Grapeseed oil appears to be an excellent source of linoleic acid. Gattuso et al. (14) examined 17 samples of oil from fresh grapeseed and reported average values of 70.8% for linoleic acid and 88.6% for total unsaturates. Studies performed by Mattich and Rice (15) on several varieties of native American and hybrid grapeseeds showed levels of linoleic acid in excess of 70%, which are in agreement with our results of 72.2% and with results summarized by Kinsella (16) on *V. vinifera* varieties.

The fatty acid composition and physical and chemical characteristics of melonseed oil obtained in our study were in general agreement with results obtained by Girgis and Said (4), Oyenuga and Fetuga (11) and by Chowdhury et al. (17). In these earlier studies, however, the levels of linoleic acid reported ranged from 52-58%, compared to 65% in the present study.

The data presented here suggest that watermelon and grapeseeds may constitute useful products with good nutritional value. The seeds could be extracted for their oil and used for edible purposes, and the meal could be used for animal and poultry feed or as a soil conditioner or fertilizer.

A large number of other plant seeds have been investigated for their amino acid (18-21) and fatty acid (22) compositions. Research should be conducted on the economic feasibility of utilizing these by-products, although it should be recognized that only in areas where these by-products are produced in large quantities and the materials recovered are in limited supply will there be any real benefit.

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Determination of Colored Substances in Soybean Lecithin

ALBERTO LEZEROVICH, Molinos Río de la Plata, Laboratorio de Investigaciones, 1323 Buenos Aires, Argentina

ABSTRACT

Methods to determine carotenoids, chlorophylls and pheophytins in lecithin by derivative spectrometry were developed. Determinations of those as well as of brown substances were made on commercial soybean lecithins, and the effects of bleaching and powder manufacturing upon color are discussed.

INTRODUCTION

The main classes of colored substances which account for the color of soybean lecithin are carotenoids, chlorophyllic pigments and brown substances (1). The principal carotenoid substance present can be β-carotene (2) or lutein (1,3). Among the chlorophyllic pigments, pheophytin A, a degradation product of chlorophyll A, is supposed to be the main constituent (4) which is related to the maximum at 670 nm in soybean lecithin and in soybean oil. The brown substances have the characteristics of aldehyde-amine reaction products and probably are formed in the oil during the solvent stripping operation (1). It also has been suggested (5) that browning in lecithin is the result of aldolic condensations catalized by bases, phosphatidylcholine being the main condensating agent.

Soybean lecithin usually is single bleached with hydrogen peroxide and double bleached with hydrogen peroxide alone or followed by benzoyl peroxide. In order to study the process of bleaching of commercial lecithin and find out its extension on each class of colored substance present, methods were developed and some determinations were made on nonbleached, single and double bleached lecithins. The effect of the acetone extraction upon the color of the insoluble phosphatides also was considered.

METHODS

Although there is an AOCS official method for chlorophyll applicable to refined and bleached oils (6) and two methods were proposed for brown substances (7), no method to determine carotenoids in lecithin without separation was found. The spectrophotometric methods for β-carotene (8) require a chromatographic separation which cannot be avoided in this case because of the absorbance at 400-500 nm due to the brown substances. As brown substances show no maxima in the visible range, their interference easily can be overcome by means of derivative spectrometry. On this base methods to determine carotenoids as well as chlorophylls and pheophytins were developed.

When reference to the "AOCS" method is made in this paper, it means a method similar to the AOCS Official Method Cc 13d-55 for chlorophyll in oils.

The graphic representation of the quotient $dA/d\lambda$ (A:absorbance, λ: wavelength) over the wavelength of interest is called a derivative spectrum, and the technique is referred to as derivative spectrometry (9,10). Theoretical aspects and performance of this method have been reviewed by O'Haver and Green (11,12). Provided some conditions are met, a useful characteristic of derivative spectrometry is that the difference between the maximum and minimum in a derivative spectrum, generated by an absorbance band, keeps constant even if the absorbance itself is affected by a background absorbance due to an interference or turbidity.

Carotenoids. The three typical peaks of carotenoids in the region of 400-500 nm (Fig. 1) correlate to three pairs of maxima and minima in the plot of the first derivative spectrum. The relationship between the fall, $\Delta ca = (dA/d\lambda)_{436} - (dA/d\lambda)_{454}$, (where the subscripts indicate wavelength) and concentration was found to be linear (correlation coefficient = 0.9998). β-Carotene was taken as a standard to determine carotenoids. The absorbance at 448 nm of crystalline β-carotene solutions in n-hexane was measured, and their concentrations were calculated (Table I) from the value $E^{1\%}_{1\,cm} = 2590$. The differences Δca also were measured in the same solutions. Plotting concentration against Δca a straight line resulted (correlation coefficient = 0.9996) with intercept 0.034 $\mu g.ml^{-1}$ and slope 103.8 $\mu g.ml^{-1}.\Delta ca^{-1}$. These values were used throughout the analyses of lecithin samples as described in the experimental section. The same result was obtained from a commercial sample of β-carotene in oil. In both cases Δca was calculated as $\Delta ca' = (dA/\lambda)_{440} - (dA/\lambda)_{458}$, due to the shift of the maximum from 448 nm (β-carotene) to 442 nm (carotenoid in lecithin) in n-hexane.

Chlorophylls-Pheophytins. In the case of chlorophyllic pigments the difference, $\Delta ch = (dA/\lambda)_{664} - (dA/\lambda)_{680}$, can be taken to account for their concentration. In Figure 2 the spectrum of a lecithin sample in absorbance and its first derivative can be seen. The relationship between Δch and concentration was found to be linear (correlation coefficient = 0.9983) as it was so the one between absorbance–calculated according to the "AOCS" method, i.e. $(A_{670} - (A_{630} + A_{710})/2)/0.1$, and concentration (correlation coefficient = 0.9989). As no standard of chlorophyll or pheophytin was available for a procedure similar to that of carotenoids, Δch could give only relative values of concentration between samples. Nevertheless, for a set of samples Δch values were plotted against concentration of chlorophyll as calculated by the "AOCS" method (Fig. 3), and the intercept and slope of the best straight line were taken to calculate concentration measuring Δch, as described in the experimental section.

Brown substances. The amount of brown substances was evaluated according to Scholfield and Dutton (7), measuring absorbances at 365 and 456 nm and calculating the absorbance due only to brown substances as $A^{B}_{365} = (6.5\ A_{365} - A_{456})/6.36$.

TABLE I

Absorbance and Δca of β-Carotene in n-Hexane and Values of Concentration Calculated From Them

		c(μg/ml)		
A_{448}	Δca X 10^4	From A_{448} [a]	From Δca [b]	Error %
1.529	559	5.903	5.836	-1.1
1.354	512	5.229	5.348	2.3
1.222	450	4.718	4.705	-0.3
0.959	349	3.703	3.657	-1.2
0.731	266	2.822	2.795	-1.0
0.622	225	2.402	2.370	-1.3
0.472	171	1.822	1.809	-0.7
0.440	164	1.698	1.736	2.2
0.369	133	1.425	1.414	-0.8
0.285	103	1.100	1.103	0.3
0.237	86	0.915	0.927	1.3
0.180	65	0.695	0.709	2.0

[a]Taking $E^{1\%}_{1\,cm} = 2590$.

[b]Calculated as 103.8 Δca + 0.034: Regression computed from data of columns 2 and 3. Estimated standard deviation of y values 0.0025 μg/ml.

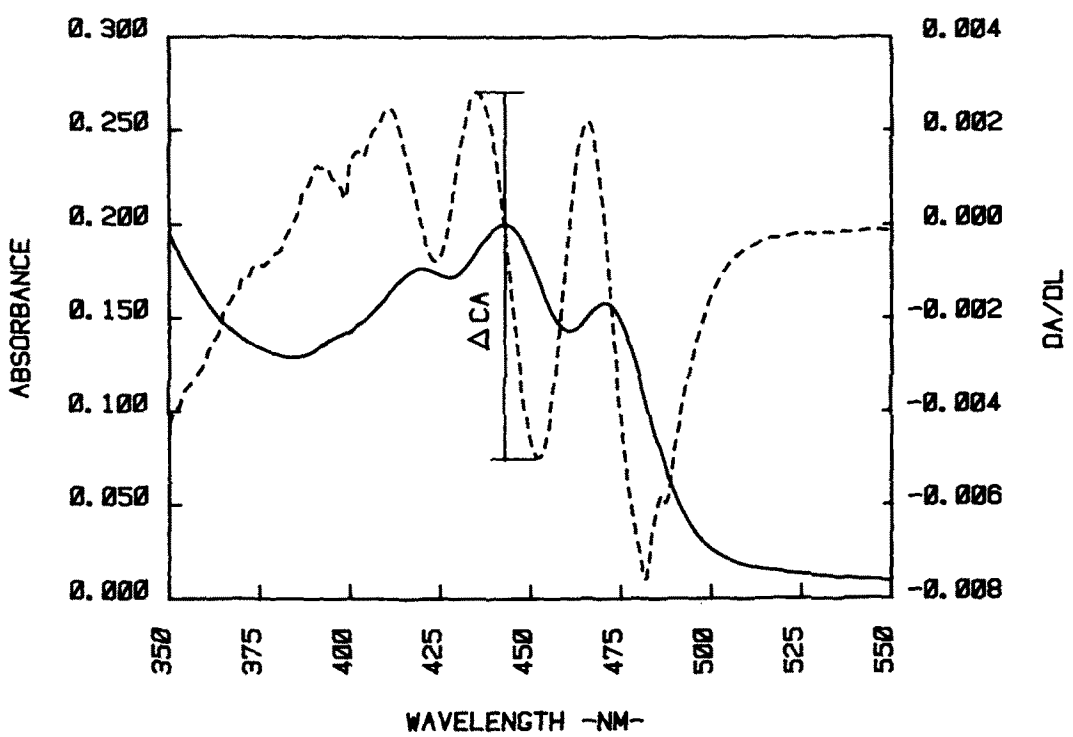

FIG. 1. Spectrum of nonbleached soybean lecithin (6.4 mg/ml n-hexane) (—) and its first order derivative (----).

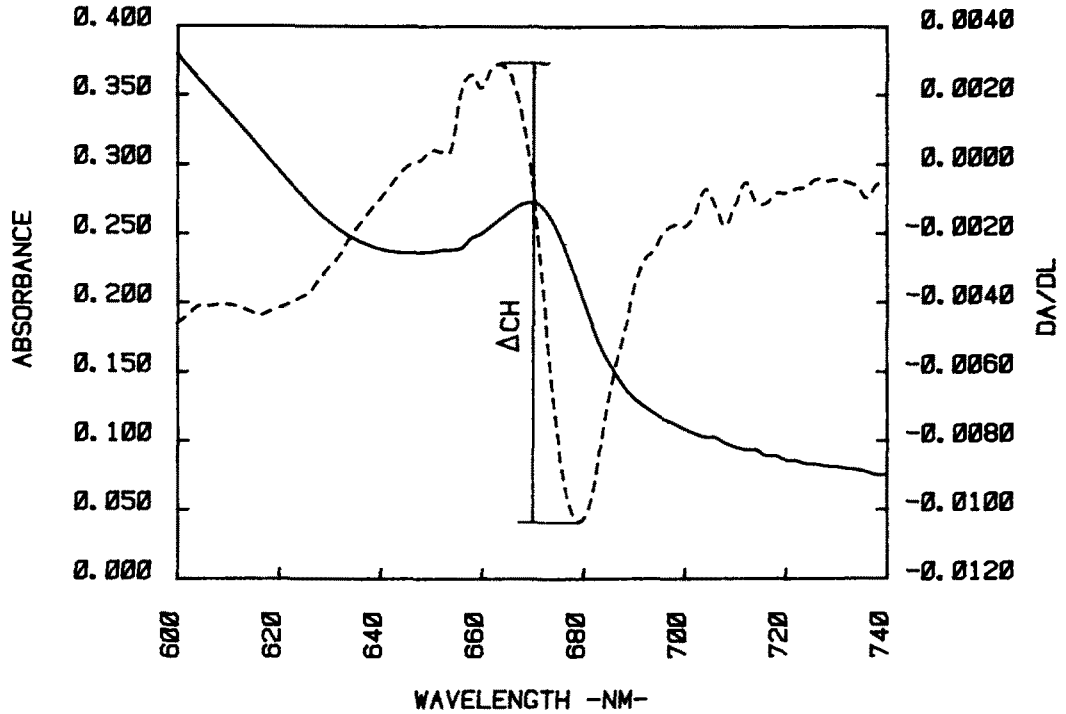

FIG. 2. Spectrum of nonbleached soybean lecithin without solvent (—) and its first order derivative (----).

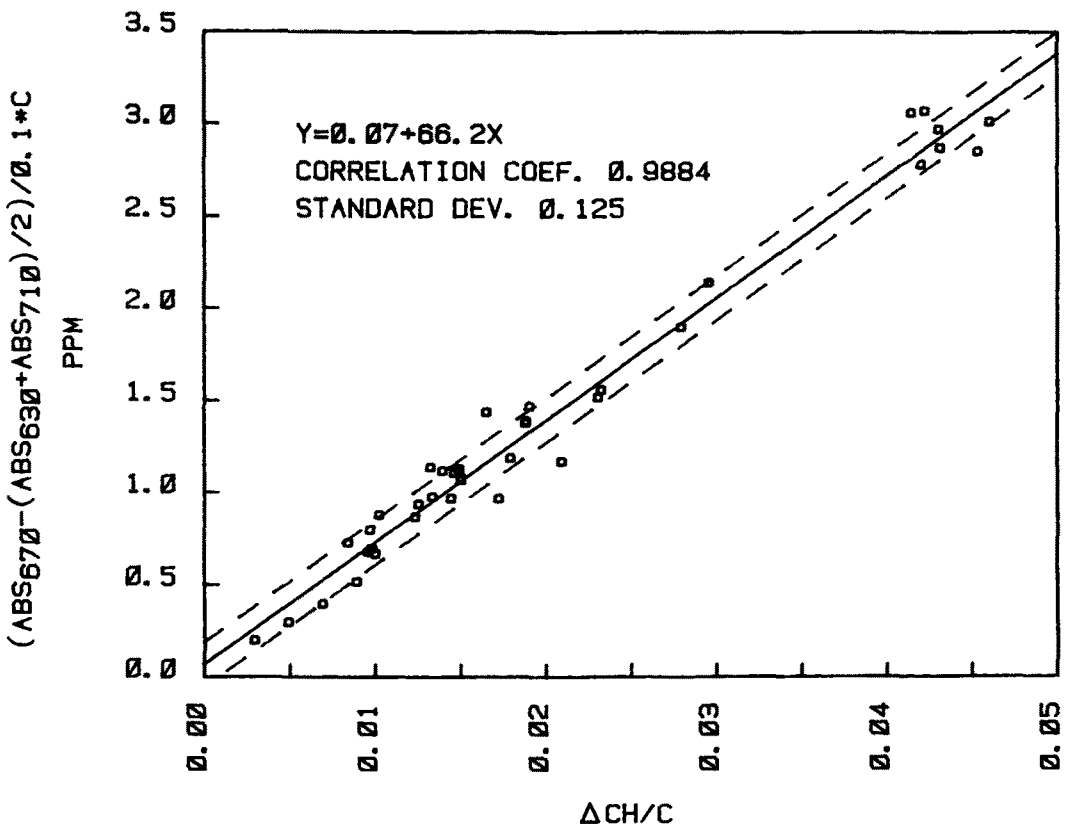

FIG. 3. Relation between concentration of chlorophyllic pigments as function of absorbances and Δch/c.

EXPERIMENTAL

β-Carotene was obtained from Sigma Chemical Co. and n-hexane spectroscopic grade from Merck. Samples of commercial soybean lecithins from different producers were studied.

Spectral absorbances and their first derivatives were measured and plotted using a Hewlett Packard 8450A spectrophotometer with 1 cm cells and a Hewlett Packard 7225B graphics plotter. To serve as a comparative reference when the derivative conditions correspond to other derivative devices or methods, the spectrum of a holmiun oxide glass in absorbance and its first derivative are shown in Figure 4.

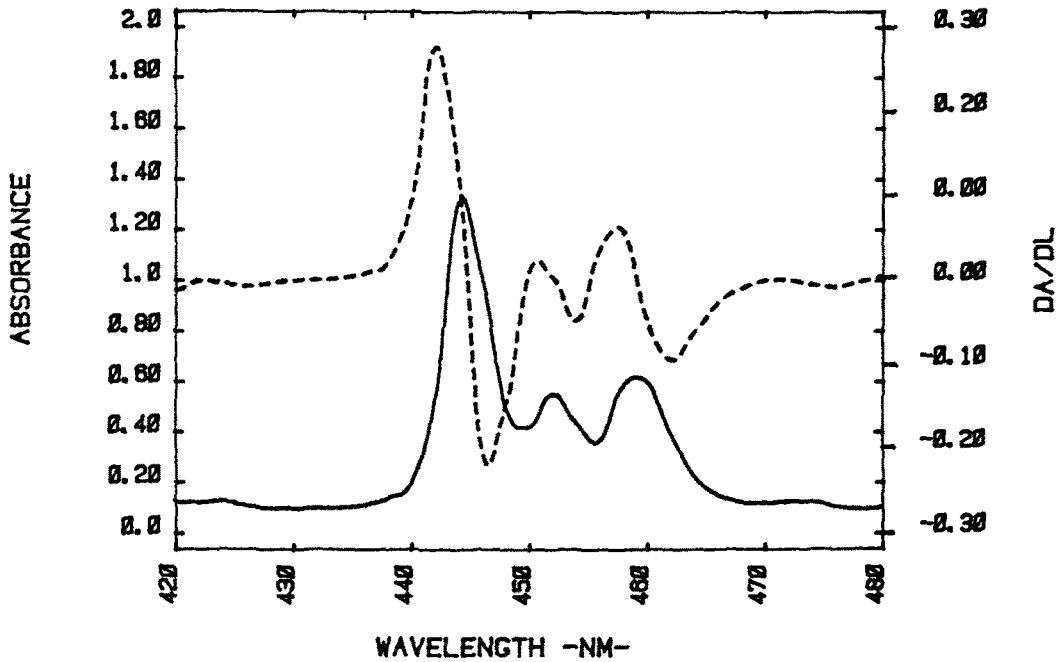

FIG. 4. Spectrum of a holmiun oxide glass in absorbance (—) and its first order derivative (----).

TABLE II

Concentration of Colored Substances, Gardner Color and Acetone Insoluble Matter in Soybean Lecithin

Sample Type	#	Carotenoids (ppm)	Chlorophyllic pigmts. (ppm) a	b	Brown subst. (u)	Gardner color	Acetone insol. (%)
Nonbleached	1	26	1.1	1.0	35	14	68.1
	2	63	0.7	0.7	18	13	68.8
	3	39	1.7	1.8	71	>18	68.4
	7	127	1.0	0.9	20	15	61.8
	16	44	2.1	2.0	32	17	63.7
	17	108	1.4	1.3	19	15	65.2
	18	84	0.4	0.6	20	15	53.8
	26	35	2.9	2.9	41	18	65.6
	29	50	0.4	0.7	33	17	62.9
	35	22	0.4	0.7	41	18+	71.2
Single bleached	27	35	1.4	1.3	24	14	64.1
	30	42	1.4	1.3	27	14	61.4
	37	17	0.6	0.7	29	15	67.7
Double bleached	31	8	0.3	0.4	25	12+	62.7
	38	0	0.2	0.2	24	12	67.0
Powder	11	20	c	c	31		100
	12	11	c	c	10		100
	15	10	0	0	64		100
	24	12	0.1	0.3	48		100
	25	18	0.1	0.3	26		100
Hydroxylat.	19	0	0.8	0.7	47	13	49.8
	20	0	0	0.1	24	12+	56.4
Hydrolyzat.	4	59	1.0	1.3	86	>18	59.9
High viscosity	8	61	1.1	1.1	38	17	62.5
	21	48	1.5	1.6	46	18+	67.2
	22	85	1.1	1.0	32	16+	67.1
	23	18	0.7	0.6	28	12	70.3

[a]Calculated from $(A_{670} - (A_{630} + A_{710})/2)/0.1$ c.
[b]Calculated from 0.07 + 66.2 Δch/c.
[c]Sample not freely soluble.

TABLE III

Change in Carotenoids and Brown Substances Levels in Soybean Lecithins Due to Deoiling

Sample #	Carotenoids (ppm) in sample		Brown substances (units) in sample	
	Whole	Deoiled	Whole	Deoiled
1	26	3	35	47
2	63	0	18	22
3	39	3	71	86
4	59	4	86	125
5	41	4	36	47
6	51	2	29	61

To determine carotenoids and brown substances, solutions of lecithin of about 100 mg (accurately weighed) increased to 10 ml with n-hexane were prepared and the values of $(dA/d\lambda)_{436}$, $(dA/d\lambda)_{454}$, A_{365} and A_{456} were measured. The concentrations were calculated by means of: Carotenoids (as β-carotene)(ppm) = (103800 Δca + 34)/c; and Brown substances (units) = 1000 (6.50 A_{365} - A_{456})/ 6.36 c, where c is the concentration of the sample in mg/ml.

To determine chlorophylls-pheophytins no solvent was used, except when absorbance was higher than 0.8. In these cases, solutions of about 5 g (accurately weighed) increased to 10 ml with n-hexane, clarified by centrifugation if necessary, were measured and the concentrations calculated as: Chlorophyllic pigments (ppm) = (A_{670} - (A_{630} + A_{710})/2)/ 0.1 c and Chlorophyllic pigments (ppm) = 0.07 + 66.2 Δch/c, where c is the concentration of the sample in g/ml. When no solvent was used, the concentration, c, was taken as 1 g/ml.

Precision and Accuracy

Both methods for chlorophyllic pigments gave the same estimated standard deviation (0.05 ppm), calculated from data of 15 samples run in duplicate. Nevertheless, at very low concentrations, the derivative method seems to be more precise. Thus, six determinations on a sample with <0.5 ppm gave -0.07 ± 0.04 ppm (per cent standard deviation = 53.4) with the "AOCS" method and 0.38 ± 0.01 ppm (per cent standard deviation = 2.4) with the derivative method. The ratio of variances (15.8) showed a significant statistical difference within the 95% level of confidence. For carotenoids the estimated standard deviation of the method was 1.5 ppm, calculated from data of 12 samples run in duplicate. Errors of values calculated from Δca, taking as correct those calculated from $E^{1\%}_{1\,cm}$, were lower than 2.5% (Table I).

RESULTS AND DISCUSSION

The proposed methods are precise, simple and quickly fulfilled. Neither reagents nor separation media are needed. The data obtained from several samples of commercial soybean lecithins about concentration of colored substances together with Gardner color and acetone insoluble matter are shown in Table II. Although it was not the aim of this work to study the effect of bleaching on lecithin but to procure some analytical procedures to do it, several conclusions can be withdrawn from the data obtained.

The level of chlorophyll-pheophytins in the analyzed samples are low, ranging from nil to 2.9 ppm with an average of 0.8 ppm. An important reduction takes place in the cases of powder and double bleached lecithins, but single bleached ones still have values of the same order as nonbleached lecithins. With regard to carotenoids, they are absent or their concentrations are very low in powders. The analyzed native lecithins contain up to 127 ppm with an average of 60 ppm. Also in this case a noticeable effect is reached only with double bleaching. Data about brown substances show from 10.5 to 86 units with a mean value of 36 units. Although a reduction also is observed, the effect of bleaching is less noticeable than in the former classes of colored substances.

Data in Table III show that when acetone insoluble powders (deoiled lecithins) are compared with the corresponding whole lecithins, a reduction in the level of carotenoids and an increase in the concentration of brown substances is observed.

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