saponification and methyl esterification of the fatty acid were done according to Christie's method⁽¹¹⁾ and Metcalfe's method⁽¹²⁾, respectively.

4. Quantative determination of capsaicin in oil

Many methods have been investigated to determine capsaicin in fruits.⁽¹³⁻¹⁸⁾ However, there has been no precise and accurate method for quantitative determination of capsaicin in oil. Hence, it was required to modify Gonzalez's method⁽¹⁶⁾ to determine capsaicin in the seed oil accurately.

a. Sample preparation

One ml of the oil and 9 ml of 99.5 % ethanol were mixed thoroughly and kept for 20 min at 70°C followed by centrifugation at 10,000 r.p.m. for 15 min. After settling for 24 hr, 5 ml of ethanol were decanted. Ethanol extraction was prepared once more following the above procedure, combined together and concentrated to 1 ml.

b. Thin layer chromatography

Thin layers of silica gel G was spread on 20×20 cm glass plate, and the adsorbent was activated for 1 hr at 105°C before use. The plates were marked out into 4 cm lanes. The standard capsaicin solution was applied to one lane and the sample solution to another. The plate was developed in chloroform-ethyl acetate (1:1, v/v). After 60~90 min, the plate was removed, air-dried for a few minutes, and heated briefly to remove traces of solvent. The outside reference lane was examined after spraying with 1 % KMnO₄ in 2 % Na₂CO₃ solution, and spots corresponding to capsaicin were scraped from the plate, and transferred to mini-column (1×15 cm). Capsaicin in silica gel was extracted with 4 ml isopropanol, and measured the absorbancy at 281 nm. The absorbancy

of several known amounts of pure capsaicin in isopropanol were measured at 281 nm. Standard curve was obtained for capsaicin content *vs* absorbancy.

Results and Discussion

Proximate analysis

Table 2 shows proximate analysis of the red pepper seeds. Oil content of the seed was 26.3 %, higher than that of other seeds such as soybean, sunflower and cotton seeds.

General characterization of crude and finished oils

Chemical and physical characteristics of both crude and finished oils were shown along with those for crude soybean oil in Table 3. As shown in the table, specific gravity, refractive index and phosphatides for the crude red pepper seed oil were lower than those for crude soybean oil. It was also shown that the phosphatides in the crude seed oil was removed completely during the degumming, and caustic refining process and possibly during clay bleaching processes. The red pigments were substantially removed during clay bleaching (with 4 % activated clay) and deodorization processes. Specific gravity and refractive index for the finished seed oil were higher than those for the crude oil. Acid value for the crude oil was 1.18, whereas that for the finished oil was 0.12. Before caustic refining, we need to know acid value (or free fatty acid) for determination of amount of caustic lye to be added to degummed oil. However, it was very difficult to detect the end point for determination of the acid value. The reason was due to the deep red color of the degummed oil caused by adhesion of red pepper powder on the seed and red pepper particles passed through No. 7 mesh sieve and subsequent extraction of the red pigments along with the crude oil from the crushed seed. Therefore, it was necessary to devise a method for acid value determination; 100 g of whole seed was washed in 200 ml of the boiling *n*-hexane for 2 hr to remove the pigments adhered to the seeds. The washed seeds were separated from *n*-hexane, dried, crushed with a Wiley mill and then extracted with *n*-hexane at 55°C. The crude oil

Table 2. Proximate composition of red pepper seed

	Content (%)
Crude fat	26.3
Crude protein	18.5
Crude fiber	33.9
Moisture	9.0
Ash	2.8
Nitrogen free extract	9.5

Table 3. Chemical and physical characteristics of crude and finished red pepper seed oils

	Crude oil	Finished oil	Crude soybean oil
Specific gravity (25/25°C)	0.897	0.919	0.917
Refractive index (25/25°C)	1.4649	1.4738	1.470~1.476
Acid value	1.18	0.12	1.0
Iodine value (Wijs)	129.5	131.5	120~141
Unsaponifiables (%)	1.34	1.35	1.5
Phosphatides (%)	0.25	0	1.5~2.5
Lovibond color*	Y : 5.0 R : 61.0	Y : 20.0 R : 2.0	— —

* Crude oil color measured in 1" cell and finished oil color measured in 5 1/4" cell.

Table 4. Neutral lipid composition of red pepper seed oil

Lipid	Content (weight %)
Triglyceride	95.87
Sterol ester	1.55
Free fatty acid	0.78
Monoglyceride	1.45
Diglyceride	0.20
Sterol	0.15

Table 5. Comparison of fatty acid composition by HPLC and GLC

No. of carbon	HPLC (weight %)	GLC (weight %)
14	0.22	0.37
16	15.27	15.82
16 : 1	0	0.43
18	0.57	2.46
18 : 1	2.93	10.30
18 : 2	78.79	70.10
18 : 3	0.63	0.53
20	0.45	0
22	1.14	0

obtained this way was light in color enough to determine the acid value. Extraction loss of the oil from the whole seeds during this process was only 0.07 %. Hence, this procedure can be incorporated into quality control procedure for determination of acid value (or free fatty acid) so that the caustic refining can be done efficiently.

Determination and removal of capsaicin in oil

The crude oil not only showed deep red color, but also had hot burning taste. Fig. 1 shows that capsaicin

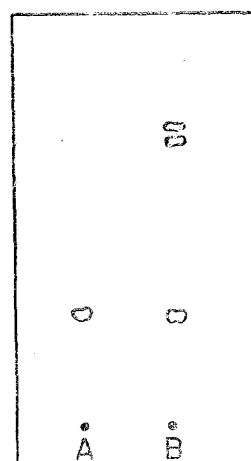


Fig. 1. Thin layer chromatogram

A : Standard capsaicin

B : Capsaicin from red pepper seed oil

was separated from the oil. The R_f value of capsaicin in solvent system of chloroform and ethylacetate (1 : 1, v/v) was 0.35. The small traces of oil and pigments were developed near to solvent line. The capsaicin band on the silica gel was scraped off, extracted with isopropanol and measured absorbance at 281 nm. Throughout the processing, the oil was flavor-panelled at each step for detection of hot burning taste and capsaicin was also determined to find out which step would remove the capsaicin. It was found that clay bleaching was the main step that removed the capsaicin and level of clay affected the removal of capsaicin. For example, capsaicin content of the crude oil was reduced from 2.76 mg/ml to 0.06 mg/ml after caustic refining and clay bleaching with 2 % clay. However, the capsaicin content decreased to only 0.42 mg/ml, when the

bleached oil was deodorized.

However, when the deodorized oil was rebleached with another 2 % clay, we could not detect the hot burning taste. Therefore, it could be concluded that the capsaicin was adsorbed onto the clay and removed from the oil, whereas the effect of deodorization for removal of capsaicin was not significant.

Consequently, a series of tests was conducted to find out the relationship between capsaicin content in oil and level of the clay used in bleaching. Fig. 2 shows the results which indicate that capsaicin

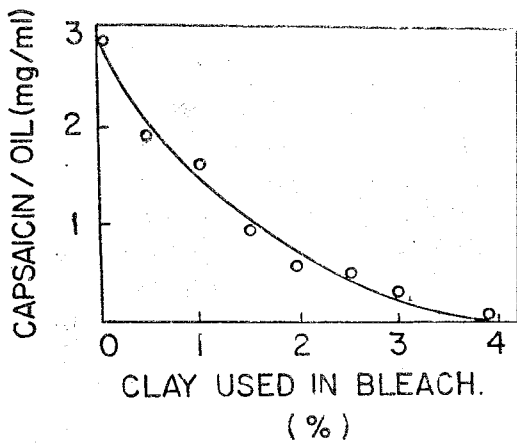


Fig. 2. Bleaching test of capsaicin on red pepper seed oil

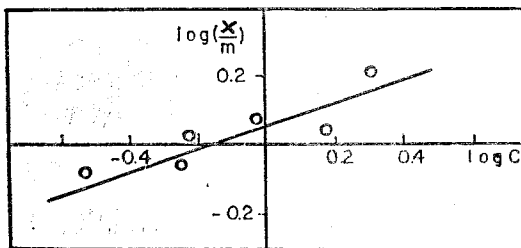


Fig. 3. Adsorption isotherm for the capsaicin

content in the oil decreased exponentially with increasing level of the clay and Freundlich equation can be applied :

$$\frac{x}{m} = kc^n \dots\dots\dots(1)$$

- where x : amount of capsaicin adsorbed (mg/ml),
- m : amount of activated clay (%)
- c : amount of residual capsaicin in oil (mg/ml)
- k and n : constants.

This equation may be written in the following form :

$$\log\left(\frac{x}{m}\right) = n \log c + \log k \dots\dots\dots(2)$$

In Fig. 3, $\log\left(\frac{x}{m}\right)$ vs $\log c$ was plotted and a straight line was drawn by using least square method. The k and n values of this equation were 1.1 and 0.31, respectively.

Neutral lipid composition

Calibration curve of each neutral lipid by potassium dichromate reduction method is shown in Fig. 4.

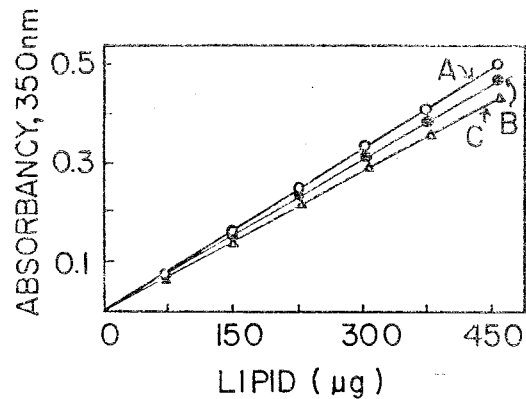


Fig. 4. Lipid standard curve
 A : Sterol, sterol ester
 B : Triglyceride
 C : Diglyceride, free fatty acid, monoglyceride

The standard lipids were oleic acid, triolein, dipalmitic acid, monoolein, cholesterol and cholesterol oleate. Reduction of dichromate was found to be linearly proportional to the amount of lipid present. The amount of dichromate reduced by cholesterol and cholesterol ester was more than those reduced by another lipids. A standard of oleic acid gave results similar to those obtained with dipalmitin and monoolein.

Each standard lipid and the oil sample were developed on silica gel plate (Fig. 5). In the solvent system of petroleum ether-ether-acetate (100 : 15 : 1, v/v), phospholipid remained at the origin. Sterol ester and triglyceride were separated completely. Table 4 shows that the contents of triglyceride and sterol ester were 95.87 % and 1.55 %, respectively.

Fatty acid composition

The total fatty acid content of the red pepper seed oil was 79.1 % by weight. High performance liquid chromatogram of the fatty acids of red pepper seed oil is shown in Fig. 6. Each peak of the chromato-

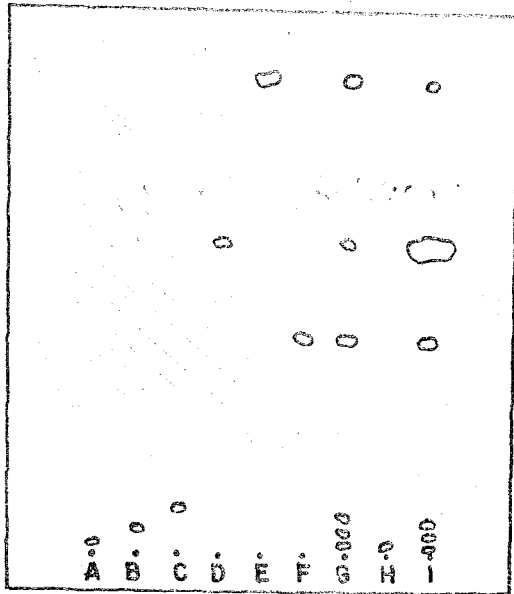


Fig. 5. Chromatoplates illustrating lipid class separation
 A : Monoglyceride F : Fatty acid
 B : Diglyceride G : Standard mixture
 C : Sterol H : Phosphatidyl ethanolamine
 D : Triglyceride
 E : Sterol ester I : Red pepper seed oil

gram had very sharp end. In this chromatogram, the main peak was identified as linoleic acid. Table 5 shows the fatty acid composition of red pepper seed oil by gas chromatography and liquid chromatography. Even though some differences in fatty acid compositions were noticed between the results of GLC and HPLC, the major fatty acid components of the red pepper seed oil were linoleic acid, palmitic acid, and oleic acid in both GLC and HPLC. Palmitoleic acid was not detected in HPLC. Arachidic and behenic acid were not detected in GLC. It seemed that the differences between GLC and HPLC were due to the properties of column, detector and the way of sample preparation. As shown in Table 5, the red pepper seed oil contained a large amount of linoleic acid and relatively small amount of linolenic acid indicating that the oil is not only stable, but also very good from nutritional view point. The results of HPLC also indicate that the content of linoleic and oleic acids were 78.79 % and 2.93 %, respectively. The content of palmitic acid was considerably high (15.27 %).

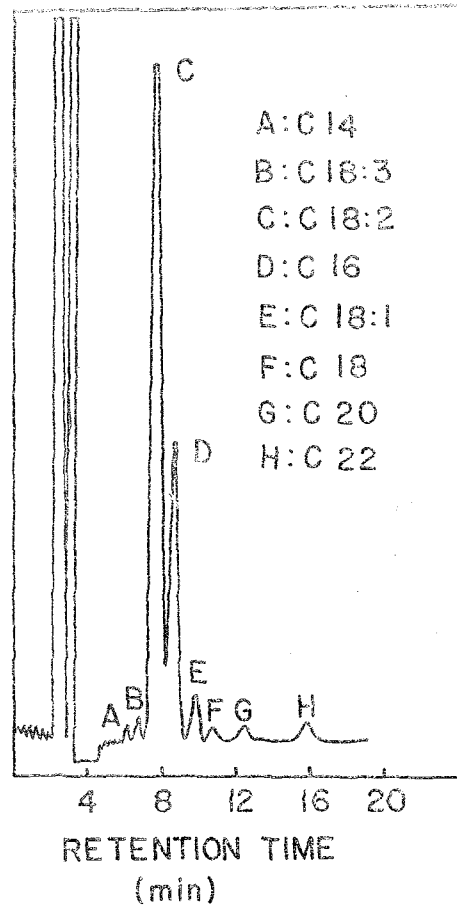


Fig. 6. Liquid chromatogram of fatty acid in red pepper seed oil

요 약

n-hexane으로 추출한 고추씨 기름에 함유되어 있는 붉은 색과 매운 맛의 제거를 시도하였다. 예상한 대로 붉은 색은 탈색 및 탈취 공정중에서 제거되었으나 매운 맛을 내는 capsaicin의 제거에는 탈취 공정은 별 영향이 없었고 탈색 공정이 아주 효과적임을 알았다. 4%의 산성 백토를 가지고 탈색을 했을 때 capsaicin은 완전히 제거 되었다. 또한 기름 중의 capsaicin의 산성 백토에의 흡착은 산성 백토의 양을 증가함에 따라 대수적으로 감소했고 다음과 같이 Freundlich 식을 이용할 수 있음을 알았다: $\frac{x}{m} = 1.1 c^{0.31}$, 여기서 *x*는 백토에 흡착된 capsaicin의 양 ((mg/ml), *m*은 산성 백토의 양 (%), 그리고 *c*는 탈색 후의 잔존 capsaicin의 양 (mg/ml)이다.

고추씨 기름의 증성 지질의 구성을 보면 95.87 %

triglyceride, 1.55 % sterol ester, 1.45 % monoglyceride, 0.78 % 유리 지방산, 0.2 % diglyceride와 0.15 % sterol이었다.

또한 HPLC에 의한 고추씨 기름의 지방산 조성을 보면 리놀산 (78.79 %), 팔미틴산 (15.27 %)과 올레인산 (2.93 %)이 주 지방산이었고 리놀렌산은 0.63 %로 극히 적은 양이었다.

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