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STUDYING OMEGA-3 AND OMEGA-6 CONTENT OF NATIVE WALNUT AND STORAGE EFFECT ON IT

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ABSTRACT: Walnuts from Northwest region of Syria was collected to determine Omega-6 (linoleic acid (LA, 18:2n-6)) and Omega-3 (α -linolenic acid (ALA, 18:3n-3)) in their oils, also called essential fatty acids (EFAs). This work was carried out using two different portions of samples. First samples were collected at harvest and second samples stored for one year under normal conditions. The extraction of the oil contained in Walnuts was done with hexane by ultrasonic technology, extract contained fatty acid and these fatty acids were esterified. Fatty acid methyl esters were analyzed by gas chromatography with flame ionization detection (GC-FID). Syrian Walnuts major fatty acids were 18:2n-2 (linoleic acid) and 18:3n-3 (α -linolenic acid). Regarding n-6/n-3 ratio corresponded to the desired profile; Syrian Walnuts could be of potential interest due to their high nutritive value and lipid content.

INTRODUCTION: Over the last few years, several oils from nuts have been studied. To make sure that they are suitable for commercial use, it is very important to investigate their chemical and physical properties¹.

Nuts may be considered an important component of a healthy diet. In general, they are nutrient-dense foods and provide protein, fat (mostly unsaturated fatty acids), dietary fiber, and many bioactive constituents such as vitamins, minerals, antioxidants, and other phytochemicals. Given such variety of bioactive compounds, extensive research has focused on potential health effects of higher nut consumption on the development of heart disease and prostate cancer².

Omega-3: unsaturated fatty acids have double bond at carbon atom number 3, if we begin enumeration from methyl fraction, also called n-3 or e-3.

Omega-6: unsaturated fatty acids have double bond at carbon atom number 6, if we begin enumeration from methyl fraction, also called n-6 or e-6.

Plants can synthesize Omega-6 and Omega-3, while human and animals cannot synthesize but they take them from wild plants as algae and plankton³.

Oil fish is considered an important resource of Omega-3 where content:

EPA (Eicosapentaenoic acid) $C_{19}H_{31}COOH$

20: 5(n-3), Omega-3, all-cis-eicosa-5, 8, 11, 14, 17 pentaenoic acid

DHA (Docosahexaenoic acid) $C_{21}H_{34}COOH$

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22:6 (n-3), Omega-3, all-cis-docosa-4, 7, 10, 13, 16, 19-hexaenoic acid.

The major constituents of oils are their fatty acids, which may include saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) that contribute to human physiology in different ways⁴. Dietary factors are associated with increasing levels of cardiovascular disease, certain types of cancers, stroke, obesity, non-insulin dependent *diabetes mellitus*, and atherosclerosis⁵.

Extensive research has shown that PUFAs are indispensable for the proper functioning of the brain, the eyes, and the entire nervous system. PUFAs are often classified into two families, n-3 and n-6, according to double bond positions. In spite of the important biological functions of n-3 and n-6 groups, humans lack the enzymes required to form double bonds beyond the $\Delta 9$ position. To overcome this limitation, the primary PUFAs precursors, linoleic acid (LA, 18:2n-6) and α -linolenic acid (ALA, 18:3n-3), also called essential fatty acids (EFAs), must be obtained from a diet⁶.

Since, saturated fatty acids increase the risks of cardiovascular diseases, cancer and autoimmune disorders, oils are of more nutritional value if they have proportionally more unsaturated than saturated fatty acids⁴. The determination of fatty acids is a routine method in food chemistry and food control. The lipid phase is extracted with organic solvents, and the fatty acid profile is determined after trans-esterification into fatty acid methyl esters (FAMES), followed by gas chromatography-flame ionization detector (GC-FID) analysis. GC-FID is the method of choice for the determination of relative contributions of individual fatty acids to the fatty acid pattern due to the relative constant response factors irrespective of the chain length and number of double bonds^{7,8}.

Food composition databases that are comprehensive and representative of available foods provide detailed information on nutrients and dietary factors in foods⁵. Thus, the aim of this study was to determine Omega-6 and Omega-3 profile of walnuts from the Northwest region of Syria, so as to provide reliable data on the nutritional composition of Syrian native walnuts.

MATERIALS AND METHODS:

Chemical and reagents: Hexane, methanol, (all of HPLC-grade), Phenol (99.6%) were purchased from MERCK. Ethanol (99.8%), KOH and anhydrous Na_2SO_4 were of analytical grade. Standards of linoleic methyl ester and linolenic methyl ester were purchased from SIGMA.

Samples: This work was carried out using walnuts obtained from the Northwest region of Syria, which were harvested from the native trees of this region. All samples were collected from annual harvest (2010). Approximately 0.5 kg of walnuts was collected from harvest. Samples were divided into two portions, sample1 was used for analysis and sample-2 stored for one year with holding external peeling under normal conditions. **Table 1** shows the fruits and nuts assayed in this study.

Sample preparation: The pulp of each sample (harvested) was grinded and homogenized in a food processor and divided into portions (5 g each one) for immediate analysis. Each portion was distributed into plastic bags. The air was removed from the plastic bags,

Total lipids extraction: The extraction of the oil contained in walnuts (pulp) was done with hexane by using ultrasonic for 20 minutes. Then, the extract was filtered. This process was repeated three times, so as to avoid the use of organochlorinated solvents in the sample treatment. The oil was obtained by hexane evaporation in a rotary evaporator at 60°C under vacuum till 10 ml. The oily fraction resulting from the cold extraction was transferred to a test tube with cap.

Fatty acids determination: About 5 ml of oil sample was weighed in a flask. Next, a methanolic solution of KOH 0.5 mol.L^{-1} was added, and the mixture was shaken for 5 minutes, to convert free fatty acids into methyl esters. Subsequently, the upper organic phase that contained the fatty acids methyl esters was collected and transferred to a test tube containing Na_2SO_4 . 1ml of the organic phase containing methyl esters was added to 1ml from phenol 1g/100ml (internal standards) and kept in flasks with caps and 1 μl of this solution was injected in the gas chromatograph. The samples were analyzed in duplicate.

Methyl esters Gas Chromatography: Fatty acids methyl esters were determined by gas chromatography using a GC2010 (Shimadzu) gas chromatograph equipped with a flame ionization detector (FID), a split/splitless capillary inlet system, and a CP-Sil 88 WCOT fused silica capillary column (Agilent) with dimensions of 60 m × 0.25 mm id × 0.25 μm film thickness (Figure 1). After several assays were carried out the operation parameters used were: injector temperature: 250°C; detector temperature: 250°C; column temperature starting at 115°C (holding for 5 minutes), and then increasing 15°C/minutes to 220°C (holding for 15 minutes) - total analysis time: 27 minutes; carrier gas: nitrogen (N₂) at a linear velocity of 30 cm.s⁻¹; and split ratio of 1:50. Retention time and peak areas were registered by means of Chromatopack 3.1 software (Shimadzu).

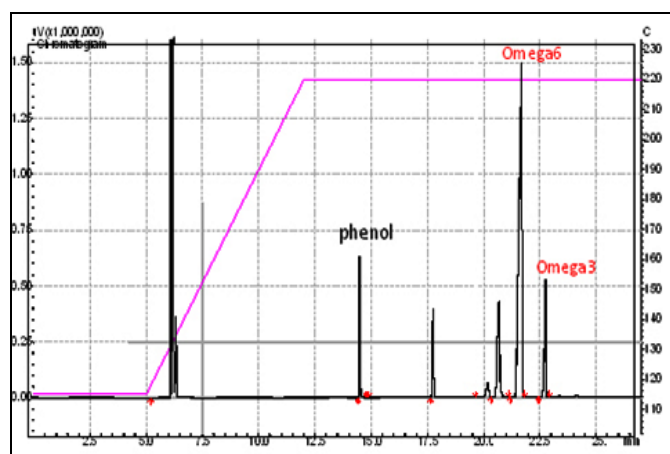


FIGURE 1: METHYL ESTERS GAS CHROMATOGRAPHY

Fatty acid identification: Fatty acids methyl esters were identified by comparing the retention time of the samples and appropriate fatty acids methyl esters standards, purchased from Sigma (Germany).

RESULTS: As can be seen in Table 1, Syrian walnut major fatty acids were 18:2 (linoleic acid) and 18:3n-3 (α-linolenic acid). Both linolenic acid and linoleic acid are essential fatty acids since linolenic acid is precursor of eicosapentaenoic acid (EPA) and LA is precursor of arachidonic acid (Which are precursors of prostaglandins, thromboxanes, and leukotrienes in humans as mentioned by Tapiero et al., 2002⁹). Also it can be seen in Table 1 the quantitative differences between fresh native walnuts and storage native walnuts.

The results obtained in this study showed the influence of the storage on Syrian walnuts content. The content of omega-3 and omega-6 were lowered in half during storage. While the storage did not influence of the ratio of n-6 to n-3, it stayed nearly the same.

TABLE 1: CONTENT OF OMEGA-3, OMEGA-6 AND OMEGA-6/OMEGA-3 RATIO OF FRESH NATIVE WALNUTS AND STORAGE NATIVE WALNUTS

Sample	OMEGA 6 g/100g =	OMEGA 3 g/100g =	OMEGA 6 OMEGA 3
Fresh native walnuts (1)	35.51	9.12	3.9
Storage native walnuts (2)	17.51	4.29	4.1

DISCUSSION: Syrian walnuts studied in this research are of potential interest due to their high nutritive value and lipid content. In addition, the ratio n-6/n-3 was 4 and this corresponds with nutritive recommendations. It is useful to prepare nutritive forms from walnuts which are suitable to eat in schools and regions which are poor in local walnuts. But walnuts must be eaten fresh or stored in special conditions (packed and stored in cool and dark place). The cultivation of these plants, which has been traditionally carried out by the natives since ancient times, should be encouraged.

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