

INVESTIGACION

Studies on carotenoids and oxidative stability of winter squash seed and soybean oils

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RESUMEN

Estudios sobre carotenoides y estabilidad oxidativa de aceites de semilla de calabaza de invierno y de soja.

Se han extraído con hexano comercial aceites de semilla de calabaza y de soja. Usando análisis espectrofotométrico y cromatografía en capa fina se han estudiado en estos aceites tres tipos de pigmentos: carotenoides, principalmente luteína y β -caroteno, clorofilas y otros no identificados. Los contenidos en carotenoides fueron 70, 60, 0 ppm en aceite de semilla de calabaza de invierno crudo, refinado y decolorado, y 80, 65, 0 ppm en aceite de soja crudo, refinado y decolorado respectivamente.

Se ha evaluado la estabilidad de aceites de semilla de calabaza de invierno, soja y una mezcla 1:1 de ambos, en sus estados crudo, refinado y decolorado. La mezcla de semilla de calabaza de invierno con aceite de soja aumentó la estabilidad de este último. El aceite crudo mostró una mayor estabilidad que los aceites refinados y decolorados.

PALABRAS-CLAVE: Aceite de semilla de calabaza de invierno - Aceite de soja - Carotenoide - Clorofila - Estabilidad oxidativa.

SUMMARY

Studies on carotenoids and oxidative stability of winter squash seed and soybean oils.

Winter squash seed and soybean oils were extracted with commercial hexane. Carotenoids and other pigments in the oils were studied using spectrophotometric and thin layer chromatographic analysis. Three types of pigments were identified: carotenoids, mainly lutein and β -carotene, chlorophyll and some unidentified pigments. Carotenoids content were 70, 60, 0 ppm in crude, refined and bleached winter squash seed oil, and 80, 65, 0 ppm in crude, refined and bleached soybean oil respectively.

Stability was evaluated for crude, refined and bleached winter squash seed and soybean oils and an 1:1 admixture. Mixing winter squash seed oil with soybean oil increased the stability of soybean oil. Crude oil showed greater stability than refined and bleached oils.

KEY-WORDS: Carotenoid - Chlorophyll - Oxidative stability - Soybean oil - Winter squash seed oil.

1. INTRODUCTION

Economic value of the seed oil protein of gourds "squashes" "*Cucurbita* sp."; has been recognized (Shahani, 1951). The oil is characterized as a semi-drying oil similar to soybean oil (Bolley, 1950). Although the crude oil has a dark colour, processed oil has good stability and does not undergo flavor reversion. Oil content of squash-

es ranges from 35-41% has properties similar to commercial vegetable oils (Vasconcellos, 1980).

Pigments of the winter squash seed oil "*Cucurbita maxima*" were investigated and the oil was shown to be rich in carotenoids (Helmy, 1990). Preliminary experiments indicated that processing conditions could be selected to produce an oil equivalent in colour to commercial vegetable oils (Vasconcellos, 1982).

The purpose of this investigation was to more fully characterize the carotenoids in detail and to study their effect on the oxidative stability of crude, refined and bleached oils of winter squash seed, soybean and an 1:1 admixture.

2. EXPERIMENTAL

Oils of winter squash seed (*Cucurbita maxima*) and soybean (*Glycine max.*) were extracted for 6 hrs from the ground sieved fine particles of the seed kernels, with commercial hexane in a Soxhlet apparatus. The solvent was then stripped from the miscella under vacuum at 50°C in a rotary evaporator.

Refining and Bleaching of Oil: Crude winter squash seed and soybean oils and an 1:1 admixture were refined and bleached according to the AOCS Official Methods (1980).

Refining: Fifty gm each of crude oils were placed in 200 ml beakers and heated to 60°C. The calculated amount of NaOH solution (18°Bé) was added to crude oil during stirring for 15 min., centrifuged at 3000 rpm, washed with warm water, recentrifuged, then decanted.

Bleaching: Forty gm each of the refined oils was taken in 200 ml flask and heated in an oil bath at 110°C. Bleaching earth "Tonsil" was added at 3% of the oil weight while stirring for 10 min. The clay was separated by centrifugation after cooling and the oil was recovered by decantation.

Spectrophotometric Analysis: Absorption spectra of crude, refined and bleached oils of winter squash seed, soybean and an 1:1 admixture was measured in a Shimadzu UV-Visible Spectrophotometer, Model UV-240 Graphcord (Tokyo, Japan). Wavelength range from 300-

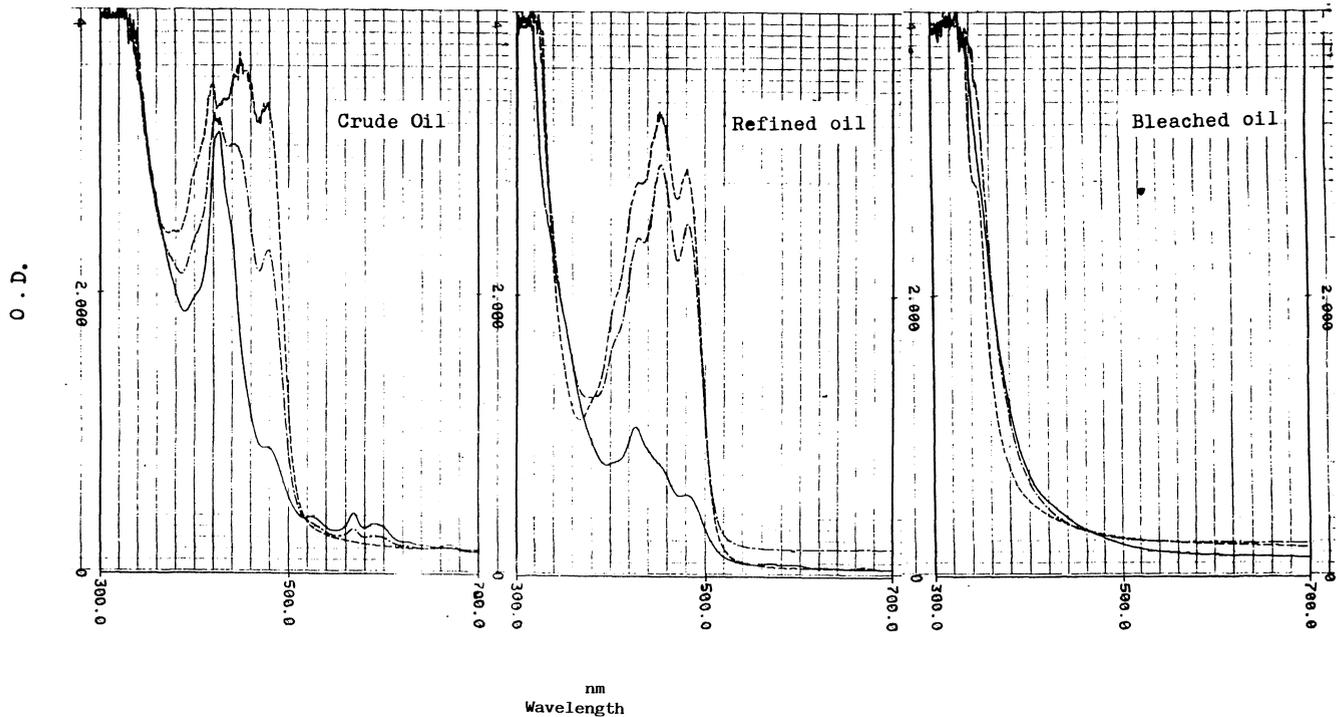


Figure 1
Absorption spectra of crude, refined and bleached winter squash seed (—), soybean (---) oils and on 1:1 admixture (— · —).

700 nm was used. Total carotenoids were determined according to Franzke (1973).

Color Evaluation: Oil color was evaluated in a Lovibond Tintometer using an 1 cuvette. The color was expressed in yellow (Y) and red (R), as described in the AOCS method.

Thin Layer Chromatographic Analysis (TLC): Carotenoids of winter squash seed and soybean oils were identified using t.l.c. chromatographic plates (10 x 20 cm) coated with silica gel G and developed with a solvent mixture of benzene-ethyl acetate-ethanol (80:20:5, v/v/v) as described by Seliskar (1966).

Oxidative Stability: Crude refined and bleached oils of winter squash seed, soybean and an 1:1 admixture, were placed in 100 ml beakers and subjected to heating in an oven at 100°C for 4 days. Peroxide value was determined daily according to the Official AOCS Method.

3. RESULTS AND DISCUSSION

Absorption spectra of soybean and winter squash seed oil is illustrated in figure 1.

Winter squash seed oil exhibits three types of pigments, carotenoids, chlorophyll and unidentified pigments.

The carotenoids appeared at the wavelengths 425,455 and 480 nm. They were mainly lutein and β -carotene. The chlorophyll appeared at the wavelength 680 nm. The unidentified pigments appeared at 525,570 and 590 nm. They may belong to flavonoids (O'Connor, 1949).

Soybean oil exhibits same carotenoids (lutein and β -carotene) at the wavelengths 425,455 and 480 nm, and same chlorophyll at 670 nm. Yet it didn't contain the unidentified pigments appeared in winter squash seed oil.

Total content of carotenoids was 70, 60 and 0 ppm in crude, refined and bleached winter squash seed oils against 80, 65 and 0 ppm in crude, refined and bleached soybean oil respectively. It can be noticed that refining

Table I
Oil Color in Lovibond Units

Oil	Crude	Refined	Bleached
Winter squash (W)	13Y + 1,1R*	12Y + 0,8R	2Y + 0,1R
Soybean (S)	10Y + 2R	10Y + 0,4R	1Y + 0,1R
1:1 (W-S)	13Y + 2R	11Y + 0,8R	1Y

* Y = Yellow, R = Red.

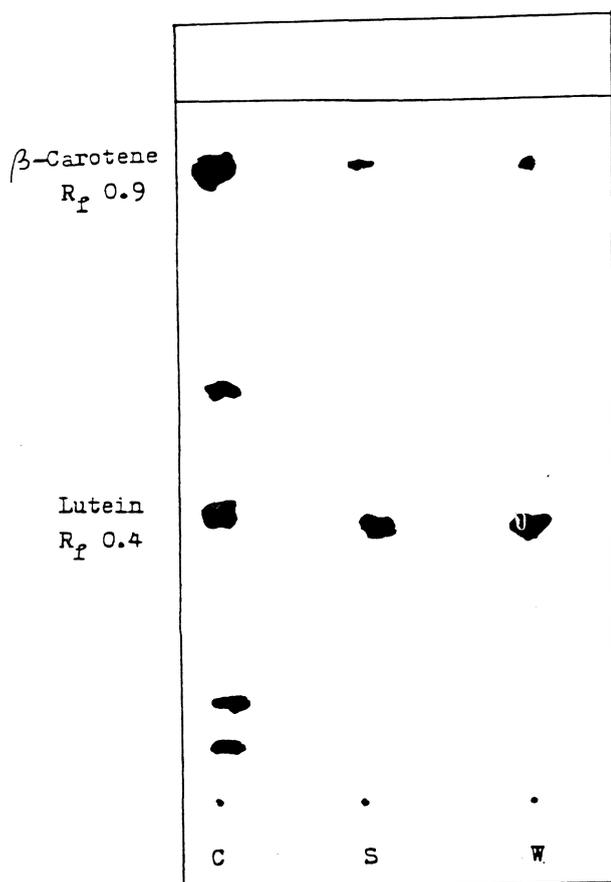


Figure 2

TLC of Carotenoids of Soybean Oil (S) and Winter Squash Seed Oil (W) and Reference of Carrot Extract (C).
Coating Agent: Silica Gel G Type 60 for TLC (Merck).
Developing Solvent: Benzene-Ethyl acetate-Ethanol (80:20:5 v/v/v).

slightly lessened carotenoids, whereas bleaching completely removed them.

Color of the crude, refined and bleached winter squash seed and soybean oils and of an 1:1 admixture in Lovibond units is illustrated in table I.

Color of winter squash seed oil approached color of soybean oil. Lovibond color of the bleached oils ranged between 1Y to 2Y + 0,1R. The refined oils were slightly lesser in color than the crude oils, particularly in red units which gave 2R for crude against 0,4R for refined oils.

Carotenoids in winter squash seed and soybean oils were further characterized by TLC. The results are shown in figure 2.

Two components of carotenoids were identified in winter squash seed and soybean oils; lutein with R_f value of 0.4 and β -carotene with R_f value of 0.9.

Oxidative stability was determined for crude, refined and bleached oils of soybean, winter squash and an 1:1 admixture. The purposes of using the oils in the form of crude, refined and bleached are 1) to remove the effect of the fatty acid composition on the oxidative stability, because refining and bleaching of crude oils do not change fatty acid composition and 2) to magnify the effect of carotenoids in the oils, where carotenoids were 80 and 70 ppm in crude w.squash and soybean oils and 0 ppm in their bleached oils. Oxidative stability evaluated is illustrated in table II.

Results revealed that:

1. In crude soybean oil peroxide value jumped from 62,7 to 386,7 (516,7% increase) after 3 days of heating against 101,3% increase in crude winter squash seed oil and only 22,6% increase in the admixture of soybean/winter squash seed oil. Admixing crude winter squash seed oil with crude soybean oil potentiated stability of the later.

After 4 days of heating, peroxide value decreased in crude soybean in from 386,7 to 208,0, where rate of decomposition of formed peroxide increased than rate of formation. At same period, peroxide value did not decrease in crude winter squash seed oil and in soybean/winter squash seed oil admixture. This mean that their stability was more higher than crude soybean oil.

2. Refined soybean oil showed 202,0% increase in peroxide value against 378,3% increase in w.squash seed oil and 209,5% increase in soybean/w. squash seed oil admixture, after 2 days of heating. Then curves of peroxide value began to decline at the third day owing to the increase in the rate of decomposition of peroxide than the rate of formation.

3. Bleached soybean oil showed the highest peroxide value (496,1) after two days of heating. Peroxide value was also high in w.squash seed oil (456,1) and in soybean/w. squash seed oils (486,6). After then peroxide value began to decrease.

Table II
Effect of Accelerated Storage on the Oxidative Stability of Winter-Squash Seed and Soybean Oils

Oil Days Storage	Winter squash			Soybean			1:1 admixture		
	C	R	B*	C	R	B	C	R	B
Fresh	7,5	8,9	9,5	6,3	8,5	8,8	7,0	8,1	9,3
1	53,4	76,5	138,0	60,1	141,7	162,1	51,4	135,1	165,0
2	62,3	365,9	456,1	62,7	427,9	496,1	57,4	418,2	486,8
3	125,4	364,4	298,9	386,7	373,4	325,8	70,4	350,6	191,7
4	172,2	346,7	291,0	208,0	289,5	311,9	161,1	277,8	151,7

* C = Crude, R = Refined, B = Bleached.

4. The rancidity began to appear clearly when the peroxide value reached to the maximum, where the carbonyls started to increase largely forming rancid odour. This characteristic can be explained as follows:

a) In winter squash seed oil rancidity began to appear in the refined oil after two days of heating (365, 9 P.V.), and in the bleached oil after two days (456,1 P.V.), and did not appear after four days of heating in the crude oil.

b) In soybean oil rancidity began to appear at 386,7 P.V. in the crude oil after three days of heating against 427,9 and 496,1 for the refined and bleached oils after two days.

c) Same was noticed for the 1:1 admixture where rancidity began to appear in the refined mixture at 418,2 P.V. after two days of heating and at 486,8 in the bleached mixture. This rancid odour was not appear in the crude oil.

Previous results revealed that crude oil is more stable than refined and bleached oils. Bleached oil was the lowest in stability, therefore it's advisable to add antioxidant to bleached oil. The results, also, revealed that winter squash seed oil was more stable than soybean oil, therefore mixing previous oils increased stability.

Figure 3 showed that carotenoids in the crude oils were destroyed after four days of heating except a small amount of residual lutein in the crude oil of winter squash seed at 425 nm. It is possible that lutein (3,3 dihydroxy α -carotene) is more stable than β -carotene, because it contains two hydroxy groups. Another small portion of the unknown pigment appeared at 600 nm.

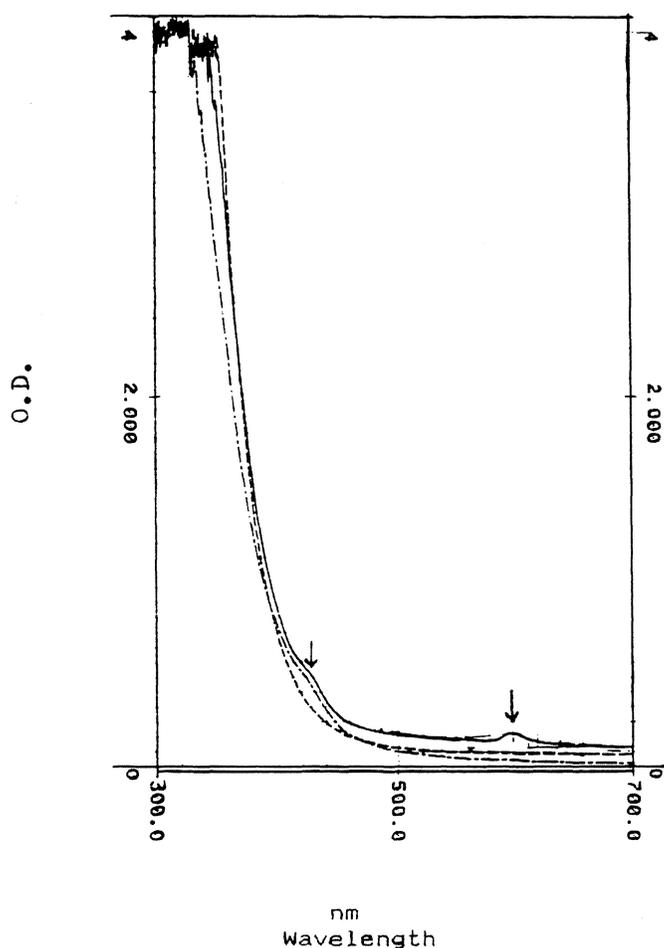


Figure 3

Absorption spectra of crude winter-squash seed (—), soybean (---), oils and a 1:1 mixture (---) after four days of 100°C heating.

REFERENCES

- Bolley, D.S., McCormack, R.H. and Curtis, L.C. (1950).— "The utilization of the seeds of the wild peremiat gourds".— *J. Am. Oil Chemists' Soc.* **27**, 571.
- Franzke, Cl., Grunert, S. and Kroschel, H. (1973).— "Importance of carotenoid and chlorophyll coloring agents in fat industry".— *Nahrung* **17**, 587.
- Helmy, H.E. (1990).— "Studies on the pigments of some citrus, prune and cucurbita seed oils when processed with or without cottonseed oil".— *J. Am. Oil Chemists' Soc.* **67**, 376.
- O'Connor, R.T., Field, E.T., Jefferson, M.E. and Dollear, F.G. (1949).— "The influence of processing on the spectral properties of vegetable oils".— *J. Am. Oil Chemists' Soc.* **26**, 710.
- "Official and Tentative Methods of the AOCS" (1980).— 3rd. ed., AOCS, Champaign, IL.
- Seliskar, C.J. (1966).— "Separation of phytolated and non-phytolated chlorophylls by TLC".— *Anal. Biochem.* **17**, 174.
- Shahani, H.S., Dollear, F.G., Markley, K.S. and Quimby, J.R. (1951).— "The Buffalo gourd, a potential oilseed crop of the Southwestern Drylands".— *J. Am. Oil Chemists' Soc.* **28**, 90.
- Vasconcellos, J.A., Berry, J.W., Weber, C.W., Bemis, W.P. and Scheerens, J.C. (1980).— "The properties of cucurbita foetidissima seed oil".— *J. Am. Oil Chemists' Soc.* **57**, 310.
- Vasconcellos, J.A. and Berry, J.W. (1982).— "Characteristics of laboratory processed cucurbita foetidissima seed oil".— *J. Am. Oil Chemists' Soc.* **59**, 79.

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