Studies on the lipid constituents of grape seeds recovered from pomace resulting from white grape processing

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RESUMEN

Estudios sobre los constituyentes lipídicos de semillas de uva recuperadas de la pulpa resultante del procesado de uva blanca

Se han realizado estudios sobre los constituyentes lipídicos de semillas de uva producidas como subproducto del procesado de uva blanca. La cromatografía gas-líquido se usó para determinar la composición de ésteres metilícos de ácidos grasos y silil derivados esteroles del aceite. Se observó que el aceite contenía cantidades apreciables de ácidos grasos insaturados, principalmente ácidos oleico y linoleico, no siendo sin embargo detectado el ácido linolénico. Se encontraron en cantidades razonables ácidos grasos saturados, principalmente, palmítico y esteárico. Como componentes minoritarios se detectaron los ácidos mirístico, palmitoleico, eicosanoico y eicosadienoico. El isofucosterol apareció junto a otros esteroles, principalmente, campesterol, estigmasterol y beta-sitosterol. El análisis por HPLC del aceite para la determinación de tocoferoles, mostró la presencia de alfa- y gamma-tocotrienoles, y alfa- y gamma- tocoferoles en cantidades de 53'2, 28'6, 16'4 y 1'8 respectivamente. El contenido en tocoferoles totales del aceite fue de 470 ppm. En la harina desgrasada de semillas de uva se vio un contenido del 24'6% en proteína, con amplias proporciones de todos los aminoácidos esenciales, determinados mediante analizador de aminoácidos.

PALABRAS-CLAVE: Composición lipídica - Proceso tecnológico - Semilla de uva - Subproducto.

SUMMARY

Studies on the lipid constituents of grape seeds recovered from pomace resulting from white grape processing

Studies on the lipid constituents of grape seeds, produced as by-product from white grape processing, were carried out. Gas liquid chromatography was used to determine the composition of fatty acid methyl esters and sterol silyl derivatives of the oil. The oil was found to contain appreciable amount of unsaturated fatty acids, namely, oleic and linoleic acids, however, linolenic acid was not detected. Saturated fatty acids, namely, palmitic and stearic were found to be present in reasonable amounts. Myristic, palmitoleic, eicosanoic and eicosadienoic acids were detected as minor components. Isofucosterol was found among the other sterol constituents namely, campesterol, stigmasterol and beta-sitosterol. HPLC analysis of the oil for determination of tocopherols, showed the presence of alpha-and gamma-tocotrienols, and alpha-and gamma-tocopherols to the extent of 53.2, 28.6, 16,4 and 1,8% respectively. The total tocopherols content of the oil was 470 ppm. The defatted meal of grape seeds was found to contain 24,6% protein which contained large proportions of all the essential amino acids as determined by amino acid analyzer.

KEY-WORDS: By-product - Grape seed - Lipid composition - Technological process.

1. INTRODUCTION

Different kinds of grapes are locally cultivated in Egypt and large amounts are consumed as fresh fruits, however, some grape varieties are mainly cultivated for producing wine, raisins, juices and other unfermented beverages. Grape seeds are considered to be a potential source of oil which constitutes about 15% of the weight of the seeds (1).

Several publications, concerning foreign grape seed varieties, dealt with the determination of lipid and protein constituents of the seeds (2-22). Only few publications on the Egyptian grape seed varieties (namely, Fayoumi, Bezzelanza and Moskat) were concerned with fatty acid composition and determination of some triglyceride components with the help of argentation and partition thin layer chromatography (23) (24). Hassan *et al.* (25) studied only the fatty acid composition of Egyptian white and red grape seed varieties with ultraviolet spectroscopy and chromatographic methods of analysis.

It was the objective of the present work to carry out detailed studies on grape seeds that are produced in tonnages as by-product from white grape processing. The studies were chiefly concerned with elucidation of fatty acid, sterol and tocopherol composition using gas liquid and high performance liquid chromatography. Additional studies on the response of the crude oil to refining and bleaching processes, were also carried out. With the aim of throwing light on the possibility of using the defatted seeds as feed components, protein and the amino acid constituents were quantitatively determined.

2. EXPERIMENTAL

The pomace, produced as by-product of white grape processing, was obtained from the Egyptian Vineyards and Distilleries Company (Ginaclis), season 1990. The fresh pomace (containing about 50% grape seeds) was placed on trays and subsequently dried in air and oven. The grape seeds were sieved out from dry pomace and solvent extracted by commercial n-hexane. The solvent was removed in a rotary evaporator and the oil was kept in stoppered bottles in the refrigerator.

Physical and chemical characteristics of the oil were determined using the official methods of analysis (26).

Nitrogen content of the defatted meal was determined

by applying a semimicro Kjeldahl procedure and the protein content was calculated as N x 6,25 (27). Quantitative determination of the individual amino acids, in protein hydrolyzate, was carried out with the help of automatic amino acid analyzer (28) (29).

Crude oil was alkali refined with 20°Be NaOH (30) and the refined oil was then bleached with 3% Tonsil N clay of the weight of the refined oil. The oil and clay mixture was heated at 110°C for 10 minutes under N_2 atmosphere while stirring. Colours of the crude, refined and refined-bleached oils were evaluated spectrophotometrically (Shimadzu Spectrophotometer UV-240) at wave lengths ranging from 300 to 700 nm. The colour index for the three oils was also calculated following the recommendation of Pons *et al.* (31).

Tocopherol constituents were measured directly in the oil using Toyo Soda CCPM high pressure liquid chromatograph (HPLC) instrument coupled to Hitachi-650-10 S fluorescence detector with an excitation and emmission wave lengths of 290 and 325 nm respectively. A column (150 x 4,6 mm) packed with silica gel YMC-A-012 SIL was eluted with the solvent hexane: isopropyl alcohol (100:0,5 v/v) at flow rate 1-2 ml/min. Sample size of 20 microliter of 1-1,5 ml oil dissolved in 100 ml hexane was injected (32).

Sterol constituents were determined by gas liquid chromatographic analysis (GLC) as TMS derivatives. Hewlett-Packard HP 5890 A gas chromatograph was used under the following operating conditions: column, 0,53 mm x 5 m coated with DB-1 phase in a film thickness of 1,0 micron; column temperature, 200-280°C with a rate of 4°C/min; injection and detector temperature, 290°C, and carrier gas was helium at flow rate of 8,0 ml/min. Preparation of pure sterols mixture and their silyl derivatives, and gas chromatographic analysis were conducted according to Slover *et al.* (33) and El-Shami (34).

GLC analysis of fatty acids, of grape seed oil in comparison with those of cottonseed and soybean oils was carried out using the same instrument but under the following operating conditions: column, 30 m x 0,32 mm filled with DB-23 in a film thickness of 0,25 micron; column temperature, 150-230°C, 3°C/min; injection temperature, 230°C; detector, flame ionization; carrier gas, helium at 1-3 ml/min and split ratio of 1:100.

3. RESULTS AND DISCUSSION

Grape seeds contain reasonable amounts of oil and protein (14,6% and 24,6% respectively) which are generally lower than the other conventional oil seeds (1). The iodine value of the oil is 128 (Table I) which indicates that this oil belongs to the class of semi-drying oils. It is also observed that the crude oil contains free acidity of 3,45% (as oleic acid) and peroxide value of 8,5. It can be concluded that acidity and hydroperoxides may arise from some enzymatic reactions taking place during grinding of the seeds although crude oil was extracted from the seeds directly after grape pressing.

Table I
Grape Seed Oil Characteristics

Refractive Index	1,4690
Iodine value	128,0
Saponification value	212,0
Unsaponifiable Matter %	3,1

Free acidity (Oleic %) 3,45%, Peroxide value: 8,5

To throw light on the autoxidation potential of the oil, it was advisable to determine quantitatively tocopherol constituents by HPLC which shows that the oil contains a considerable amount of tocopherols and tocotrienols amounting to 470 ppm (mg tocopherol/kg oil). Alpha- tocotrienol is the most predominant compound and constitutes 53,2% of the total tocopherols and tocotrienols (Table II). Gamma-tocotrienol, alpha-and gamma-tocopherols are found at the levels of 28,6, 16,4 and 1,8% respectively.

Table II
HPLC Analysis of Tocopherols Constituents

Total tocopherols	470 ppm
Alpha-Tocopherol	16,4 %
Gamma-Tocopherol	1,8 %
Alpha-Tocotrienol	53,2 %
Gamma-Tocotrienol	28,6 %

Considering the fact that grape seed oil can be used mainly for edible and pharmaceutical purposes, refining as well as bleaching were carried out to obtain an oil of more acceptable and desirable colour to the customers and to get rid of the pigments that can play roles as prooxidants. The response of the oil to refining was followed by measuring the colour by both Lovibond and spectrophotometric methods. The colours of the crude, refined and refined-bleached oils are recorded in Table III. It can be noticed that the pigments respond quite well to bleaching and therefore the colour is more greatly reduced by the bleaching than by the refining process alone. The influence of both refining and bleaching processes on the removal of pigments was followed spectrophotometrically.

Table III
Studies on the Colour of Crude, Refined and Bleached Grape Seed Oil

Colour [A]	Crude Oil	Refined oil	Bleached oil
Yellow (y)	12	12	0,1
Red (R)	1,1	0,7	—
Blue (B)	0,1	—	—
Y + 10R	23,0	19,0	0,1
R + B	1,2	0,7	—
Colour Index [B]	486,8	273,8	16,08

A: As Lovibond Units, B as Colour Index (Spectrophotometrically 400-550 nm).

The absorption curve of the crude oil shows clearly maxima at about 670 and 610 nm which are characteristic to chlorophylls and/or their derivatives. Other maxima at 535 and 560 nm, contributed by flavonoids, also appear. In addition, the absorption curve also shows absorption at lower wave lengths of 415, 435, 455 and 485 nm which are characteristic to carotenoid pigments. It can be noticed from the absorption spectra, that refining alone could not remove the chlorophylls and carotenoids completely, while refining followed by bleaching removed effectively all the absorption bands belonging to the different pigments. Therefore refining followed by bleaching removes effectively the pigments that enhance autoxidation reactions, thus avoiding any prooxidant activity in the oil (35) (36).

The fatty acid pattern of grape seed oil shows that it is rich in both oleic and linoleic acids, however, linolenic acid is not detected. Comparing the fatty acid patterns of grape seed, cottonseed and soybean oils (Table IV) it is found that the oil has a similar composition to cottonseed oil and therefore problems encountered with flavour instability, arising from linolenic acid, is avoided.

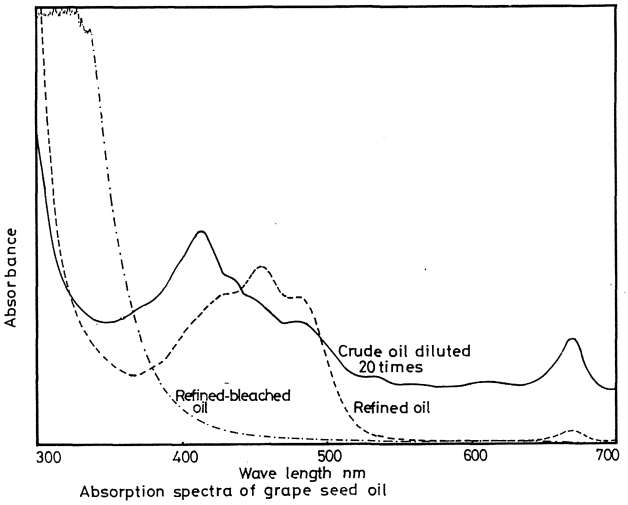

Figure 1
Absorption spectra of grape seed oil

Table IV
Fatty Acid Pattern of Grape Seed Oil in Comparison With Cottonseed and Soybean Oils

	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$	$C_{20:2}$
Grape seed oil	0,4	12,8	0,2	7,9	28,9	49	—	0,2	0,6
Cottonseed oil	1,0	25,2	0,9	3,0	20,3	48,7	—	0,3	—
Soybean oil	—	12,6	—	6,5	30,1	44,4	6,4	—	—

To complete the picture, it was of interest to determine the sterol constituents of the oil by GLC analysis. Pure sterols, isolated from the unsaponifiable matter, were analyzed by GLC as their TMS derivatives. The analysis shows four sterols i.e., campesterol, stigmasterol, beta-sitosterol and isofucosterol at the levels of 10,6, 13,7, 73,8 and 1,9%, respectively (Table V).

Table V
GLC Analysis of Sterols Constituents

Campesterol	10,6%
Stigmasterol	13,7%
Beta-Sitosterol	73,8%
Isofucosterol	1,9%

In an attempt to throw light on the possibility of utilization of the meal of grape seeds as a potential source of protein, the defatted seed meal was evaluated for its protein content and amino acid composition. It was found that the protein content of the defatted meal amounts to 24,6% which is lower than those found in cottonseed and soybean meals (1). Table VI illustrates amino acid, composition of grape seed meal which contains large proportions of essential amino acids, but they are generally lower in their quantities than those in the other oil seed meals. Therefore, it is recommended that grape seed meal can be used in a mixture with other vegetable proteins to give better amino acid balance for different feeding purposes.

Table VI
Amino Acid Constituents of Grape Seed Meal

Amino Acid constituents	Amino Acid g / 16g N_2
Lysine	3,57
Histidine	1,72
Arginine	4,0
Threonine	1,45
Valine	3,46
Methionine	0,9
Cystine	0,0
Isoleucine	1,97
Leucine	—
Tyrosine	2,15
Phenylalanine	2,60

It is generally concluded that grape seeds can be used as potential source of oil and protein. The absence of linolenic acid in the oil and the presence of high levels of tocotrienols with considerable amounts of tocopherols can improve the oil stability. Owing to the fact that grape seeds, locally produced as by-product, have not been previously studied in details, the present investigations will assist to increase its economic utilization for different purposes.

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