BIOCHEMICAL STUDIES ON PECAN KERNELS CULTIVATED IN EGYPT

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دراسات بيوكيميائية على لب البكان

ملخص البحث

وجد أن لب البكان يحتوى على ٦٨٪ ليبيدات ، ١١,٢٪ بروتين ، ١٦,٣٪ كربوهيدرات ، ١,٨٥٪ رماد .

وكانت خواص الزيت كالآتى : رقم التصبن ١٨٩,٥ ـ العدد اليودى ١٠١,٥ ـ معامل الانكسار على درجة ٢٥°م ١,٤٦٩ والكثافة على درجة ٢٥°م ٠,٩١٧ ـ والمواد الغير متصبنه ٠,٤٦٪ .

وقد أجرى فصلا كروماتوجرافيا للزيت على طبقة من السليكاجيل (TLC) وكانت الجليسريدات الثلاثية هى المكون الرئيسى للزيت بينما ظهرت الجليسريدات الثنائية بكمية بسيطة .

أثبت التحليل الكروماتوجرافى الغازى لاستر ميثايل الزيت وجود ٩ أحماض دهنية وكانت الأحماض الدهنية الغير مشبعة (الاوليك واللينوليك والبالميتوليك وميريستوليك) هى المكون الأساسى للزيت حيث بلغت حوالى ٨٤,١٩٪ بينما وصلت نسبة البالميتك ١٢,٦٪ وحمض الاستياريك ٢,٠١٪ .

أما أحماض الكبريك والميريستك واللوريك فقد ظهرت بتركيزات منخفضة جدا .

وقد وجد أن كسب البذرة يحتوى على ١٨ حامض أمينى وقد أظهر البروتين نقطة تعادل كهربى على درجة pH ٤,٥ وأخرى على درجة pH مختلفة وهى ٧,٨ .

ABSTRACT

The chemical composition of pecan kernels showed that kernels contained about 68% lipids, 11.2% proteins, 16.3% carbohydrates and 1.85% ash (dry wt.). The oil characterized by sap. value 189.5, iodine value 101.5, refractive index (n^{25}) 1.469, specific gravity (d^{25}) 0.917 and 0.46%unsaponifiable matter.

TLC of lipids showed that the triglycerides were the major class in the investigated oil. While mono and diglycerides were of minor existance.

GLC of fatty acids showed the presence of nine fatty acids from which the unsaturated fatty acids, oleic, linoleic, palmitoleic and myristoleic contributed 84.19% of the total fatty acids. Palmitic and stearic acids were 12.60% and 2.01% respectively. Capric, myristic and lauric acids were detected.

Eighteen amino acids were detected in the defatted meal by using Amino acid Anlyzer. The protein solubility of defatted meal at different pH values showed two iso electric points, one at pH 4.5 and the other at pH 7.8.

INTRODUCTION

In Egypt production of nuts is inadequate, Pecan or Black nut (Juglans nigra) is recently cultivated. El-Hamady et al. (1984) reported that pecan fruit is a nutritious and a good source of energy, its oil generally showed low acid value and high saponification value. They also mentioned that the oil is digestable and can be used safely in human nutrition. Meredith (1975) studied the chemical composition of five varieties of pecan. He found that the oil percent ranged from 61% to 75% and protein percent from 6.6 to 12.4%. He added that eighteen amino acids were detected through which glutamic acid was the highest and cysteine was the lowest.

Youssef et al. (1967) reported that pecan oil contained negligible amount of volatile fatty acids and low content of saturated fatty acids. Oleic acid was the major component contributed 70.37%

of total fatty acids, linoleic was the second, while linolenic was undetectable.

August et al. (1967) studied the concentration of the major glycerid types component in cotton seed and pecan oils at selected iodine values. They found that the relative concentrations of the glycrides changed markedly with increasing iodine value. They added that pecan oil consisted mainly, of oleic, linoleic, stearic and palmitic acids. It is worthy to indicate that Wood and Mc Means (1982) mentioned that the major glycerides fractions were oleate, linoleate, palmitate, linolenate, stearate, myristate and arachidate.

He stated that the major sugars in pecan kernels were fructose, glucose, sucrose and inositol.

The work embodied in this study was carried out to investigate the fatty acids composition together with amino acid content in pecan kernels cultivated in Gharbia province, Egypt.

MATERIAL AND METHODS

Samples of pecan fruits (Money maker) were obtained during the season of 1986. The kernels were removed by manual cracking. The kernel percentage was calculated (60.5% ± 4.2%). The kernels were ground in mill to pass through a 20 mesh sieve and stored at 0°C to be used for analysis.

Analytical methods: Total proteins, crude lipids were determined according to the procedure described by AOAC (1975). The protein content was calculated by using the factor 6.25. The total carbohydrates were determined by the method of Dubis et al. (1956).

Specific gravity and refractive index were estimated at 25°C. Acid, saponification and iodine values were determined following the methods described by AOCS (1973).

TL- chromatography: The nonpolar lipid fraction was cleaned up. via silicic acid adsorption chromatography similar to Zederiowski and Soususki (1978).
Twenty gm sample of the above lipid was dissolved in 250 ml $CHCl_3$ and shaked for 20 min with 100 gm of activated silicic acid (100 mesh, activated by heating at 120°C for 24 hr). The mixture was poured into a filtered disc funnel to separate the nonpolar fraction.

The isolation of such nonpolar fraction to its classes was carried out by thin layer chromatography using silica gel G plates 20x20 cm, layer thickness 0.25 mm (E. Merck, Darmatat, W. Germany). Whereas 0.5 gm of the above nonpolar fraction was dissolved in 5 ml $CHCL_3$, then 10 µl sample was applied on the origin line of the plate. The nonpolar fraction of cotton seed and maize oils, previously prepared as forementioned. The charged plates, in dublicate, were developed ascendingly at room temperature for 45 min, using the solvent system : pet. ether : diethyl ether : acetic acid 80/20/1 by volume. One of the air dried plates was visualized in iodine vapors to locate the separated spots with the aid of the reference samples.

Triglycerides sample was prepared by spotting the nonpolar fraction in bands on dublicate plates, developed under the above conditions. One of the developed plates was visualized in iodine vapors whereas the triglycerides band on the other developed plate could be easily located. The located triglycerides band was scraped and extracted with minimum amount of $CHCL_3$ and kept in refregirator ready for analysis.

The triglyceride groups in the above glyceridic mixture were fractionated, according to their unsaturation, via the argentation TLC technique. Whereas plates of the forementioned thickness were impregnated with silver nitrate according to Kaufman and Wessels

(1964) by vertical developing in a 12.5% methanolic silver nitrate solution till the end of the plates, then dried in an oven at 100°C for 3 hrs and stored in dark.

Samples of the above triglycerides mixture in chloroform were applied, as a 2-cm streak, on the plates impregnated with silver nitrate. The charged plates were developed, assendingly at room temperature in the solvent system: CCl_4 : $CHCl_3$: CH_3COOH : C_2H_5OH (60/40/0.5/0.4 by Volume). Then the air dried plates were visualized by spraying with 50% H_2SO_4 solution and chering on hot plate.

GLC analysis:

The methyl esters of pecan oil fatty acids were prepared according to the procedure described by Ibrahim et al. (1964). The methyl ester produced were injected into Pye Unicum chromatographic apparatus. The column was packed with 10% PEGA an acid washed diatomit (100-120 mesh); length (150 cm X 4 mm) and carrier gas flow of nitrogen (30 ml/min) hydrogen (33 ml/min) air (330 ml/min), detector and injection temperature 220°C, column temperature 190°C.

Amino acid analysis: The amino acid composition of the protein hydrolyzate was determined by means of L K B Amino acid analyzer (AAA). Whereas cystine and methionine were determined by PC of acidic hydrolysis of protein adapting the procedure of Bloc et al. (1958) Tryptophan was determined according Blauth et al. (1963).

Protein pH solubility profile: Representative samples (one gm) of the fine defatted meal were dispersed in known vol. of distilled water, then series of pH treatments were adjusted to be in the range of 1-12 by using a molar solutions of either HCl or Na OH. Such solutions were shaken mechanically at room temperature (25°C) for 1 h. The soluble nitrogen fraction of each of the above treatments were separated by centrifugation at 1260 xg for 30 min. The clear

supernatants were subjected to nitrogen determination via kjeldahl method where the protein content could be deduced by using the factor 6.25.

RESULTS AND DISCUSSION

The crude lipids content of pecan kernels was found to be 68% dry wt. in good agreement with the previous reported data of Meredith (1975).

Table (1): The physico-chemical properties of oil extracted from Pecan nuts.

Physico-chemical properties	Value
Refractive index 25°C	1.469
Specific gravity 25°C	0.917
Acid value	0.220
Iodine value	101.500
Saponification number	189.500
Unsaponifiable Matters %	0.460

Table (1) showed low acid value, high iodine number and high saponification value, results are very close to that of El-Hamady et al. (1984).

The actual chromatogram Fig. (1) refers to the separated lipid classes of pecan oil (A) comparable with cotton seed (B) and corn oils (C). It could be noticed that the investigated oil showed similar fractions when compared with those of both, cotton seed and corn oils. It is of interest to indicate that pecan nut oil consists, mainly of triglycerides while the monoglycerides were of minor existance.

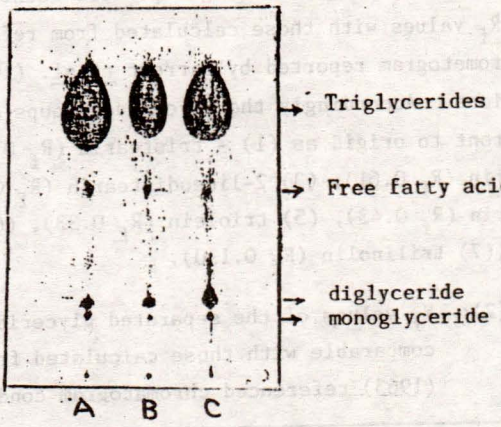

Fig. (1): TLC of pecan oil (A), cotton seed oil (B) and corn oil (C).

The triglycerides fraction was isolated and fractionated to its glyceridic composition via argentation TLC technique (Fig. 2). Such fraction achieved the presence of seven glyceride groups varied in their R_f values.

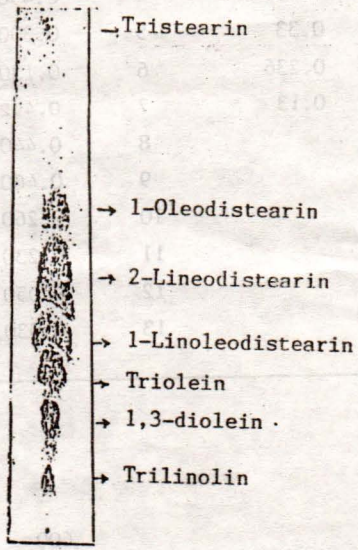

Fig. (2): Argination TLC of triglycerides of pecan oil.

The separated glyceride groups were identified by comparing their R_f values with those calculated from reference compounds in the chromatogram reported by Barrett et al. (1963) under similar conditions. Accordingly the glyceride groups could be identified from front to origin as (1) - tristearin (R_f 0.95), (2) 1- oliodistearin (R_f 0.61), (3) 2-lineodistearin (R_f 0.50), (4) 1-linoleodistearin (R_f 0.43), (5) triolein (R_f 0.33), (6) 1,3 diolein (R_f 0.236) (7) trilinolin (R_f 0.130).

Table (2): R_f values of the separated glyceride compositions comparable with those calculated from Barrett et al. (1963) referenced chromatogram conditions.

Found				Barrett et al.
Comps.No. front to origin	R_f	Compos.No. front to origin	R_f	Glycerides fraction
1	0.95	1	0.950	Tristearin
2	0.61	2	0.690	2-oleodistearin
3	0.50	3	0.620	1-oleodistearin
4	0.43	4	0.490	1-stearodiolin
5	0.33	5	0.300	triolein
6	0.236	6	0.130	Trilinolin
7	0.13	7	0.492	2-linoleodistearin
		8	0.440	1-linoleodistearin
		9	0.460	1,3-distearin
		10	0.260	1,2-diolein
		11	0.230	1,3-diolein
		12	0.050	monostearin
		13	0.030	monoolein

GLC analysis of the methyl esters of pecan nut kernels revealed the presence of nine fatty acids Table (2) showed the relative concentration of the fatty acid contents of the investigated oil. It is worthy to notice that pecan oil was rich in oleic acid (59.62%) followed by linoleic acid (14.63%). However Youssef et al. (1967) indicated that oleic acid ranged between 69.51-70.37%, such variations may be due to variatial and other environmental conditions. Palmitoleic and myristoleic were also detected among the unsaturated fatty acids.

The saturated fatty acid contents were 15.81%, of which palmitic acid was the major saturated fatty acid (12.6%) followed by stearic (2.01%). It is of interest to notice that the TU/TS ratio was 5.3.

Table (3): Relative concentrations of the fatty acid contents of Pecan muts oil calculated from GLC.

Fatty acids	relative concentrations
Capric	0.51
Lauric	0.31
Myristic	0.37
Myristoleic	0.56
Palmitic	12.60
Palmitoleic	9.36
Stearic	2.01
Oleic	59.62
Linoleic	14.63
Total saturated fatty acids (TS)	15.80
Total unsaturated fatty acids (TU)	84.17
TU/TS ratio	5.30

Fig. (3) represented the solubility index of pecan muts proteins. The solubility in the alkaline region was much higher than that under the acidic condition, whereas it was 85% and 37% at pH 10 and 2 respectively. It is interesting to note the presence of two minimum solubility regions, i.e. pH 4.5 and 7.8. Such observation was reported for mustard seed proteins by Rao et al. (1978) who reported that mustard seed protein showed two isoelectric points the first at pH 4.0 and the second at pH 8.0. Also Thompsm et al. (1976) found two isoelectric points for rapeseed meal proteins.

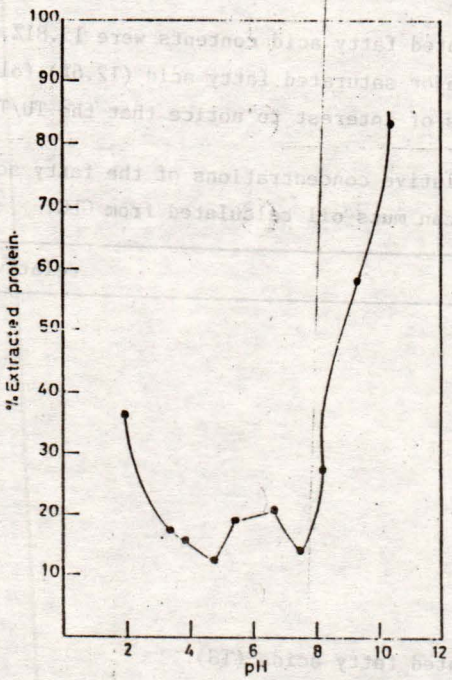

Fig. (3): Solubility Index of Pecan nut Protein.

Table (4): Amino acid composition of pecan nut kernels.

Amino acid	Mg amino acid/g. dry defatted meal
Essential amino acids:	
Therionine	3.78
Valine	4.65
Methionine	3.05
Isoleucine	3.90
Ieucine	7.41
Phenylalanine	4.07
Lysine	3.49
Tryptophan	3.60
Non-essential amino acids:	
Aspartic acid	12.73
Serine	6.34
Glutamic acid	18.70
Proline	3.65
Glycine	2.89
Alanine	5.35
Cystine	2.15
Tyrosine	2.64
Histidine	2.89
Arginine	10.70
Ammonia	1.75

Table (4) showed the presence of appriciable amounts of eight essential amino acids in the protein hydrolyzate of pecan protein. Also, ten non-essential amino acids were detected of which glutamic acid was predominant (18.7 mg/g.).

It could be concluded that pecan protein is a good source for most amino acids required in diet.

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