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The Effects of cultivation area and altitude variation on the composition of fatty acids of *Laurus nobilis* L. berries in Nothern Turkey and Abkhazia

Bilge Yılmaz[*1], İlhan Deniz[2]

[*1] *Department of Fiber and Paper Technology , Forest Industry Engineering, Forest Faculty, Karadeniz Technical University, 61080, Trabzon, Turkey. E-posta: bilgekarasakal@gmail.com*
[2] *Department of Fiber and Paper Technology , Forest Industry Engineering, Forest Faculty, Karadeniz Technical University, 61080, Trabzon, Turkey. E-posta: ideniz@ktu.edu.tr*

Corresponding author: bilgekarasakal@gmail.com

Abstract

The aim of the study was to determine and compare the fatty acids contents of Laurel berries (*Laurus nobilis* L.) grown in Trabzon, Bartın, Samsun and Abkhazia. Fleshy parts of laurel berries and seeds were analyzed separately to reveal yields of fixed oils. Three altitude ranges were determined for the study and samples were collected from the ranges that can be found from these elevations where the altitude is 0-100m, 100-300m and 300-600m. Automatic extraction technique was used for getting fixed oil and the amount and the composition of the oil was identified by using GC-FID. The results showed that the fixed oil yield of fleshy parts of berries ranged between 28.37% and 42.08%. The highest amount of fixed oil yield in fleshy part was obtained in Bartın (0-100 m). The fixed oil in of seeds ranged between 16.26% and 22.81%. The highest amount of fixed oil yield in seeds was obtained in Bartın (100-300 m). According to GC-FID results, oleic acid (27.06 % - 48.93%) was the most abundant fatty acid, lauric acid (0.49% -1.35 %) was the least abundant fatty acid in the fleshy parts of laurel berries. In seeds, lauric acid was the most common fatty acid (32.37%-44.49%) and arachidic acid (0.87%-1.17%) was the least fatty acid. According to results, it is thus deduced that, the amount and content of fixed is affected by cultivation area, altitude variation and the parts of laurel berry
Keywords: Fatty acid, fleshy, laurel berry, seed.

Özet

Çalışmanın amacı Trabzon, Bartın, Samsun, Hatay ve Abhazya'da yetişen defne meyvelerinden elde edilen sabit yağın içeriğinin belirlenerek çıkan sonuçların yetişme yeri ve yükseklik farkına göre karşılaştırmaktır. Çalışma için 0-100m, 100-300 m ve 300-600 m olarak üç ayrı yükseklik aralığı belirlenmiş ve bu yükseltilerden bulunabilen aralıklardan örnekler toplanmıştır. Defne meyvelerinde etli kısımlar ve tohum kısımları ayrılarak çalışılmıştır. Çalışmada sabit yağlar otomatik ekstraksiyon tekniği ile elde edilmiş olup, sabit yağların içeriklerinin belirlenmesinde GC-FID cihazı kullanılmıştır. Sonuçlara bakılacak olunursa, defne meyvesi etli kısmından elde edilen sabit yağ miktarı %28.37 ile %42.08 aralığında değişmektedir. En yüksek sabit yağ oranı etli kısım için Bartın 0-100m'den elde edilmiştir. Tohumlarda ise sabit yağ miktarı %16.26 ve %22.81 aralığında değişmektedir. En yüksek sabit yağ oranı tohum için Bartın 100-300m'den elde edilmiştir. GC-FID sonuçlarına göre, oleik asit (%27.06 - %48.93) defne meyveleri etli kısmında en yüksek miktarda bulunan yağ asitlerinden olmuş olup laurik asit ise (%0.49 - %1.35) oranı ile defne meyveleri etli kısım örneklerinde en düşük miktarda bulunan yağ asitlerinden olmaktadır. Tohum sonuçlarına bakıldığında ise laurik asit (%32.37 - %44.49) ile tohumda en yüksek miktarda bulunan yağ asitlerinden olmuş olup, araşidik asit (%0.87 - %1.17) oranı ile tohum örneklerinde en düşük miktarda bulunan yağ asidi olarak saptanmıştır. Çalışmanın sonuçları defne meyvesinin etli kısmında ve tohum kısmında bulunan sabit yağ miktarının ve içeriğinin defne meyvesinin yetişme yeri ve yüksekliğe göre değiştiğini desteklemektedir.
Anahtar Kelimeler: Defne meyvesi, etli kısım, tohum, yağ asidi.

Introduction

Since ancient times the leaves and berries of the *Laurus nobilis* L. have been known. Laurel is a very important medicinal and aromatic plant in Turkey and nearby regions. Laurel is an evergreen tree, 3-10 m in height and the leaves have an aromatic odor. The leaves about 5-10 cm long and 2-5 cm broad. The shapes of the leaves are firm and like a spearhead. The edges of leaves are wavy and short-handed (Baytop 1999). The shape of laurel berry is elliptical, and it is 1.5 cm long and about 1 cm thick, containing a single seed. Berries are one seeded olive or chickpea size. The weight of the kernel varies with the weight of the whole berry, and this ratio is generally around 70%. In the most inner part of the berry, the core consists of the endocarp, the fleshy part (mesocarp) and the outer shell (pericarp) between the core and the fleshy part (Yazıcı 2002). Leaves of this plant have essential oil and berries have fixed oil and a little bit essential oil. The chemical content of these oils were closely examined, and it was found that volatile compounds and non-volatile fatty acids have many benefits for human health. Because of this, this plant have been used for medicinal applications from ancient times (Yazıcı 2002).

Fixed oil is semi-solid in hot seasons and has a special aroma. It melts at about 32-36 °C (Riaz and Asraf 1987, Baytop 1999).The chemical composition of laurel berry fixed oil comprises from saturated fatty acids and unsaturated fatty acids. There is a high amount of lauric acid in the structure of the laurel berry. The areas of usage of lauric acid are food and cosmetic industry (Erickson, 1990, Baytop 1999). Due to the fact that the pericarp and the nucleus do not develop in a proportional manner during berry development the fatty acid composition does not change much until maturing period but there are huge variations in fatty acid ratios (Timur 2001). Laurel berry fixed oil occurs ending of the esterification reactions glycerin and fatty acids, and it is liquid and solid. It is insoluble in water but soluble in organic solvents. In many countries, laurel berries are used as folk medicines for the treatment of various disease. Conventionally, laurel berries using for stimulating blood flow in the pelvic area and uterus and treating hysteria and also crushed fresh berry is consumed a lot for the treatments of hemorrhoidand ulcer (Simic et al 1989, Tuzlacı and Erol 1999, Tuzlacı and Erol, 2000).

The fragrant oil obtained from the berry is used in veterinary, pharmaceutical and perfumery industries. When laurel berry is mixed with palm kernel oil and coconut oil, it can treat skin diseases (Baytop 1983, Gang et al, 1993). Laurel fixed oil is commonly used on the treatment of sprains, rot and rheumatism and also fixed oil has parasiticidic property (Hoppe 1944, Leyel 1984, Boukef 1986, Tuzlacı and Erol 1999). Laurel berries are generally used in perfumed soap and hair care products since having antidandruff activity and also laurel oil treats eczema, skin eruptions and scaling (Hafızoğlu and Reunanen 1933).

Although there are many studies related with isolation and biological activity of laurel leaf essential oils, there has been little work on its berries. From this point of view, the present paper aims to determine fatty acids compositions of laurel berries which are grown in eastern, western and central Karadeniz region and Abkhazia.

Material and Methods

Plant material

The areas where the laurel berries were collected are given in Table 1. It was about 500 gr samples were collected for each altitudes. Fleshy berries were collected in September and October, 2014. They were stored at +4 ºC until analyzes. Collected berries were separated into seeds and fleshy parts. Since they have high moisture content, they have been oven-dried at 70 ºC for 3 days. After that, the berries

were weighed and moisture contents were determined separately. After they were dried seeds and fleshy parts (mesocarps) of laurel berries were ground with plant grinder (Waring and Retsch-ZM 200).

Table 1. Locations and altitudes of collected laurel berries.

Location	**Altitude (m)**		
	0-100	**100-300**	**300-600**
Trabzon	20	-	-
Samsun	77	-	-
Bartın	10	200	400
Abkhazia	50	-	-

Extraction

Fixed oils were obtained by using automatic Foss Soxtec Extraction device. 2.5-3 gr samples were subjected to extraction procedure (seeds and pericarps). Hexane was used as a solvent for the extraction (70-90 ml). Extraction was completed in 4 stages. First stage was boiling of solvent, it was about 25 min. Second, extraction, it was about 30 min. Third, ending of extraction and recovery of solvent, it was about 15 min and last was drying, it was about 10 min. Total time for extraction of fatty oil was about 80 minutes. Then the fatty oil were dried and stored in the dark at room temperature until use.

Fatty acids methyl esters

Because of the polar nature of the fatty oil of laurel, In order to analyze fatty oil in GC-FID, It is necessary to perform the fatty oil methylation process. For methylation process, firstly 0.1 gr fixed oil was added into the glass tubes and 500 ml CH_3OH+65 gr KOH solution was prepared.1 ml of methanolated KOH was added to each of 0.1 g fixed oils and vortexed. After the saponification reaction was started, 10 ml hexane was added each of tubes in two steps and each tube was vortexed and mixed. The methyl esters were centrifuged at 7000 rpm for about 5 minutes to pass to the solvent phase. The top phase was taken to be injected into the GC-FID.

GC-FID analysis

The GC–FID analysis was carried out with Shimadzu GC 2025 Technocroma capiller TRCN 100 column (60mx0.25 mmx0.25 μm) was used with helium as carrier gas (0.8ml/min) for analysis of fatty acid methyl esters. GC oven temperature was kept at 80°C and programmed to 140°C at a rate of 3°C/min, and kept constant at 140 °C for 1 min and then programmed to 240 °C at a rate of 3 °C/min and kept constant at 240 °C for 5 min. The injector temperature was set at 250°C and FID detector temperature was 250°C.In the analysis, the split ratio was determined as 50:1. Total duration time was 61 minutes. Prior to the analysis, a Supelco Fame 37 Internal standard was used and a calibration chart of each fatty acid component was drawn and retention times were determined. The obtained chromatograms were evaluated in the internal standard frame given in advance to the device. (Özkaya et al 2014)

Results

Fixed Oil Yield of Fleshy Parts of Berries and Seeds

The amount of fixed oil obtained from the fleshy parts of laurel and seeds are shown in Table 2.

Table 2. The average yields fixed oil of fleshy parts and seeds of *Laurus nobilis* L. berries

Fleshy Part		Seed	
Sampling area	Total Amount of Oil (ml/100 gr)	Sampling Area	Total Amount of Oil (ml/100gr)
Bartın 10 m, B_1	42.08±0.3450	Bartın 10 m	19.15±0.0120
Bartın 200 m, B_2	34.89±0.1783	Bartın 200 m	22.81±0.3100
Bartın 400 m, B_3	28.37±0.0328	Bartın 400 m	16.53±0.1197
Abkhazia 50 m, A_1	29.28±0.3979	Abkhazia 50 m	19.69±0.0713
Trabzon 20 m, T_1	29.31±0.4705	Trabzon 20 m	16.26±0.1804
Samsun 77 m, S_1	29.80±0.1849	Samsun 77 m	21.31±0.1125

GC-FID Analysis

The results of the GC-FID analysis of fleshy parts and seeds of laurel berries fixed oils are shown in Table 3 and Table 4.

Table 3. Fatty acid composition of flesh parts of berries.

FLESHY PART **Fatty Acid**		**RT***	**T_1**	**S_1**	**A_1**	**B_1**	**B_2**	**B_3**
C-12:0	Lauric Acid	17.58	1.30	0.49	1.35	-	0.57	0.62
C-16:0	Palmitic Acid	28.67	32.61	20.70	25.74	30.22	29.53	33.07
16:1 Δ^9	Palmiteloic Acid	29.97	1.14	0.83	0.41	1.48	0.78	1.33
C18:0	Stearic Acid	33.63	2.11	1.32	2.68	1.23	1.05	0.81
18:1 Δ^9	Oleic Acid	34.75	30.19	48.93	36.43	35.21	36.56	27.06
18:2 $\Delta^{9,12}$	Linoleic Acid	36.56	29.37	24.80	30.05	29.21	29.18	34.39
18:3 $\Delta^{9,12,15}$	α-Linolenic Acid	38.64	2.15	1.68	2.19	1.76	1.42	2.03

RT*: Retention Time

Table 4. Fatty acid composition of seeds of berries.

SEED **Fatty Acid**		**RT***	**T_1**	**S_1**	**A_1**	**B_1**	**B_2**	**B_3**
C10:0	Capric Acid	12.08	-	0.83	0.52	-	0.96	0.72
C12:0	Lauric Acid	17.58	32.37	42.94	33.06	36.1	44.49	37.07
C14:0	Myristic Acid	23.04	1.14	1.73	1.24	1.45	1.61	0.94
C16:0	Palmitic Acid	28.67	8.81	9.84	10.90	7.59	6.80	6.85
C16:1Δ^9	PalmiteloicAcid	29.97	-	0.45	0.75	-	1.33	-
C18:0	Stearic Acid	33.63	1.30	2.39	3.06	1.30	1.03	0.99
18:1 Δ^9	Oleic Acid	34.75	29.50	23.87	26.99	29.39	24.46	26.15
18:2 $\Delta^{9,12}$	Linoleic Acid	36.56	23.73	15.45	20.49	20.8	18.27	24.35
18:3 $\Delta^{9,12,15}$	α-LinolenicAcid	38.64	0.91	0.58	0.70	0.72	0.48	-
C20:0	Arachidic Acid	39.04	0.92	0.87	1.10	1.04	0.95	1.17

RT*: Retention Time

Table 5. Total % fatty acid composition of fleshy parts of berries

TFA*(%)	**T_1**	**S_1**	**A_1**	**B_1**	**B_2**	**B_3**
∑SFA (Saturated Fatty Acid)	36.02	22.51	29.77	31.45	31.15	34.50
∑USFA (Unsaturated Fatty Acid)	31.33	49.76	36.48	36.69	37.34	28.39
∑PUFA (Polyunsaturated Fatty Acid)	31.52	26.48	32.24	30.97	30.60	36.42

TFA*: Total Fatty Acid (%)

Table 6. Total % fatty acid composition of fleshy parts of berries.

TFA*(%)	**T1**	**S1**	**A1**	**B1**	**B2**	**B3**
∑SFA (Saturated Fatty Acid)	44.54	58.60	49.88	47.48	55.81	47.74
∑USFA (Unsaturated Fatty Acid)	29.50	24.32	27.74	29.39	24.46	26.15
∑PUFA (Polyunsaturated Fatty Acid)	24.64	16,30	21.19	21.52	18.75	24.35

TFA*: Total Fatty Acid (%)

Discussion

The fixed oil yield of fleshy parts of berries range between 28.37% and 42.08%. The highest amount of fixed oil yield in fleshy part was obtained in Bartın 10 m. The fixed oil yield of seeds ranges between 16.26% and 22.81%. The highest amount of fixed oil yield in seeds was obtained in Bartın 200m.

According to the results of analysis, the most abundant fatty acids for fleshy parts of berries were oleic acid (27.06%-48.93%) and linoleic acid (29.18%-34.39%) from unsaturated fatty acids and also palmitic acid (20.70%-33.07%) from saturated fatty acids. The least amount of fatty acids were lauric acid (0.49%-1.35%) and palmitic acid (0.41%-1.48%) from saturated fatty acids.

According to the fixed oil analysis obtained from the seeds of laurel berries, lauric acid (32.37%-44.49%), oleic acid (23.87%-29.50%), linoleic acid (15.45%-24.35%) were the most abundant fatty acids. α-Linoleic acid (0.48%-0.91%), palmiteloic acid (0.45%-1.33%) were the least amount of fatty acids. Tables 5 and 6 show the total amount of fatty acids according to the types of fatty acids.it is observed that the amount of unsaturated fatty acids is higher for all regions in fleshy parts of berries. Table 6 shows that saturated fatty acids predominantly exist in laurel seeds.

When the study areas are examined within itself, it can be said that the oil yield is affected by the altitude. As the height increases for Bartın, the amount of fixed oil in flesh part decreases. The situation is little bit different for seeds as seen from the results of fixed oil yield for seeds. As the height (altitude) increases the amount of fixed oil in seeds, first increased and later decreased. The highest fixed oil yield in seeds was obtained in Bartın in the range of 200 m with fat yield 22.81%. The least amount was obtained as 16.26% in Trabzon 20 m. There are lots of factors affecting the production of fatty oil in plants. It can be explained as an environmental, geographical, physiological, genetic, political and social factors (Bozan and Karakaplan 2007).

Nurbaş and Bal reported that, the extraction yields of laurel berries which were purchased from İzmir, 32.12% for whole berry. Beis and Dunford reported oil yields for Muğla region as varied from 14 to 28% and also they reported that, lauric (43.10%-44.80%), oleic (37.2%,37.3%) and linoleic acid (14.7%,13.3%) most abundant fatty acids for the berries of laurel for two methods respectively (supercritical CO_2 extraction and solvent extraction). They indicated that oil yield depends on the method and particle size used for oil recovery. As long as particle size increases, extraction yield decreased (Beis and Dunford 2006).

Bozan and Karakaplan (2007) reported fixed oil yield as 16.30% for pericarp, 23.40% for seed. Bal et al. reported for whole berry as 34.80% saturated fatty acids, 62.40% unsaturated fatty acid. Ozcan et al. (2010) reported that 40.79% linoleic acid, 38.08% lauric acid as major fatty acids for laurel seeds. Marzouki et al (2008) reported that lauric acid (27.7%) was the most abundant fatty acid for whole berry by Supercritical CO_2 extraction technique.

The results obtained with regard to fat yields and amount of fatty acids in our study are consistent with the literature, but the data obtained in present study is partially superior than previous reports. In this study characterization of fatty acids composition of laurel berry fat was different because we analyzed berry in two part: fleshy part and seed. According to the results of analysis, unsaturated fatty acids and polyunsaturated fatty acids are predominant in the fleshy part of laurel berry and saturated fatty acids are mostly found in the seed part. Therefore, with automatic solvent extraction method, the extraction time was shortened and the solvent was recovered with this method. Environmentally sensitive work has been performed by recovering the solvent.

Conclusions

There is a first report for Abkhazia and Trabzon. The chemical compositions of seeds and fleshy parts of berries were determined. For the present study the overall results indicated that the extraction of the laurel berry (fleshy part and seed) can be successfully performed by automatic extraction technique. Thanks to this technique, extraction time is shortened and solvent is recovered. Thus, it can be driven a profit both time and chemicals. So this method can be called environmentally friendly. On the other hand, Bartın is a convenient city for Karadeniz region for manufacturing laurel berry oil. Because the fixed oil yields are the highest for both seed and fleshy parts and also chemical characterizations of fixed oils are non-negligible.

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