E-ISBN 978-9975-75-893-2

COMPOZIŢIA CHIMICA A ŞROTULUI DIN MIEZ DE NUCI JUGLANS REGIA L.

Dr., conf. univ., Carolina GROSU

Academy of Economic Studies of Moldova,
Republic of Moldova, Chisinau, 61 Mitropolitul Banulescu-Bodoni street
Phone: (+373 22) 22 41 28; Web site: www.ase.md

Abstract

Walnut kernels contain a large amount of fat (> 50% of weight), 11% protein, 5% carbohydrates and is highly caloric (about 525 kcal per 100g. product) [18]. Of special importance is the walnuts and byproducts (fr: co-produits, English: by-products) thorough processing, primarily of the oilcake resulting from oil extraction [7].
Oilcake resulting from oil extraction is only used for animal feed, therefore study of the nutritional and sensory quality and identification of its processing and use conditions in nutrition is necessary.

Key words: *walnut oilcake Juglans Regia L., chemical composition, nutritional value, sensory quality, optimal condition, proteinaceous substances, dietary fibres.*

JEL CLASSIFICATION: Q16

INTRODUCTION. Walnut crop occupies a significant place in Moldovan agriculture and is a strategic branch in the country's national economy. Moldova is positioned on a favorable geographic position concerning propitious conditions, as climate so and soil for growing walnuts, being counted among the top ten producers of walnuts kernel and walnuts in shell of world production volume reaching about 10 to 13 thousand tons per year [4].

The interest for walnuts is determined and by it’s nutritional value, walnuts fat are rich in omega-3, omega-6 fatty acids, which play an essential role for the body functioning, but can’t be synthesized by the human body. Walnuts are one of the few foods that contain melatonin - the hormone involved in the regulation of circadian rhythm and resveratrol - polifenol with strong anti-aging and protective effect on the cardiovascular system [1]. They have also amounts of dietary fiber, vitamins (E, B3, B5 B6) and minerals (potassium, phosphorus and magnesium). The chemical composition of the walnut kernels is influenced by genetic and environmental factors [5].

A special importance has the byproducts processing, mainly the processing of walnut oilcake which results from oil extraction. Oilcake resulting from the extraction of oil is not processed further, being used only for animal feed [8]. As for the general information regarding the chemical composition, nutritional and biological value of walnuts grown in Moldova, these are very limited and fragmented and totally missing for the oilcake. Study of the chemical composition and nutritive value for the oilcake is necessary because it will be used in confectionery products.

The practical application of oilcake for producing food products with improved nutritional profile requires a thorough study of the chemical composition and nutritive value of oilcake.

MATERIALS AND METHODS. Main raw materials used for this research were the walnut kernels (*Juglans Regia L.)*- GOST 16832—71, the harvest of years 2011-2014 and walnut oilcake obtained in laboratory conditions.

The fat content was determined by the Soxhlet method by repeated extraction with diethyl ether or with petroleum of fatty substances. Assessing the Acid Index is the amount of KOH required to neutralize a gram of fat (mg KOH / g or mg NaOH 1N / l00 g fat). Determining the total number of microorganisms allows to detect the number of colonies arising from cells microorganisms.

The protein content in each fraction was determined by the Kjeldahl method. Walnuts Kernel was previously milled and defatted flour was obtained. Protein fractions were determined using the degree of solubility in various solvents. Determination of the amino acid constituents of proteins is

E-ISBN 978-9975-75-893-2

possible by hydrolysis of proteins - decomposition into constituents amino acids under acid action of (HCl – 6N, 110^0C, 12-48 hours) [12].

Establishment of protein digestibility is based on the changing pH after protein hydrolysis with a multienzyme combination.

The entire mineral elements presents the residue obtained after calcination of a product sample at 525 ± 25 ° C and is called "ash". Ash content is a measure of the total amount of the mineral, while the content of mineral substances is a measure of the amount of specific inorganic elements present in the food. The content of mineral elements of the kernel and walnut oilcake was determined by atomic absorption spectrophotometry (AAS) after ashing the samples and solubilization of the resulting ash in hydrochloric acid [3].

The fatty acid content in the kernel and walnut oilcake were determined by gas chromatography using gas chromatograph, ECHP with a flame ionization detector [16].

Experimental results are expressed as average ± SD (standard deviation).

RESULTS AND DISCUSSION. The main spare component of walnuts are lipids. The total lipid content of the samples is shown in Table 1.

Table 1. The total lipid content in the walnut kernel and oilcake

Variety	**% from dry matiere (DM)**			
	Călărași		**Cogălniceanu**	
	kernel	oilcake	kernel	oilcake
Total Lipids	64,55±0,51	39,90±0,41	65,13±0,42	40,85±0,51

**Sources: Grosu C.Valorificarea şrotului de nuci şi obţinerea produselor de cofetărie. Teza de doctor în tehnică.C.Z.U.: [664.143 + 664.68] : 634.51 (478)(043.2),2016.*

From the results it is noted that the lipid content in the samples studied ranged between 64.55 and 65.13% DM for Walnuts variety Calarasi / Cogîlniceanu and from 39.90 to 40.85% DM for oilcake variety Calarasi / Cogîlniceanu. Fractionation of protein extracts of the oilcake variety Calarasi and Kogalniceanu was achieved through sequential extraction in different solvents (tab.2).

Table 2. Results for walnut oilcake protein fractionation

Protein fraction Nitrogen fraction	Oilcake content, % DM		Distribution of protein fractions,% of total protein	
	Călărași	Cogălniceanu	Călărași	Cogălniceanu
Total protein, including:	**25,69±0,48**	**26.25±0,50**	**100**	**100**
• Albumins	1,28±0,13	1.28±0,13	4,98	4,87
• Globulins	5,12±0,63	4.76±0,62	19,92	18,13
• Glutelins	14,46±0,13	15.02±0,15	56,28	57,21
• Stroma (constitutional proteins)	4,17±0,13	4,67±0,16	16,23	17,79
Total nitrogen, including:	**4,09**±0,19	**4,16**±0,20	100	100
• Protein’s nitrogen	3,34±0,11	3,34±0,12	81,66	80,28
• Extractive nitrogen	0,08±0,03	0,12±0,02	1,95	2,88
• Stroma’s nitrogen	**0,67**±0,01	**0,75**±0,01	16,38	18,02

**Sources: Grosu C.Valorificarea şrotului de nuci şi obţinerea produselor de cofetărie. Teza de doctor în tehnică.C.Z.U.: [664.143 + 664.68] : 634.51 (478)(043.2),2016.*

The major protein fraction in oilcakes from Calarasi and Cogalniceanu Walnuts are glutelins constituting 56.28 and 57.21% respectively, being followed up by globulins stroma and albumins. Comparable results were reported by Sze-Tao (2000), Mao (2014) and Pirnazarov (1975) [17]. The

E-ISBN 978-9975-75-893-2

amino acid composition and chemical index of the walnut kernel and oilcake proteins variety Calarasi and Cogălniceanu are shown in Figure 1 and 2.

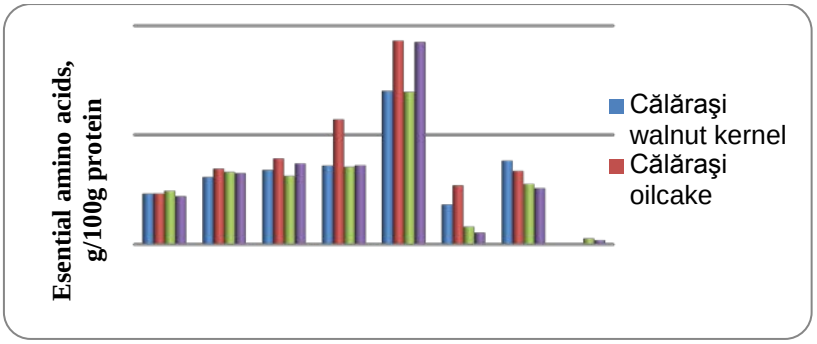

Figure 1. *The content of amino acids in the walnut kernel and oilcake protein*

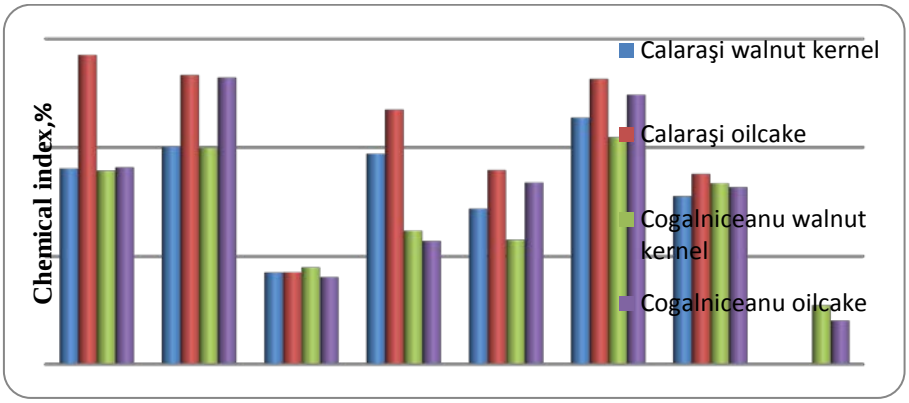

Figure 2. *The chemical index of the walnut kernel and oilcake protein, %.*

**Sources: Grosu C.Valorificarea şrotului de nuci şi obţinerea produselor de cofetărie. Teza de doctor în tehnică.C.Z.U.: [664.143 + 664.68] : 634.51 (478)(043.2),2016.*

Analysis of the results from Figures 1 and 2 show that the majority of the protein amino acids of walnuts kernel and oilcake are glutamic acid, aspartic acid and arginine - common feature for all vegetable. The given values of the amino acid content are comparable to those reported by Ruggeri and others (1998) [15]. One of the quality characteristics of plant proteins is the content of lysine which is 2.33 / 2.46 for walnut varieties Calarasi / Cogălniceanu and 2.34 / 2.21 for walnut oilcake variety Calarasi / Cogălniceanu.

Lysine importance stems from the fact that it is an essential amino acid, but playing a significant role in chemical and technological behavior of proteins that amine function from aliphatic chain can participate in various chemical reactions, including grafting and crosslinking proteins. The proportion of essential amino acids is approximately 30%.

Walnut proteins contain appreciable amounts of aspartic acid constituting 7,40 / 11,21 for walnut varieties Calarasi / Cogălniceanu and 7.65 / 12.57 for the oilcake from walnut varieties Calarasi / Cogălniceanu and glutamic acid which constitutes 15,72 / 20,11 for kernel and 19,20 / 17,59 for the oilcake that are considered essential in protein metabolism [14].

It is worth noting, and the high content of arginine, which is an important regulator of blood pressure in the cardiovascular system [9]. Finally, it is worth mentioning and the high content of the flavour amino acids and (glutamic and aspartic acid, alanine and glycine), which proportion constitutes 30-40% 30-40% .

Limiting amino acids are lysine with a chemical score of 42., / 44,9% for kernel (Calarasi / Cogălniceanu) and 42,6 / 40,3% for oilcake (Calarasi / Cogălniceanu), threonine – 77,4 / 83, 5% for kernel (Calarasi / Cogălniceanu) and 87,7 / 81,6% for oilcake (Calarasi / Cogălniceanu). To fill the gap of certain amino acids, it is necessary to combine walnuts with other food.

E-ISBN 978-9975-75-893-2

For example, to recoup the shortfall in lysine walnuts can be combined with : meat, fish, eggs, dairy products, vegetables, soybeans, corn and fermented foods (eg sauerkraut). To compensate the shortfall in methionine, walnuts can be combined with: egg products, fish, garlic, lentils, meat, onions, soy, milk and dairy products.

All of the mineral elements are shown in Table 3 and they have a 95% confidence interval.

Table 3. The mineral content in the walnut kernel and oilcake variety Calarasi

Mineral elements	Content, mg/100 g		
	Oilcake	**Kernel**	**USDA**
Potassium	528,3±31,69	356,3±21,3	**441**
Sodium	1,65±0,09	1,33±0,07	2
Magnesium	198,8±11,92	146,6±8,7	**158**
Calcium	180,9±10,8	136,1±8,1	**98**
Iron	8,59±0,51	7,09±0,42	**2.91**
Zinc	3,79±0,22	2,91±0,17	3,1
Copper	1,96±0,11	1,56±0,09	**1,6**

**Sources: USDA National Nutrient Database for Standard Reference, Release 21 (2008)*

Depending on their content in kernel and oilcake (mg / 100g) mineral elements form the series: K> Mg> Ca> Zn> Fe> Na> Cu. Analyzing the results with those stipulated by USDA and published by other authors, it is obvious that mineral content of the oilcake is higher than in the kernel, which indicates that the mineral elements are not uniformly distributed in the mass of the kernel and that their content in lipid fraction is smaller (with the exception of phosphorus), and their use in human food would provide the required amount of minerals in a well-balanced diet. The walnut kernel contains 52-70% fat [6; 13; 16]. The major constituents of the oil are triglycerides walnut with large amounts of monounsaturated fatty acids (mainly oleic acid) and polyunsaturated (linoleic and linolenic acid).

The proportion of fatty acids is an important indicator for assessing the quality of the oil. The high content of fatty acids linoleic and linolenic may result in a lower oxidative stability and a shorter shelf life of oils. The presence of tocopherols (a powerful antioxidant) has a protective effect against oxidation.

Walnuts also contain phytosterols (substances considered as nutraceuticals), inhibiting intestinal absorption of cholesterol and several minor unsaponifiable substances, such as carbohydrates [11]. Knowing the chemical composition of lipid is necessary for the evaluation of the nutritional quality and to encourage the consumption of walnuts and derived products, and to identify the feasibility of using them in food production.

The average content of total fat in walnut kernel was 64.85% and 39.90 ± 0.41% oilcake. Average values and standard deviations of the fatty acid composition are shown in Figure 3.

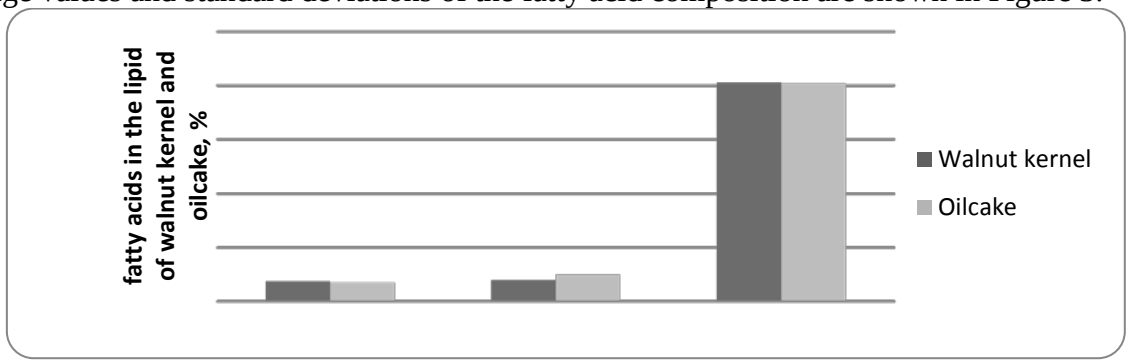

***Figure 3.** The content of fatty acids in the lipid of walnut kernel and oilcake, %*

**Sources: Grosu C.Valorificarea şrotului de nuci şi obţinerea produselor de cofetărie. Teza de doctor în tehnică.C.Z.U.: [664.143 + 664.68] : 634.51 (478)(043.2),2016.*

E-ISBN 978-9975-75-893-2

As it is illustrated in Figure 3, the proportion of saturated, mono- and polyunsaturated fatty acids of the kernel lipids is respectively 7,53 – 7,93 and 81.1% and 7,04 to 10.30 and 80,79 for the oilcake lipids . The results are comparable to those reported in the literature [10; 18].

Total number of germs expressed in cells / 1g oilcake, was determined by Agar and Sabouraud culture media. The results are shown in Table 4.

Table 4. Influence of storage conditions on the total number of microorganisms in oilcake kept in different conditions

The storage period of oilcake	**Sample Names, culture media, and the total colony count (cells / 1 g oilcake)**					
	Vacuum packed frozen oilcake		**Chilled oilcake**		**Frozen oilcake**	
	Agar (Bacteria and yeasts)	**Sabouraud** (Fungi)	**Agar** (Bacteria and yeasts)	**Sabouraud** (Fungi)	**Agar** (Bacteria and yeasts)	**Sabouraud** (Fungi)
Initial	$2,0*10^3$					
1 month	$4,6*10^3$	-	$3,2*10^4$	-	$2,1*10^4$	-
2 month	$5,0*10^3$	$4,8*10^2$	$4,6*10^4$	$4.2*10^4$	$3,8*10^4$	$3,8*10^4$
3 month	$5,8*10^3$	$5,6*10^2$	$7,2*10^4$	$6,8*10^4$	$5,8*10^4$	$6,4*10^4$
Identified microflora	Bacteria: g.Pseudomonas Fungi: g. Aspergillus g. Micrococus. g. Penicillium g. Bacillus g. Mucor g. Fusarium					

**Sources: Grosu C.Valorificarea şrotului de nuci şi obţinerea produselor de cofetărie. Teza de doctor în tehnică.C.Z.U.: [664.143 + 664.68] : 634.51 (478)(043.2),2016.*

From the experimental data set (tab. 4) it can be seen that during the oilcake storage, the total number of microorganisms has a specific evolution, due to environmental conditions, to storage period and to type of packaging.

Speed multiplication of microorganisms is higher in chilled oilcake and lower in frozen and in one packed under vacuum, perhaps due to increased generation time, and to reduced number of cell divisions. This way experimental results show that packaging under vacuum may allow the extinction of oilcake shelf life reducing the risk of microbiological spoilage.

Bacteriostatic effect exerted by vacuum packing, manifested in particular by slowing down the multiplication of aerobic bacteria is due to changes of gaseous medium composition inside the package or change of the ratio of oxygen and carbon dioxide based on oxygen reduced partial pressure of and increasing the partial pressure of carbon- dioxide – gas with microbiostatic properties.

The digestibility of kernel protein and walnut oilcake are shown in Figure 4.

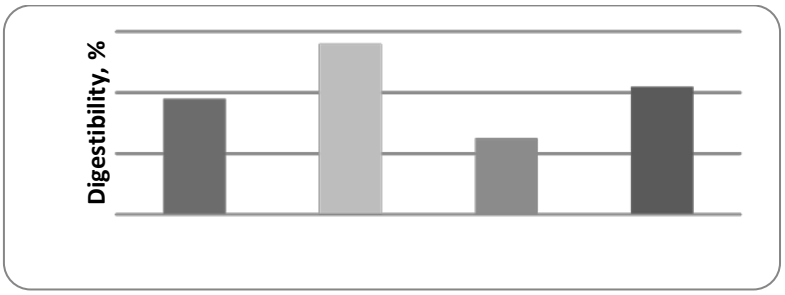

Figura 4. *The digestibility of kernel protein and walnut oilcake*

**Sources: Grosu C.Valorificarea şrotului de nuci şi obţinerea produselor de cofetărie. Teza de doctor în tehnică.C.Z.U.: [664.143 + 664.68] : 634.51 (478)(043.2),2016.*

E-ISBN 978-9975-75-893-2

The results show that walnut kernel and oilcake protein digestibility are significant, being slightly higher for oilcake proteins.

This effect is caused by heat treatment (even slightly) and pressing, after which proteins denature, and resulting in better exposure of cleavage sites to digestive enzymes. At temperatures above 70^0C occur phenomena of oxidation and proteins aggregation that conceal these sites and their digestibility decreases [2].

CONCLUSIONS

In the paper were identified and characterized protein fractions, protein digestibility and amino acid composition of the walnuts kernel and oilcake.

By comparing the chemical composition of the walnuts kernel and oilcake resulted after oil extraction by cold pressing was found a oilcake protein concentration to 60% (on dry basis), carbohydrates, dietary fiber, minerals and vitamins. The lipid content in the oilcake is around 38-40%, and the degree of oil extraction from the walnuts varies between 60-70%.

Proteins contain all essential amino acids required for normal development of the body's metabolism and their digestibility is 70,0 to 73,6%. Walnut kernel and oilcake lipids contain significant amounts of mono- and polyunsaturated fatty acids, the proportion of which is about 90% of total fatty acids. Good ratio between omega 3 and omega 6 places the walnut oil in the category of lipids with high biological value. The walnut kernel and oilcake contain relatively large amounts of K, Mg, Ca, Zn, Fe, Cu, and their use in human food would provide the required amount of minerals in a well balanced diet.

By the biological value, oilcake compete walnut kernel as a product with a great potential for use in the food industry, especially in bakery and confectionery as filler in the production of bread, biscuits, cakes etc.

REFERENCES

1. Abbasi M.A., Raza A., Riaz T., Shahzadi T., Aziz-ur-Rehman, Jahangir M., Shahwar D., Siddiqui S.Z., Chaudhary A.R., Ahmad N. Investigation on the volatile constituents of Juglans regia and their in vitro antioxidant potential. Pakistan (2010). Acad. Sci., 47:137-141.
2. Bax M.L., Aubry L., Ferreira C., Daudin J.D., Gatellier P., Remond D., Sante-Lhoutellier V., 2012.Cooking temperature is a key determinant of in vitro meat protein digestion rate: investigation of underlying mechanisms. J Agric Food Chem 60, 2569-2576
3. Ермаков, А. И., Арасимович, В. В., рош, Н. П. Методы биохимического исследования растений. Ленинград: В.О. Агропромиздат, 1987, 408 с.
4. Gajim C. Tainele nucului. C.C.R.E. „Presa”, Chişinău, 2005, 127 p.
5. Gemma Brufaua, Josep Boatellaa and Magda Rafecasa. Nuts: source of energy and macronutrients. British Journal of Nutrition. British Journal of Nutrition /Volume 96/ Supplement S2 / November 2006, p. S24-S28.
6. Greve, L.C., G. McGranahan, J. Hasey, R. Snyder, K. Kelly, D.Goldhamer, and J.M. Labavitch, Variation in PolyunsaturatedFatty Acids Composition of Persian Walnut, J. Am. Soc. Hort.Sci. 117:518–522 (1992).
7. Jenac A., Migalatiev O., Caragia V., Soboleva I. Caracteristica CO2- extractului din firimituri de miez de nucă. Institutul Ştiinţifico-Practic de Horticultură şi Tehnologii Alimentare. Decembrie 2013, 82-87 p.
8. Habeanu M., ş.a. Efectul suplimentării cu enzime a reţetelor de nutreţcombinat pe bază de porumb, şrot de soia sau rapiţă, asupra perfomanţelor porcilor în îngrăşare-finisare. Institutul de Biologie şi Nutriţie Animală Baloteşti. Analele IBNA vol.22, 2006.
9. Lira R., Arredondo P. (2004). Oxido nitrico : un heroe disfrazado de villano. Ciencia y Cultura, 53, 11-18.
10. Martínez M.L., Maestri D.M., Oil chemical variation in walnut (*Juglans regia* L.) genotypes grown in Argentina. Eur J Lipid Sci Technol. 2008;110:1183–1189.
11. McGill, A.S., C.F. Moffat, P.R. Mackie, and P. Cruickshank, The Composition and Concentration of n -Alkanes in Retail S amples of Edible Oils, J. Sci. Food Agric. 61: 357–362 (1993).
12. Moore S. and William H. Stein. Modified ninhydrin reagent for the photometric determination of amino acids and related compounds (From the Laboratories of The Rockefeller Institute for Medical Research, New York $1, New York). Received for publication, July 6, 1954. 907-913.
13. Prasad, R.B.N., Walnuts and Pecans, in Encyclopedia of FoodScience, Food Technology and Nutrition, Academic Press, London, 1994, pp. 4828–4831.

E-ISBN 978-9975-75-893-2

14. Reeds P.J., Burrin D.G., Stoll B., Jahoor F., Intestinal glutamate metabolism. 1U.S. Department of Agriculture/Agricultural Research Service, Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA. J Nutr. 2000 Apr;130 (4S Suppl) :978 S-82 S.
15. Ruggeri, S.; Cappelloni, M.; Gambelli, L.; Nicoli, S.; Carnovale, E. Chemical composition and nutritive value of nuts grown in Italy. Ital. J. Food Sci. 1998, 10, 243–252.
16. Savage, G.P., P.C. Dutta, and D.L. McNeil, Fatty Acid and Tocopherol Contents and Oxidative Stability of Walnut Oils, J.Am. Oil Chem. Soc. 76:1059–1063 (1999)
17. Xiaoying Mao 1 , Yufei Hua 2 and Guogang Chen 1,* Amino Acid Composition, Molecular Weight Distribution and Gel Electrophoresis of Walnut (Juglans regia L.) Proteins and Protein Fractionations. International Journal of Molecular Sciences ISSN 1422-0067 www.mdpi.com/journal/ijms.
18. Zwarts, L., Savage G.P., and McNeil B.L., Fatty acid content of New Zealandgrown walnuts (Juglans Regia L.). International Journal of Food Science Nutrition, 1999 50(3), p.189-194.