PHYSICAL AND CHEMICAL COMPOSITION OF SOME WALNUT *(JUGLANS REGIA L)* BIOTYPES FROM MOLDAVIA

Antoanela PATRAŞ [1], Paula DOROBANŢU [1]

[1] University of Agricultural Sciences and Veterinary Medicine, Iaşi

Abstract

The walnut fruits *(Juglans regia)* contain many active principles which are very important for the prevention of many chronic diseases. The romanian walnut fruits are rich in nutritional compounds, with a high lipid content (between 55% and 76%), proteins (11% - 25%) and carbohydrates (16%). Polyunsaturated fatty acids are found in a ratio around 70% in the walnut oil and predominantly is the linoleic acid (about 58%). Also, walnuts contain compounds as: phytosterols, tocopherols and scvalens, which prevent cardiovascular diseases. Not all the walnuts from different areas of Romania correspond to the market standards and so they are considered inadequate for consumption. Therefore an economic variant with nutritive value is the use of this raw material for the obtaining of edible oil thus turning to good account the insufficiently used resources. In this research we analyzed the fruits from several local populations of the Moldavian territory: Targu Neamt, Vrancea, Galati, Iasi, Bacau, Vaslui. We analyzed morphological aspects, physical and chemical parameters of the kernel and of the oil (refractive index, oil density, iodine value, acidity). We found that the fruits we studied are medium and large, weighing between 8-15 g, the kernel ratio has values between 32% and 54%, total lipid content is between 55% - 72% and the protein content is quite high, with values between 14% - 20%.

Key words: walnuts, morphology, proteins, fatty acids

The walnut fruits grown in our country come into a large morphological variety in terms of size, shape, color, valve surface, thickness, hardness, endocarp ornamentation etc. At the same time, the protein and oil content of seeds and the chemical composition of oil vary within quite large limits from one biotype to another (Cociu V, 2003, Pomologia R.S.R., 1967). This paper is part of a complex study where we analyzed a total number of 49 walnut sources from different Moldavian regions, fruits that were harvested during the autumn of 2007. At this stage of the research, we have studied 8 walnut sources and we examined both morphological and chemical composition of the walnut kernel.

The walnut fruits were collected from: Hupca, county Vaslui; Săbăoani, county Neamţ; Goruni, county Iaşi; Andreiaşu de Jos, county Vrancea; Dumbrava Gura Văii, county Bacău; Umbrăreşti Deal, county Galaţi; Săuceşti, county Bacău and Darabani, county Botoşani.

MATERIAL AND METHOD

The material we used is represented by the walnut fruits harvested in 2007 from the sources mentioned above and by the oil obtained by pressing process. We analysed 10 fruits for every source. The following parameters were analysed for each sample: length, equatorial diameter, the endocarp thickness, nut weight, kernel ratio, kernel moisture, protein and oil content, fatty acid composition, oil acidity, iodine value, refractive index and the oil density.

The walnut oil was obtained by pressing, using a mini lab press. Dried nuts were shelled manually and whole kernel (1 kg per every source) was crushed, roasted for 1 hour at a temperature of 60 °C and then subjected to pressing. The oil was collected, decanted and kept in dark bottles, at a temperature of 4 °C.

The determination of moisture and volatile matter content in kernel was effectuated according to the European Standard EN ISO 662/2002 by the drying process in a drying chamber at the temperature of 103 °C [8].

Oil content was determined in a Soxhlet apparatus, extracting the lipids from kernel sample with petroleum ether, followed by extract evaporation to dryness and gravimetric determination (STAS 145 / 20 – 88).

Protein content was analysed by Kjeldahl method.

Fat and protein index were calculated using the formula**:** (kernel ratio x oil content)/100, respectively (kernel ratio x protein content)/100.

Free acidity was determined by titration of the dissolved oil in a mixture of alcohol-ether (1:2) with an aqueous solution of sodium or potassium hydroxide (Standard EN ISO 660.

Iodine value was performed by Hannus method, according to STAS 145 / 19-67.

Refractive index of walnut oil was determined by using the Abbe refractometer.

Oil density was determined by pycnometry method, according to STAS 145 – 67.

Fatty acids composition of the walnut oil was determined by gas chromatography in the laboratory of Food Technology of Fulda University, Germany.

RESULTS AND DISCUSSIONS

The fruit weight range between 8.05 g (Darabani, Botoşani) and 14.79 g (Umbrăreşti, Deal, Galaţi; Huşi, Vaslui), and they are considered to be of middle size *(table 1)*.

The kernel ratio (kernel weight (g) / fruit weight (g) x 100) is low, ranges between 31% (Andreiaşu de Jos, Vrancea) and 53% (Umbrăreşti Deal, Galaţi) *(tab. 1)*. Otherwise, as it is known, the kernel content of the walnut biotypes in Romania varies between very large limits (25-60%) (Pomologia R.S.R., 1967). The walnuts considered to be valuable for fresh consumption or to be used in confectionary must contain more than 50% kernel (Pomologia R.S.R., 1967).

Table 1

The physical parameters of walnut biotypes

Nr. crt.	Source	Fruit weight (g)	Kernel weight (g)	Kernel ratio (%)	Shell thickness (mm)
1	Săbăoani, Tg. Neamţ	9.34	3.64	38.97	1.2
2	Hupca, Vaslui	11.42	4.74	41.50	1.2
3	Goruni, Iaşi	11.37	4.76	41.86	1.2
4	Andreiaşu de Jos, Vrancea	12.14	3.81	31.38	2.0
5	Dumbrava, Gura Văii, Bacău	10.58	4.65	43.95	1.0
6	Umbrăreşti Deal, Galaţi	14.79	7.95	53.75	2.2
7	Săuceşti, Bacău	8.05	3.30	40.99	1.3
8	Darabani, Botoşani	8.32	3.84	46.15	1.4
Average		10.75	4.58	42.31	1.43
Standard deviation		±2.21	±1.46	±6.34	±0.42
Percentage variation %		20.58	32.0	14.98	29,73

Endocarp thickness is between 1.0 mm (Dumbrava, Gura Văii, Bacău) and 2.2 mm (Umbrăreşti Deal, Galaţi), in most of the cases the endocarp is quite thick and hard *(table 1)*.

Fruit length is between 29 mm (Săuceşti, Bacău) and 38 mm (Hupca, Vaslui). **Fruits' equatorial diameter** was measured in three portions. It has values between 27 mm (Andreiaşu de Jos) and 30 mm (Goruni, Iaşi) *(table 2)*.

Table 2

The morphometric parameters of walnut biotypes

Nr. Crt	Source	Joining line (mm)	Nut length (mm)	Nut diameter (Ø) (mm)			Point	Basis
				midle	1/3 inferior	1/3 superior		
1	Săbăoani, Neamţ	2.0	33.1	29.5	26.4	25.1	cut-off	cut-off
2	Hupca, Vaslui	3.0	38.3	32.9	30.9	28.8	pointed	rounded
3	Goruni, Iaşi	2.7	37.2	30.7	32.0	27.8	cut-off	cut-off
4	Andreiaşu de Jos, Vrancea	2.7	37.2	27.6	23.7	22.6	rounded, rounded-rostrate,	cut-off-rostrate
5	Dumbrava, Gura Văii	2.2	38.5	29.7	26.7	24.8	rounded-cut off	rounded
6	Umbrăreşti Deal, Galaţi	2.6	35.9	31.7	29.4	24.8	rounded	cut off
7	Săuceşti, Bacău	2.0	29.5	29.3	27.1	23.5	cut-off-emarginate	cut-off
8	Darabani, Botoşani	>1.4	36.5	29.8	28.1	27.8	rounded	rounded
Average		2.32	35.77	30.15	28.03	25.65	-	-
Standard deviation		±0.52	±3.04	±1.61	±2.67	±2.23	-	-
Percentage variation %		22.37	8.51	5.35	9.52	8.69	-	-

The evaluation of the alimentary value of walnuts can't be done only by considering separately the kernel content, the oil content or the protein content; it is needed to take into account the connection between all these parameters (Pomologia R.S.R., 1967). All the analysed walnut biotypes have high oil content, between 55% - 68% *(table 3)*. It is known that the valuable walnut biotypes (authorized cultivars) must contain minimum 65% oil (Pomologia R.S.R., 1967). Previous researches mention 61% - 70% oil in the walnuts from different geographical regions (Chisholm A., J. Mann, 1998, Gulcan Ozkan, 2005, Lavedrine F., D. Zmirou, 1999, Marcela L. Martínez, 2008, Pomologia R.S.R., 1967). The fat index of the analyzed walnuts is high, ranging from 19.52 to 34.98 *(table 3)*. For the Romanian authorized walnut cultivars, the fat index is 16.48 – 41.42 (Pomologia R.S.R., 1967).

Protein content of the studied walnuts ranges between 14.5% – 18.34% *(table 3)*. The proteins content of the Romanian walnut types is between 12% - 24% (Pomologia R.S.R., 1967). Previous worldwide researches mention 6% - 22% protein content (Chisholm A., J. Mann, 1998, Gulcan Ozkan, 2005, Lavedrine F., D. Zmirou, 1999, Marcela L. Martínez, 2008, Savage G.P, 1999). Protein index varies between 5.39 -7.80 *(table 3)*. For the Romanian authorized walnut cultivars, the protein index is 4.55 -12.60 [6]. Regarding the moisture content of the studied walnuts, it is between 3.3% – 4.3% *(table 3),* corresponding to the Romanian Standard 1288/ 2004 concerning the quality of the walnuts in shells.

Table 3

Chemical composition of the walnut kernel

Nr. Crt.	Walnut oil Source	Moisture %	Oil content %	Fat index	Protein content %	Protein index
1	Săbăoani, Neamţ	4.31	55.00	21.43	16.80	6.54
2	Darabani, Botoşani	3.78	60.18	27.77	16.36	7.55
3	Hupca, Vaslui	3.60	61.04	25.33	15.67	6.50
4	Goruni, Iaşi	4.30	61.68	25.81	18.34	7.67
5	Andreiaşu de Jos, Vrancea	4.50	62.23	19.52	1720	5.39
6	Dumbrava, Gura Văii, Bacău	3.30	63.47	27.89	17.76	7.80
7	Umbrăreşti Deal, Galaţi	3.96	65.08	34.98	14.50	7.79
8	Săuceşti, Bacău	3.74	68.32	28.00	14.80	6.06
Average		3.93	62.12	26.34	16.42	6.91
Standard deviation		±0.40	±3.87	±4.68	±1.36	±0.91
Percentage variation %		10.39	6.22	17.79	8.33	13.27

The fatty acids identified in the walnut oil, by gas chromatography method are: palmitic acid (6.88% - 7.64%) stearic acid (2.32% - 3.40%) and predominantly: oleic acid (14.99% - 22.72%), linoleic acid (57.24% - 60.88%) and linolenic acid (5.31% – 12.13%) *(table 5)*. Polyunsaturated fatty acids (oleic acid, linoleic acid and linolenic acid) are found in a ratio of about 80% in the walnut samples. All these values are in good agreement with previous researches (Chisholm A., J. Mann, 1998, Gulcan Ozkan, 2005, Lavedrine F., D. Zmirou, 1999, Marcela L. Martínez, 2008, Savage G.P, 1999).

Oil acidity has low values, between 0.20% and 0.31% oleic acid, and corresponds to present standards (Standard 1288 / 2004.

Iodine value is between 142 and 157 and is in good agreement with literature (Marcela L. Martínez, 2008).

Walnut oil **density** is between 945 - 970 kg/m^3.

The values of the refractive index are connected to the nature and the percent of fatty acids (unsaturated fatty acids increase the index value). We obtained for the refractive index values between 1.4777 – 1.4788 *(table 5)*.

Table 4

Fatty acid composition in walnut oil (*Juglans regia*) in different areas

Areas	Oil content (%)	Palmitic acid (%)	Stearic acid (%)	Oleic acid (%)	Linoleic acid (%)	Linolenic acid (%)	Unsaturation degree
Europe	6420	7.73	2.05	19.58	57.09	12.45	155
U.S.A.	67.60	6.46	1.43	17.94	58.03	15.07	162
Canada	70.60	6.52	3.54	12.14	49.56	12.86	154
Argentine	76.65	6.94	1.50	16.51	56.45	18.58	168
Portuguese	70.59	6.62	3.07	20.22	55.51	14.02	157
Persia	59.00	5.87	3.24	15.73	57.29	15.75	161
New Zeeland	67.70	7.30	0.08	18.09	58.43	13.31	156
Turkish	69.00	5.82	1.90	22.67	51.60	17.83	162
Bulgaria	68.50	11.9	3.7	18.70	48.50	15.80	148
China	65.70	0.40	0.20	18.90	67.30	8.90	155
France	63.78	0.40	0.10	15.80	65.80	9.40	156
Hungary	65.87	0.70	0.20	19.00	61.90	5.70	154
India	65.40	1.30	0.60	25.60	54.10	9.60	151
Italy	63.78	0.70	0.20	16.20	69.90	8.60	159
Spain	67.89	0.40	0.20	18.10	66.60	9.20	156
Romania (unauthorized cultivars)	71.20	7.64	2.38	16.83	59.29	11.66	154

Table 5

Fatty acid composition in walnut oil and physical and chemical parameters

Chemical composition	Sources									
	Săbăoani, Neamţ	Hupca, Vaslui	Goruni, Iaşi	Andreiaşu de Jos, Vrancea	Dumbrava, Gura Văii, Bacău	Umbrăreşti Deal, Galaţi	Săuceşti, Bacău	Darabani, Botoşani	Average	Standard deviation
Palmitic acid %	7.64	7.07	7.50	7.13	6.88	7.63	7.43	7.04	7.29	±0.29
Stearic acid %	2.38	2.58	2.65	2.42	3.40	2.32	2.72	3.33	2.72	±0.41
Oleic acid %	16.83	14.99	16.16	19.54	22.10	17.22	15.06	22.72	18.07	±3.03
Linoleic acid %	59.29	60.88	58.70	57.24	59.62	59.14	60.35	60.42	59.45	±1.15
Linolenic acid %	11.66	12.13	11.83	11.15	5.31	11.75	11.78	4.38	9.99	±3.20
PUFA %	70.95	73.01	70.53	68.39	64.93	70.89	72.13	64.80	69.45	±3.12
PUFA /MUFA	4.21	4.87	4.36	3.50	2.93	4.11	4.79	2.85	3.95	±0.780
Acidity (% oleic acid)	0.22	0.31	0.27	0.20	0.23	0.28	0.28	0.25	0.25	±0.03
Iodine value (g I /100 g)	154.6	157.1	153.7	152.0	142.5	154.9	155.3	142.0	151.51	±5.89
Density at 25°C (kg/m^3)	970.0	965.0	945.0	946.0	968.0	956.0	960	970	960.0	±10.18
Refractive index at 25°C	1.4777	1.4783	1.4788	1.4778	1.4788	1.4787	1.4748	1.4777	1.477	±0.001

PUFA/MUFA – Polyunsaturated/ Monounsaturated fatty acids

Previous studies (Chisholm A., J. Mann, 1998) have shown that walnut oil is an excellent source of edible oil due to its high content of unsaturated fatty acids. Though the walnut oil is not described in Codex Alimentarium, its consumption has become very popular worldwide due to its beneficial effects on human health by reducing "bad" cholesterol - LDL in blood and also the triglycerides (Chisholm A., J. Mann, 1998) Lavedrine F., D. Zmirou, 1999) showing a protective factor against the risks of cardiovascular disease.

CONCLUSIONS

The studied walnut fruits, coming from different places of Moldavia (Romania), are middle size, their endocarp is quite thick and they have an average kernel ratio.

The walnut kernel has a high oil and protein content and regarding to fatty acid composition in the analyzed sources, we identified a high content of unsaturated fatty acids and the results were in good agreement with those of walnut oil from different varieties and geographic origins.

We found that not all the studied walnut fruits correspond to the existing standards for fresh consumption because of the low weight of the fruits, quite low kernel ratio, inadequate aspect and color, quite thick endocarp. Therefore, we recommend their use as raw material for the production of edible oil, thus capitalizing insufficiently used resources.

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