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SOME CHEMICAL, PHYSICAL AND PHYSIO-MECHANICAL QUALITY PROPERTIES OF COLD-ADAPTED PROMISING WALNUT GENOTYPES: TURKEY, BINGOL REGION

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Abstract: *The objective of the present study was to examine some physical and chemical shell and kernel attributes of promising walnut genotypes selected from the Bingol province in Turkey. Bingol province possesses a rich walnut population almost all of which are seedling-grown. Shell cracking resistance, kernel firmness, kernel percentage, pellicle and cotyledon color, total oil ratio, total phenol content, and antioxidant capacity as DPPH were analyzed and evaluated. Shell cracking resistance ranged from 8.73 to 34.83 kgf with an average value of 17.96 kgf. Kernel firmness was found to range from 0.87 to 1.34 kgf. Lightness (L*) of pellicle was in the range of 47.06 and 63.01 while yellowness (b*) in the range of 25.02 and 31.98. Lightness (L*) of cotyledon changed mildly from 70.76 to 76.47 with a mean of 73.57 while cotyledon yellowness was in the range of 25.49 and 30.34. The total oil ratio was found to vary between 45.04 and 56.88%. Total phenol content was in the range of 80.97 and 142.91 mg.kg^{-1} with an average value of 118.12 mg.kg^{-1}. The DPPH free radical scavenging capacity of the genotypes vaguely varied from 64.14 to 70.52% with a mean of 69.04%. Finding especially shell cracking index, kernel percentage, and pellicle color may contribute to walnut improvement programs.*

Keywords*: Juglans regia, total oil, total phenols, antioxidant activity, kernel firmness, shell cracking*

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1. Introduction

Walnut (*Juglans regia* L.) is an economically very important tree species. It contains a substantial amount of fats, carbohydrates, and proteins. Hence, walnut fruit has been utilized as a dietary food in human nutrition since antiquity [1]. The origin of the walnut includes Asia including west of the Himalayan zone and Kyrgyzstan [2]. The walnut has spread through various ecosystems like the Mediterranean which includes Turkey. The walnut is commercially cultivated in more than 60 countries including China, Iran, the United States, Turkey, France, and Brazil. With over 1.500.000 tones, China is the prime walnut producer (over 40 % of world production), followed by the USA, Iran, and Turkey [3].

Walnut kernels are consumed as fresh or toasted, additionally are used in confectionery as ingredients or additive such as Baklava and churchkhela (walnut sujuk) in Turkey. Besides filled with basic nutritional compounds, walnut kernels carry antioxidative phytochemicals like phenolics and unsaturated fatty acids. Health-giving effects of walnuts are well documented such as alleviating the occurrence of cardiovascular diseases [4], diabetes [5], and some cancer forms [6]; reducing postprandial oxidative stress [7]; declining adiposity and low-grade systemic inflammation [8]; and lowering total, LDL cholesterol, and triglyceride levels whereas raising HDL cholesterol and apolipoprotein A1 portions fraction [9,10].

Turkey is one of the prime countries in the world owing to high walnut plantation and production. With 225.000-tones walnut production, Turkey ranks 4th in the world [11]. Bingol province is located in eastern Turkey within the East Anatolian Region and is known for its famous walnuts. Walnut trees are grown everywhere in Bingol province from the province city center to the high mountains. Nearly all of the walnut production of Bingol province has been provided from walnut trees propagated by seeds. Therefore, there is a wide genetic variation adapted to the ecological conditions of the region and having different characteristics from each other. There is only a little literature related to walnuts grown in the Bingol province [12-14].

The aim of the study was to determine some physical and phytochemical properties of ten promising walnut genotypes adapted to cold in Bingol province.

2. Materials and Methods

2.1. Plant material

The present study was performed on 10 promising walnut genotypes (12AK01, 12AK12, 12AK19, 12AK22, 12YE26, 12YE28, 12YE29, 12YE33, 12YE41, and 12YE41) from Bingol province. The genotypes were selected from 50 genotypes grown from a seedling in Asagikoy and Yelesen villages of Bingol city center. Asagikoy village is located at 38° 51' 23.83" N and 40° 22' 34.97" E with an elevation of 1400-1800 m and Yelesen 38° 52' 4.13" N and 40° 19' 25.72" E with an altitude of 1400-1800 m.

2.2. Shell cracking resistance

The shell cracking resistance was the physical force (kgf) value when the shell shuttered after applying a physical force. The A50-kg force was exerted to the suture of a walnut shell with 3-mm insertion by employing a texture analyzer (TA-XT Plus Texture Analyzer, Stable Micro System Ltd., Surrey, UK)

2.3. Kernel firmness

Kernel firmness was measured with the texture analyzer by applying a 5-kg force to each half of the kernel and expressed as kgf. The probe was inserted into the kernel with a depth of 5 mm.

2.4. Kernel percentage

After weighing inshells and kernels, the percentage was calculated according to the formula of kernel weight/inshell nut wight x 100.

2.5. Pellicle and cotyledon color

Pellicle and cotyledon color was measured and expressed as L* (lightness) and b* (yellowness) using a colorimeter (Lovibond, RT 300, Amesburg, Germany).

2.6. Total oil ratio

Total oil extraction was done according to the procedure employed by [15]. Five-g granulated kernel sample with petroleum ether solvent (100 ml) was used to extract the oil at 40 – 60 °C for 4 h equipped with a Soxhlet apparatus. The total oil ratio was calculated based on the weight differences of tubes before and at the end of the process.

2.7. Total phenol content

Total phenol content was carried out according to the Folin-Ciocalteu method [16]. Methanol, deionized water, Follin-Ciocalteu reagent, and Na_2CO_3 solution were used for the quantification. The mixture was read at 745 nm on a spectrophotometer after 2-h incubation at room temperature. Total phenolic content was expressed as milligrams equivalents of gallic acid per kg.

2.8. Total antioxidant capacity

DPPH (1,1-diphenyl-2-picrylhydrazyl) method was employed for the total antioxidant capacity [17]. Methanol-diluted extract (50 µl; 1:20 w/v) was mixed with 3 ml DPHH solution (v/v) then left on the stand in the dark for 30 min in order to get desired color formation. The mixture was read at 517 at a spectrophotometer. Total antioxidant activity was reported in % (Trolox Equivalent).

2.9. Statistical analysis

Mean separation was performed due to the trees being neither on the same plot and nor treated equally; each value is expressed as only mean± standard deviation.

3. Results and Discussion

Shell cracking resistance, kernel firmness, and kernel percentage values of the genotypes are illustrated in Table 1. Shell cracking resistance ranged from 8.73 (12YE28) to 34.83 kgf (12YE29) with an average value of 17.96 kgf. In walnut processing technology, a nut with an easy-to-break shell is preferred. The genotype 12YE28 had the lowest shell cracking resistance implying that it may have o potential to become a superior walnut genotype. Walnut cracking is directly affected by shell thickness and moisture content; thin-shell structure and lower moisture content provide a better shell rapture [18,19]. Kernel firmness was found to range from 0.87 (12YE33 and 12YE41) to 1.34 kgf (12AK12). The genotype of 12AK12 distinguished itself from others by having a crispier kernel structure, which is a preferable walnut trait [20]. Kernel firmness seems to be a genetic trait [21] while greatly affected by the moisture content of the kernel [20] and mildly affected by the storage temperature [22]. Kernel percentage varied from 44.20 (12AK01) to 56.77% (12AK12) with a mean of 51.56%. The genotypes may be dived into 3 groups; group 1 with a kernel percentage under 50, group 2 with a kernel percentage between 50 and 55%, and group 3 with a kernel percentage over 55%. 12AK19 and 12AK22 genotypes have the potential to become superior genotypes since they have a kernel percentage over 55%. Kernel

percentage is a genetic trait [23] while can be influenced by growing conditions, season, altitude, moisture content, shell thickness, and inshell sections [24,25].

Table 1. Shell cracking resistance, kernel firmness, kernel percentage, and pellicle and cotyledon color (L* lightness and b* yellowness) of the walnut fruits.

Genotypes	Shell cracking resistance (kgf)	Kernel firmness (kgf)	Kernel percentage (%)	Pellicle L*	Pellicle b*	Cotyledon L*	Cotyledon b*
12AK01	15.70	1.01	44.20	52.21	28.39	73.20	26.83
12AK12	12.95	1.34	52.64	58.07	29.25	74.60	26.55
12AK19	20.31	0.97	56.77	51.80	29.84	71.98	29.23
12AK22	10.19	1.13	55.50	62.56	30.78	75.25	28.98
12YE26	15.70	1.06	48.88	47.06	25.02	70.76	29.04
12YE28	8.73	1.08	53.50	56.71	32.37	72.22	30.34
12YE29	34.83	1.09	48.51	55.38	29.17	74.16	26.68
12YE33	22.16	0.87	55.00	58.46	28.80	76.47	27.63
12YE41	17.16	0.87	51.13	61.28	31.98	73.22	25.49
12YE47	21.90	0.95	44.47	63.01	31.57	73.81	27.18
Min	*8.73*	*0.85*	*44.20*	*47.06*	*25.02*	*70.76*	*25.49*
Max	*34.88*	*1.34*	*56.77*	*63.01*	*31.98*	*76.64*	*30.34*
Avg	*17.96*	*1.04*	*51.56*	*56.66*	*29.72*	*73.57*	*27.80*

Pellicle and cotyledon color values (L* and b*) are presented in Table 1. Lightness (L*) of the pellicle was in the range of 47.06 and 63.01 while yellowness (b*) in the range of 25.02 and 31.98. The brightest pellicle was recorded on 12YE47 (63.01) whereas the lightest yellow on 12YE26 (25.02). In terms of pellicle color, the genotypes may be dived into light-yellowed genotypes (b* value under 30) and dark-yellowed (amber) genotypes (b* value over 30). Pellicle color is an important commercial trait and is greatly affected by its polyphenol content and antioxidant capacities, meaning light-yellowed pellicles comprise more polyphenolic contents and antioxidant capacities than dark-yellowed (amber) ones [26,27]. Persic *et al.* [27] suggested amber-colored pellicle may be too low phenolic contents which partially fail to prevent polyphenol oxidation. Lightness (L*) of cotyledon changed mildly from 70.76 (12YE26) to 76.47 (12YE28) with a mean of 73.57. 12AK22 and 12YE33 came out with the brightest cotyledon color over 75. Cotyledon yellowness was in the range of 25.49 (12YE41) and 30.34 (12YE28), exerting almost all the cotyledon colors were the same. No study recording cotyledon color has been published yet but Warmud and Sambeek [28] reported that walnut with amber-colored pellicle seems to have a smaller kernel than those with a yellow-colored pellicle.

Total oil and phenol content, and antioxidant capacity of the promising genotypes were shown in Table 2. Total oil content was found between 45.04 (12YE28) and 56.88% (12AK12). Apart from 12YE28 and 12YE29, the genotypes had total oil content of over 50%. Simsek et al. [13] reported a higher total oil content of 63.63-66.78% for 12 promising genotypes in Bingol but in different districts.

However, the average total oil content of the genotypes of the present study was ca. 52 % which was close to the values from several studies around the world [29-33]. We registered the highest total oil of 56.88 % from 12AK12 genotype yet a value of as high as 73.90 % was reported from 'Sorrento' cultivar in Argentina by Martinez and Maestri [34].

Table 2. Total oil and total phenolic content, and total antioxidant capacity of the walnut fruits.

Genotypes	Total fat (%)	Total phenolic content ($mg.kg^{-1}$)	DPPH (%)
12AK01	51.57	136.13±15.38	70.52±0.13
12AK12	56.88	118.24±6.93	68.41±0.23
12AK19	51.95	119.91±9.23	69.77±0.11
12AK22	53.44	133.03±27.82	64.14±0.12
12YE26	50.13	142.91±14.11	69.34±0.08
12YE28	45.04	117.53±12.87	69.66±0.11
12YE29	48.53	80.97±27.68	69.06±0.19
12YE33	53.17	91.53±25.81	69.20±0.24
12YE41	56.36	141.10±22.65	70.34±0.18
12YE47	51.55	99.85±35.81	69.91±0.25
Min	*45.04*	*80.97*	*64.14*
Max	*56.88*	*142.91*	*70.52*
Avg	*51.86*	*118.12*	*69.04*

Total phenol content was in the range of 80.97 (12YE29) and 142.91 $mg.kg^{-1}$ (12YE26) with average value of 118.12 $mg.kg^{-1}$. 12YE41 and 12YE26 stood out from other genotypes with higher total phenolic content of over 140 $mg.kg^{-1}$. Our results were sometimes fell in the range of previously published works [35,36] yet fell short of some [31,37,38]. Walnut's rich phenolic contents are mainly attributed to tannin, especially hydrolyzable tannins [39,40]. The major source of phenolic contents in the kernel is the pellicle that protects the embryo from physical, chemical, or microbial entities such as oxidation, microbes [27]. Up to 28 phenolics are identified in the pellicle [27].

The DPPH free radical scavenging capacity of the genotypes vaguely varied from 64.14% (12AK22) to 70.52% (12AK01) with a mean of 69.04%. Akin et al. [41] (2013) recorded a slightly higher average antioxidant capacity of 84.62% from walnut kernels in Turkey. Bakkalbasi et al. [42] (2013) published a work in which DPPH values for 6 commercial local walnut cultivars in Turkey ranged from 41.91 to 85.15%. In another work, total antioxidant activity determined by TAEC method from 15 genotypes and 'Sebin' cultivar in Turkey was in the range of 93 and 128 µmol Trolox eq./g [43] (Beyhan et al., 2016). When compared to the previously published studies restricted to Turkey, our results were close to the values reported by these works.

One of the reasons for the high antioxidant capacity of walnuts may be due to high phenolic contents especially located in pellicles [37, 44]. Tocopherol abundantly found in the kernels is a natural

and effective antioxidant [45]. The high antioxidant capacity of the kernel makes walnuts one of the richest dietary sources in total oxidants after dog rose fruit before pomegranate [46].

4. Conclusion

Local walnut genetic resources are very crucial for breeding programs whether utilizing them in crop improvement or conversation programs [47]. Bingol province offers an important resource in this regard. The genotype of 12YE28 with low shell cracking resistance, 12AK19 with high kernel percentage, and 12YE26 with light-yellowed pellicle may be used for walnut crop improvement programs. It is also necessary to mention that the genotype 12AK01 was rich in oils, 12YE26 in phenolics, and 12AK01 in antioxidant capacity. Also, it is thought that the findings from this study are an important step for future walnut breeding programs in the Bingol province in Turkey. Moreover, the present works add information to the database of walnut genotypes in Turkey.

Conflict of interest:
The authors declare no conflict of interest.
The compliance to Research and Publication Ethics: This study was carried out by obeying research and ethics rules.
The Declaration of Ethics Committee Approval

The author declares that this document does not require an ethics committee approval or any special permission. Our study does not cause any harm to the environment.

Authors' Contributions:
Muharrrem Ergun: Conceptualization, Methodology, Formal analysis, Writing - Original draft preparation (%50). Zahide Süslüoğlu: Conceptualization, Methodology, Resources, Investigation, Writing - Original draft preparation (%50).
All authors read and approved the final manuscript.

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