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Physical and chemical composition of some walnut (*Juglans regia* L) genotypes grown in Turkey

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RESUMEN

Composición física y química de diversos genotipos de nueces (*Juglans regia* L.) cultivadas en Turquía.

Las nueces (*Juglans regia* L.) fueron recolectadas durante los años 2000 y 2001 en diversos nogales seleccionados de Turquía y analizadas para determinar sus propiedades físicas y bioquímicas. Se seleccionaron 10 genotipos en los que fueron determinadas las propiedades de la cáscara y la semilla, la composición en ácidos grasos, así como su composición general. Las propiedades físicas son un atributo de calidad de las nueces. Los genotipos investigados mostraron una buena calidad de acuerdo a los resultados. El contenido total en aceite varió entre el 61.97 y el 70.92 % mientras que la proteína cruda fue del 15.17-19.24 %. La ceniza fue del 1.26-2.06 % y la humedad fue del 3.25-3.91 % de la semilla. Los carbohidratos totales fueron el 8.05-13.23 %. El contenido en ácido oleico fue del 21.18-40.20 % de los ácidos grasos totales, mientras que el contenido en linoleico fue el 43.94-60.12 %, y el de linolénico el 6.91-11.52 %. El palmítico fue el 5.24-7.62 % y el esteárico el 2.56-3.67 %.

PALABRAS-CLAVE: Genotipos de nueces - Juglans regia L. - Propiedades bioquímicas - Propiedades físicas.

SUMMARY

Physical and chemical composition of some walnut (*Juglans regia* L) genotypes grown in Turkey.

Walnut (*Juglans regia* L.) samples were collected during the 2000 and 2001 harvests from different selections of trees grown in Turkey. Important physical and biochemical properties of fruit were examined in these walnut types. At the end of this study, 10 types were selected and nut and kernel properties, fatty acid composition and the proximate composition of these selections which are commonly grown in Turkey were evaluated. Physical properties are attributed to the quality criteria of nuts. Investigated genotypes showed good quality according to the test results. The total oil content ranged from 61.97 to 70.92% while the crude protein ranged from 15.17 to 19.24%. Ash ranged from 1.26 to 2.06% while the moisture was found between 3.25-3.91% of the kernel. The total carbohydrate was calculated as between 8.05-13.23%. The oleic acid content of the oils ranged from 21.18 to 40.20% of the total fatty acids, while the linoleic acid content ranged from 43.94 to 60.12% and the linolenic contents from 6.91 to11.52%. It was found that palmitic acid was between 5.24 and 7.62%, while stearic acid ranged from 2.56 to 3.67%.

KEY-WORDS: Biochemical properties - Juglans regia L. - Physical properties - Walnut genotypes.

1. INTRODUCTION

Walnut (*Juglans regia* L.) is the oldest cultivated fruit in the world and grown spontaneously almost all over Turkey (Sen, 1986). The most important walnut varieties in Turkey are Sebin, Yalova 1, Yalova 2, Yalova 3, Yalova 4 and Bilecik. The walnut plant has a high nutritional value and high-quality wood. In addition, walnuts have significant economical value and medicinal importance for human health because of their biochemical composition of polyunsaturated fatty acids, especially 18:2 and 18:3 and protein value (Savage et al., 2001). People consume it in large quantities; therefore, it has a very important place in public nutritional habits. Walnut has a high calorie level, rich nutrient composition and a special value in traditional Turkish foods (Sen, 1986). Turkey has a remarkable walnut population of walnut varieties when compared to other parts of the world. Several studies have been carried out especially dealing with the quality and fruit properties of walnuts grown in Turkey (Olez, 1971; Sen, 1983; Celebioglu et al., 1988; Sen and Beyhan, 1993; Ferhatoglu, 1993; Akca and Sen, 1995; Askin and Gun, 1995; Koyuncu and Askin, 1995; Koyuncu and Askin, 1999).

The Turkish Standard Institute established physical nut and kernel properties of walnut as a quality criteria (Anonymous, 1990; 1991). These properties are nut weight, kernel weight, kernel ratio, shell shape, nut dimensions, and other shell properties. The proximate compositions of walnut are as follows. Energy, 630 kcal; protein, 18.10–13.60%; total oil, 62.60-70.30%; dietary fiber, 5.20%; ash, 1.80% (Savage, 2001). Koyuncu and Askin (1995) investigated the chemical composition of 12 walnut genotypes as follows: Protein, 20.92 –25.95%; ash, 1.68-2.06%; fat, 66.30-74.95%. In addition, Zwarts et al. (1999) evaluated the fatty acid composition of two US commercial cultivars (Tehama and Vina), three European commercial cultivars (Esterhazy, 139, G120) and five New Zealand selections (Rex, Dublin's Glory, Meyric, McKinster, Stanley). As a result, they found that the total oil content of the nuts ranged from 62.4 to 68.7%, the oleic acid content of the oils ranged from 14.3 to 26.1% of the total fatty acids, while the linoleic acid content ranged from 49.3 to 62.3% and the linolenic contents from 8.0 to 13.8%. As a result, walnuts are a rich source of n-3 and n-6 polyunsaturated fatty acids. The effects of walnut consumption on hyperlipidemia and systolic blood pressure, triglyceride and cholesterol were determined. In conclusion, the composition of the fatty acids consumed in the human diet appears to be more important than its total content (Savage et al., 2001). The benefits of walnuts in the human diet against hypercholesterolemia were studied (Savage et al., 2001). The blood profile of cholesterol, HDL-cholesterol and triglycerides were monitored before and after the addition of walnuts to the diet. Initial analyses demonstrated that the dietary supplement had a positive effect of most of the volunteers. Another study indicated that the consumption of nuts results in some protection against heart diseases (Simopoulos, 1999).

In this study, the aime was to determine the promise of walnuts and to preserve them as valuable genetic sources (*Juglans regia* L.) within existing populations, all of which consist of seedling trees. So this investigation was carried out in order to determine the physical parameters related to both the quality of fruit and the nutritional value of walnuts.

2. MATERIALS AND METHODS

2.1. Materials

The genotypes in this research are 32.YS.060, 32.YS.023, 32.YS.097, 32.YS.031, 32.YS.075, 32.YS.098, 32.YS.051, 32.YS.099, 32.YS.088 and 32.YS.119. These genotypes were collected from Isparta, a surrounding area of Turkey which is situated between the South and Middle Anatolia, in September, 2000 and 2001 at altitudes of 1050-1085 meters. The examinations were carried out for two years and samples were taken every year from the same trees which were all between 27 and 43 years old. The location of walnut tree, the sampling days and storage conditions and time until analyses were similar for all genotypes. Physical analyses were quickly determined and kernel samples were kept at –18 ^{o}C before chemical analyses. There were three repetitions in chemical analyses and ten repetitions in physical analyses.

2.2. Physical analysis

The following physical walnut analyses were performed: Nut dimensions and shape properties (nut diameter, nut length, nut thickness, nut shape, nut size), fruit properties (nut weight, kernel weight, kernel ratio, shell thickness, shell roughness) and kernel properties (kernel color, kernel fullness, kernel crinkling) were tested according to TSE 1275 and 1276 (Anonymous, 1990; 1991).

Shape index: nut length / (nut diameter + nut thickness) / 2. This index was evaluated as follows: shape index < 1.25: sample is sphere; shape index 1.25: sample is oval. Kernel ratio (%): (kernel weight / nut weight) x100. Extra: Nut diameter ≥ 27 mm for sphere, nut diameter 26 mm for oval. Class I: nut diameter 24-27 mm for sphere, nut diameter 24-26 mm for oval, class II: diameter 20-24 mm for sphere and oval. Kernel crinkling was determined as fine, medium, bad and empty; kernel colors were determined as light, medium, dark. These analyses were determined in at least 10 samples of the same genotypes.

2.3. Analysis of fatty acids

Fatty acid composition for the walnut samples was determined using the modified fatty acid methyl ester method as described by Baydar et al. (1999). The oil was extracted three times from 2 g air-dried seed sample by homogenization with petroleum ether. For fatty acid methyl esters (FAME), 1 ml of methylation reagent (80 ml methanol + 0.5 g sodium methylate + 20 ml isooctane) was added to 50 mg of oil. The mixture was vortexed and allowed to react for 24 hours at room temperature; then 0.25 ml of isooctane was added. The sample was then centrifuged for 5 min at 2400 x *g* at 5^{o}C and the liquid portion was transferred to labelled Wheaton vials and stored at 20^{o}C. The methyl esters of the fatty acids (0.5 μl) were analyzed in a gas chromatograph (Perkin Elmer Auto System XL, USA) equipped with a flame ionizing detector (FID), a fused silica capillary column (MN FFAP (50 m x 0.32 mm i.d.; film thickness 0.25 μm). It was operated under the following conditions: oven temperature program, 120^{o}C for 1 min. raised to 240^{o}C at a rate of 6^{o}C/min and than kept at 240^{o}C for 15 min); injector and detector temperatures, 250 and 260^{o}C; respectively, carrier gas, helium at flow rate of 14 psi; split ratio, 1/20 ml/min. The contents of palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids were determined by a computing integrator.

2.4. Proximate composition

The AOAC method was used in this study (AOAC, 1990). Ash (AOAC 20.013), fat (AOAC 22.034), moisture (AOAC 22.003), protein (AOAC PN-75/A-04018) were determined using standard methods. The total amount of carbohydrate was found by subtracting the amount of ash protein and fat from the total dry matter. Nitrogen was determined by Kjeldahl analyses, multiplied by 5.4 and reported as protein.

2.5. Statistical analysis

Results of the research were tested for statistical significance by one-way ANOVA. Differences were considered statistically significant at the P ≤ 0.05 level (Ozdamar, 1999).

3. RESULTS AND DISCUSSION

In our study, a statistical comparison was made among the genotypes and over the years 2000 and 2001. As the results lacked any statistical significance between both years, the mean of two years' data for chemical and physical properties was taken. Differences among fruit properties, nut dimensions and shape properties were statistically significant (Tables 1 and 2). Nut weights ranged from 11.09 g (32.YS.023) to 8.43 g (32.YS.060). Kernel weight was highest in type 32.YS.023 (6.32 g) and lowest in type 32.YS.031 (4.35 g). The kernel ratio was highest in 32.YS.060 (57.41%) and lowest in 32.YS.119 (48.89%). Kernel ratios were < 50% in 32.YS.075 and 32.YS.119, the others were > 50 %. It was determined that 32.YS.060 had superior kernel percent (57.41%) and the the kernel weight of the walnut was determined as 5 g (Table 1). These values, especially the kernel ratio, lye within fine walnut standards (Anonymous 1990; 1991). Previous studied reported kernel weight, as 5.30-10.10 g, and kernel ratio as 58.34-100% (Ölez, 1971); kernel ratio as 39.93-55.01% (Koyuncu and Askin, 1995) and nut weight as 5.45-11.42 g and kernel ratio as 39.01-57.53% (Sen and Tekintas, 1990). In addition, Germain (1988) found nut weight to be 8.00-12.00 g and kernel ratio to be 35.00-50.00 % and Serr (1962) reported kernel weight as 5.60-7.70 g and kernel ratio as 47.00-52.00 % of Plancentia, Payne, Eureka, Hartley and Franquette walnut varieties. While our results showed harmony with most of these results, better results than ours were found by Ölez (1971). This could be due to differences in the ecological and genetic properties of walnut genotypes. Shell thickness was between 0.83 mm (32.YS.098) and 1.47 mm (32.YS.119), respectively. While 32.YS.031 had smooth shells, the others were medium and rough. These values, especially shell thickness, are in compliance with fine walnut standards (Anonymous 1990). In the selections, nut thickness ranged from 33.45 mm in 32.YS.097 to 29.24 mm in 32.YS.031. Nut length was highest in 32.YS.051 (37.88 mm) and nut diameter was highest in 32.YS.023 (31.12 mm) (Table 2). The nut dimensions of all the samples could be classified as fine (above 27.68 mm) according to size standards (Anonymous, 1990). Akca and Sen (1995) showed nut length as 39.97 mm, nut diameter as 33.59 mm and nut thickness as 34.75 of the promising walnut genotype.

All the walnuts have superior kernel fullness, fine kernel crinkling and light kernel color. While kernel fullness was 100% for three samples and 90% for the others, all samples were found to be fine (100%) according to kernel crinkling properties and light as kernel color. Other authors also performed selection studies in various parts of Anatolia (Olez, 1971; Sen and Beyhan, 1993). They determined light kernel color as > 70-80%. Our results were in suitable

Table I

Fruit properties of the walnut samples (the mean of two years data)

Genotypes	Nut weight (g)	Kernel weight (g)	Kernel ratio (%)	Shell thickness (mm)	Shell roughness
32.YS.060	8.70±0.62de[1]	5.00±0.45b	57.41±2.50a	0.97±0.13de	Medium
32.YS.023	11.09±0.84a	6.32±0.60a	56.40±1.04a	1.05±0.12d	Rough
32.YS.097	8.58±0.85e	4.49±0.67de	52.34±1.09bc	0.89±0.07ef	Medium
32.YS.031	8.43±0.82e	4.35±0.50e	51.51±2.42bcd	1.31±0.07b	Smooth
32.YS.075	9.25±0.80cd	4.55±0.49cde	49.18±2.74de	1.17±0.11c	Medium
32.YS.098	8.82±0.84de	4.71±070bcde	53.30±3.01b	0.83±0.08f	Rough
32.YS.051	8.58±0.67e	4.78±0.41bcd	55.76±1.56a	0.93±0.13e	Rough
32.YS.099	9.72±1.03bc	4.90±0.54bc	50.39±2.90cde	1.40±0.18a	Medium
32.YS.088	9.81±0.72b	4.91±0.35bc	50.05±0.45cde	1.29±0.09b	Rough
32.YS.119	10.07±1.01b	4.92±0.58bc	48.89±3.24e	1.47±0.20a	Medium

[1]Differences between means indicated by the same letters are not statistically significant (Duncan's multiple range test, P ≤ 0.05).

Table II
Nut dimensions and shape properties (the mean of two years data)

Genotypes	Nut length (mm)	Nut diameter (Ø) (mm)	Nut thickness (mm)	Shape	Size
32.YS.060	34.97±1.26c[1]	29.12±0.96cd	29.53±0.92c	Spherical	Extra
32.YS.023	37.75±1.47a	31.12±1.29a	32.93±1.40ab	Spherical	Extra
32.YS.097	34.65±1.06c	27.68±0.75e	33.45±3.47a	Spherical	Extra
32.YS.031	29.72±1.82e	29.10±0.94cd	29.24±1.48c	Spherical	Extra
32.YS.075	36.29±1.45b	28.84±1.02d	29.93±2.36bc	Spherical	Extra
32.YS.098	36.42±1.14b	29.56±1.20c	30.89±1.20abc	Spherical	Extra
32.YS.051	37.88±0.83a	28.67±0.70d	30.32±2.06abc	Spherical	Extra
32.YS.099	35.74±1.01b	30.28±0.81b	30.12±2.34abc	Oval	Extra
32.YS.088	33.42±0.51d	29.00±0.55cd	31.23±0.96abc	Spherical	Extra
32.YS.119	36.01±0.67b	30.64±0.78ab	30.86±0.64abc	Spherical	Extra

[1]Differences between means indicated by the same letters are not statically significant (Duncan's multiple range test, $P \leq 0.05$).

agreement with them and the TSE 1276 standard (Anonymous, 1991).

For chemical composition (Moisture, ash, fat, protein, total carbohydrate), Statistical differences between stored and unstored kernel samples were not found. So the mean of stored and unstored data for chemical properties was taken. However, statistical differences among the genotypes were significant. Table 3 shows the highest protein quantity (19.24%) in 32.YS.088, the lowest value was found in 32.YS.075 (15.17%). The protein values were in good agreement with the literature (Koyuncu and Askin, 1995). Walnut has a high protein level like some legumes and cereals such as chickpea, pea, lentil, wheat, etc. The protein contents of these products varied between 6.30 and 22.0%. Moisture values were between 3.25% in 32.YS.075 and 3.91% in 32.YS.099 genotypes. Moisture contents were in good agreement with Koyuncu and Askin (1999). Ash content was found to be in the range of 1.26–2.06%. Sen and Beyhan (1993) and Koyuncu and Askin (1999) determined values between 1.77–2.42%. Fat contents of the sample (%) were in the range of 61.97–70.92. 32.YS.023 had the highest fat contents. A normal fat content ranges between 69.30 and 63.7%. Total carbohydrate was calculated by subtracting other nutrient contents from total weight. These values were in the range of 8.05–13.23%. Fat and other nutrient contents were in good agreement with the literature (Savage, 2001).

In this research, the fatty acid contents of walnuts were examined and statistical differences among the genotypes were found significant at the $P \leq 0.05$ level (Table 4). The fatty acid composition of walnut samples is especially important to human nutrition and biochemistry. When examining the fatty acid composition, some differences among the samples were observed. Palmitic acid values ranged from 5.24 and 7.62%. Stearic acid values were between 2.56 and 3.67%. All of them were in good agreement with literature values (Savage, 2001; Koyuncu and Askin, 1995; Koyuncu and Askin, 1999). Oleic acid ratios ranged from 21.18 and 40.20% in 32.YS.119, which had the highest oleic acid ratio. Due to the fact that harvesting dates and growing conditions are similar, differences in these values can be specific to walnut type. The fatty acid contents were affected by some of the factors mentioned above. The linoleic acid contents ranged between 43.94 and 60.12% and linolenic acid contents ranged between 6.91 and 11.51% in this study. It was determined that total unsaturated fatty acid contents such as oleic, linoleic and linolenic acids were in high levels (90%). These results were comparable to data previously reported in the literature. . Koyuncu and Askin (1995) found that the average palmitic, stearic, oleic, linoleic and linolenic contents (%) were: 7.22, 1.07, 28.51, 52.46 and 10.50 depending on harvesting time. Garcia et al (1994) found that the quantity of fatty acid compositions occurred in wide ranges. Oleic acid was in the range of 16.10-27.00%; polyunsaturated fatty acids showed differences among the genotypes as follows (%): 18:2, 51.80–61.50; 18:3, 10.00–18.50. The walnut fatty acid composition

Table III
Proximate composition of walnut genotypes (% dry weight) (the mean of two years data)

Genotypes	Moisture	Ash	Fat	Protein	Total carbohydrate
32.YS.060	3.36±0.14de[1]	1.35±0.11de	69.96±0.73b	15.50±0.24f	9.83±0.89c
32.YS.023	3.87±0.24a	1.26±0.08e	66.16±0.49d	16.67±0.20e	12.04±0.50b
32.YS.097	3.58±0.25bcd	1.88±0.10bc	70.92±0.69a	15.57±0.12f	8.05±0.82d
32.YS.031	3.85±0.08ab	1.76±0.09c	68.32±0.37c	16.43±0.12e	9.64±0.45c
32.YS.075	3.81±0.12abc	1.80±0.18bc	66.64±0.66d	17.99±0.29d	9.76±0.40c
32.YS.098	3.75±0.08abc	1.88±0.04bc	66.14±0.58d	18.17±0.06d	10.06±0.49c
32.YS.051	3.25±0.08e	1.79±0.03bc	69.98±0.84b	15.17±0.13g	9.81±1.00c
32.YS.099	3.91±0.26a	2.06±0.11a	61.97±0.44f	18.92±0.10b	13.14±0.34a
32.YS.088	3.56±0.07cd	1.46±0.12d	62.51±0.61f	19.24±0.15a	13.23±0.51a
32.YS.119	3.83±0.19ab	1.93±0.07ab	63.77±0.53e	18.54±0.16c	11.93±0.48b

** Total carbohydrate was calculated by subtracting other nutrients from total weight.
[1]Differences between means indicated by the same letters are not statically significant (Duncan's multiple range test, $P \leq 0.05$).

Table IV
Fatty acid composition of walnut (% dry matter) (the mean of two years data)

Tip No	Palmitic Acid (%)	Stearic Acid (%)	Oleic Acid (%)	Linoleic Acid (%)	Linolenic Acid (%)
32.YS.060	7.62±0.30a[1]	3.34±0.02ab	29.36±1.68b	50.56±1.52ab	9.13±0.16bc
32.YS.023	6.01±0.14bcd	2.94±0.00bc	24.26±2.15b	54.04±1.51a	11.52±0.26a
32.YS.097	5.86±0.10bcd	3.25±0.04abc	32.12±0.86ab	50.95±0.94ab	7.83±0.15cd
32.YS.031	6.32±0.11bc	2.61±0.21c	27.23±4.78b	54.71±4.73a	8.93±0.36bc
32.YS.075	5.24±0.15d	2.83±0.15bc	24.15±2.40b	56.86±2.33a	10.63±0.37ab
32.YS.098	5.58±0.09cd	3.67±0.04a	21.18±2.80b	60.12±2.58a	9.44±0.19bc
32.YS.051	6.52±0.28b	3.08±0.27abc	23.56±1.94b	57.94±1.91a	8.11±0.27cd
32.YS.099	6.06±0.34bcd	2.56±0.23c	27.74±5.45b	54.91±4.74a	8.73±0.60c
32.YS.088	5.54±0.14cd	2.96±0.31bc	25.81±2.15b	56.68±1.43a	9.00±0.88bc
32.YS.119	6.00±0.51bcd	2.75±0.36bc	40.20±3.53a	43.94±2.56b	6.91±1.02d

[1]Differences between means indicated by the same letters are not statically significant (Duncan's multiple range test, $P \leq 0.05$).

shows high contents of linoleic acid and linolenic acid which are beneficial to human health. Linoleic acid and especially linolenic acid play important roles for human health regarding the cardiovascular system (Sabate et al., 1993; Abbey et al., 1994 and Cunnane et al., 1993).

As a result; 32.YS.060, 32.YS.023, 32.YS.097, 32.YS.031, 32.YS.075, 32.YS.098, 32.YS.051, 32.YS.099, 32.YS.088 and 32.YS.119 genotypes were found to be promising walnut types and it is suggested that they should be cultivated and developed for standard genotypes in the future according to their agricultural, physical and biochemical properties. The data reported in this paper confirm that walnuts are a rich source of a number of important nutrients that appear to have a very positive effect on human health. Humans would most benefit from an increased understanding of the mechanisms of all the nutrients in walnuts.

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