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## STUDY OF ORGANIC ACIDS IN ALMOND LEAVES

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### Introduction

Almond (*Amygdalus communis*) is a stone fruit, from the *Rosaceae* family, closest to the peach. The almond is a deciduous tree, growing 4–6 m in height. The young twigs are green at first, becoming purplish when exposed to sunlight, then grey in their second year. The leaves are 8–10 cm long, 2–4 cm width with a serrated margin and a 2.5 cm petiole. The flowers are white to pale pink, 3–5 cm in diameter with five petals, produced singly or in pairs and appearing before the leaves in early spring. Almond is spread throughout the entire Mediterranean region and afterwards to the Southwestern USA, Northern Africa, Turkey, Iran, Australia and South Africa. It is sensitive to wet conditions, and therefore is not grown in wet climates. Iran is located in the semi-arid region of the world. Because of its special tolerance to water stress, almond is one of the main agricultural products in rainfed condition in Iran [1, 2].

Almond leaves have been investigated for their phenolic content and antioxidant activity. It was found that total antioxidant activity and phenolic compounds exhibited variations according to season, plant organ (leaf and stem) and variety. This could be a result from ecological, genetic and metabolic differences. Also, in the period during which almond tree has no fruit, the leaves and stems could be made use by their antioxidant activity. Phenolic compounds in the leaves of some varieties were high in October. In all varieties, phenolic compounds were lower in April and July [3]. Bioactive compounds such as phenols and flavonoids were also obtained and correlated to antioxidant activity in other works [4–6].

Organic acids have a wide range of pharmacological effects on the human body, benzoic and salicylic acids exhibit antibacterial activity, hydroxycinnamic acids and their derivatives - anti-inflammatory, malic acid promotes the absorption of iron. [7–9]. Analysis of previous research on almonds focused on investigating compounds mostly in seeds and phenolic compounds in leaves, but organic acids in leaves have not been studied.

**Aim** of this study was investigation of organic acids in leaves of almond variety which is distributed in Razavi Khorasan province of Iran.

### Materials and Methods

In August 2012 almond leaves were collected in rainfed place, orchards in a village called Divangah, Meshkan District, Razavi Khorasan province of Iran, dried

and grinded. The study of qualitative composition and quantitative determination of carboxylic acids in almond leaves was carried out by gas chromatography with mass spectrometric detection [10].

For determination organic acids content, to 50 mg of dried plant material in 2 ml vial internal standard (50 µg of tridecane in hexane) was added and filled up with 1.0 ml of methylating agent (14 %  $\text{BCl}_3$  in methanol, Supelco 3-3033). The mixture was kept in a sealed vial during 8 hours at 65 °C. At this time fatty oil was fully extracted, and hydrolyzed into its constituent fatty acids and their methylation was done. At the same time free organic and phenolcarboxylic acids were methylated too.

The reaction mixture was poured from the plant material sediment and was diluted with 1 ml of distilled water. To extract methyl esters of fatty acids 0.2 ml dichloromethane was filled up, gently shaken several times within an hour and then obtained methyl esters extract was chromatographed.

Injection of sample (2 µl) in a chromatographic column was carried out splitless, which allowed introducing the sample without flow division and significantly (10–20 time) increased sensitivity to chromatography analysis.

Content of organic acids were determined on Agilent Technologies 6890 chromatograph with mass spectrometric detector 5973; chromatography column - INNOWAX, capillary diam. 0.25 mm and a length of 30m; the rate of carrier gas (helium): 1.2 ml/min; heater temperature - 250°C; thermostat temperature was programmed from 50 to 250 °C with a speed of 4 °C / min.

For components identification, library of mass spectra NIST05 and WILEY 2007 with a total number of spectra more than 470,000 in conjunction with AMDIS and NIST programs were used.

For quantitative calculations the internal standard method was used.

Calculation of components content C (mg/kg) was carried out by the formula:

$$C = P_1 \cdot 50 \cdot 1000 / P_2 \cdot M$$

Where is:

$P_1$  - The peak area of the tested substance;  $P_2$  - the peak area of the standard; 50 – mass of internal standard (µg), injected into the sample; m – sample mass (g).

The statistical processing of results was carried out using package Statistica 6.0. The error does not exceed 5%.

### Results and discussion

32 organic acids were established in almond leaves in correspondence to library of mass spectra NIST05 and WILEY 2007 and their quantitative values were determined preliminary, using internal standard method. The research results are presented in Table 1.

Graphical result of determination of organic acids is shown on figure 1.

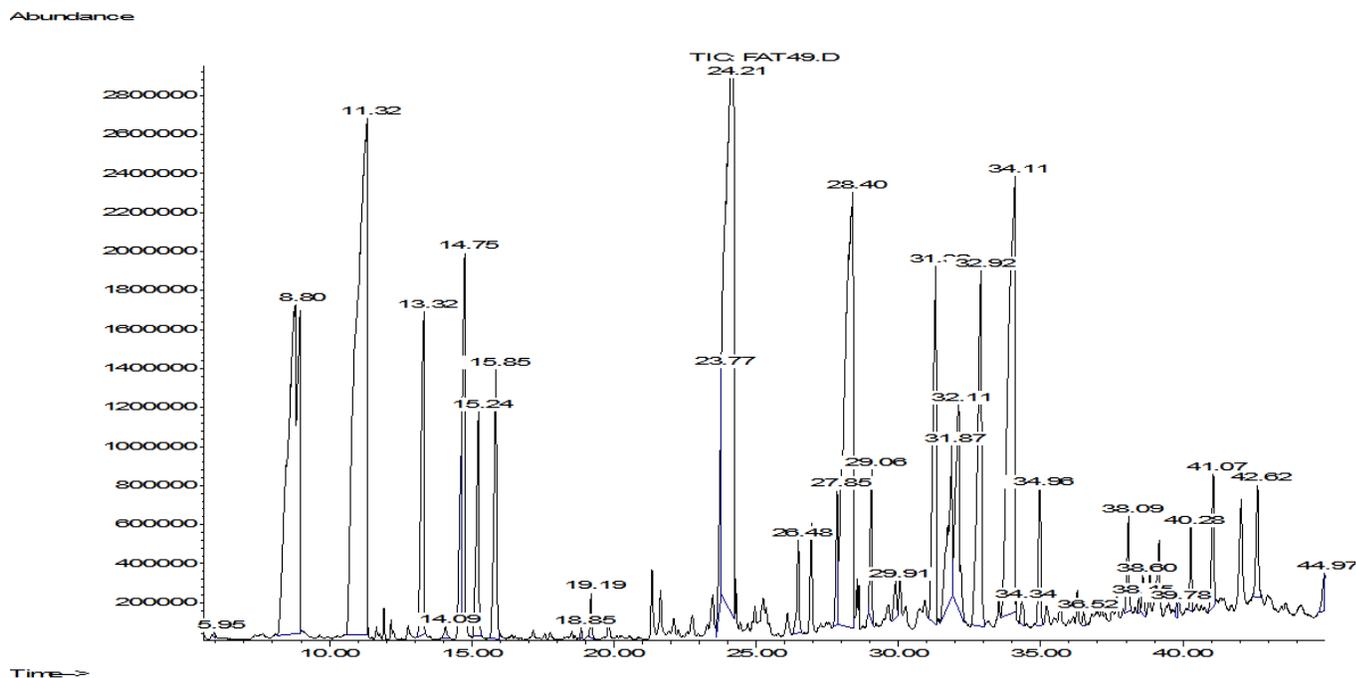


Figure 1. Graphical results of gas chromatography investigation of organic acid in almond leaves.

Table 1.- Results of determination of organic acid in almond leaves

Acid	Hold time, min	Content mg/kg	Acid	Hold time, min	Content, mg/kg
Caproic	5.943	5.43	Citric	31.318	746.55
Oxalic	11.325	3616.41	Stearic	31.87	438.37
Malonic	13.322	629.12	Oleic	32.11	575.31
Fumaric	14.086	19.86	Linoleic	32.913	945.72
Levulinic	14.755	596.65	Linolenic	34.112	1963.60
Succinic	15.246	380.98	Vanillic	34.341	43.59
Benzoic	15.848	488.43	Arachic	34.96	225.33
Phenylacetic	18.848	9.41	Heneicosylic	36.516	18.74
Salicylic	19.194	46.17	Behenic	38.089	125.60
Myristic	23.773	238.80	<i>p</i> -Coumaric	38.451	13.74
Malic	24.213	3343.03	Hexa dicarboxylic	38.596	56.01
Azelaic	26.483	127.82	Syringic	39.784	19.73
$\alpha$ -Hydroxy phenylacetic	27.849	189.24	Gentisic	40.275	94.46
Palmitic	28.402	2343.49	Lignoceric	41.067	212.53
Palmitic oleic	29.065	186.61	Ferulic	42.623	183.20
Heptadecanoic	29.907	65.57	Cerotic	44.971	49.89

Overall, plant raw material contained 1.80% of the organic acids, including (%) – 0.75 fatty; 0.95 di- and three carboxylic; 0.10 phenol carboxylic acids.

Among the carboxylic acids, oxalic and malic acid were the largest with- 3616.41 (38.23%) and 3343.03 (35.34%) mg / kg, respectively.

Palmitic and linoleic acids dominated among the fatty acids, their content was determined as (mg/kg) 2343.49 and 1963.60 that were 30.98 % and 25.96% respectively of the total fatty acids. Among phenol carboxylic acids, the

largest content was defined for benzoic acid. It was 488.43 mg / kg or 50.16% of the total amount of phenol carboxylic acids.

### Conclusion

Almond leaves were investigated by gas chromatography. 32 organic acids were established and their quantitative value were determined. It was found that almond leaves contain 1.80% of the organic acids, including (%) –

0.75 fatty acids; 0.95 di- and three carboxylic acids; 0.10 phenol carboxylic acids.

The results of this study are significant for determining beneficial compounds in almond leaves, so making use of these plant raw material as source of new medicines in the future is possible.

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Results and discussion. 32 organic acids were established in almond leaves and their quantitative values were determined, using internal standard method. Overall,

plant raw material contained 1.80% of the organic acids, including (%) – 0.75 fatty; 0.95 di- and three carboxylic; 0.10 phenol carboxylic acids. Among the carboxylic acids, oxalic and malic acid were the largest with- 3616.41 (38.23%) and 3343.03 (35.34%) mg / kg, respectively. Palmitic and linoleic acids dominated among the fatty acids, their content was determined as (mg/kg) 2343.49 and 1963.60 that were 30.98 % and 25.96% respectively of the total fatty acids. Among phenol carboxylic acids, the largest content was defined for benzoic acid. It was 488.43 mg / kg or 50.16% of the total amount of phenol carboxylic acids.

Conclusion. Almond leaves were investigated by gas chromatography. 32 organic acids were established and their quantitative value were determined. It was found that almond leaves contain 1.80% of the organic acids, including (%) – 0.75 fatty acids; 0.95 di- and three carboxylic acids; 0.10 phenol carboxylic acids.

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**Keywords:** almond, leaves, gas chromatography, organic acids.

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### ИЗУЧЕНИЕ ОРГАНИЧЕСКИХ КИСЛОТ В ЛИСТЬЯХ МИНДАЛЯ

Ленчик Л.В., Баят С., Кисличенко В. С.

Методом газовой хроматографии с масс спектрометрическим детектором был изучен качественный состав и количественное содержание органических кислот в листьях миндаля, заготовленных в богарных садах Ирана. При хроматографировании использовали хроматографическую колонку - INNOWAX, диаметром 0,25 мм, длиной 30 м. Скорость газа-носителя (гелий): 1,2 мл / мин; температура нагревателя - 250 ° С. Температура термостата программировалась от 50 до 250 ° С со скоростью 4 ° С / мин. Установлено наличие и определено количественные содержание 32 органических кислот. Всего сырье содержало 1,80% органических кислот, в том числе (%) – 0,75 жирных; 0,95 ди- и три карбоновых; 0,10 фенолкарбоновых. Среди карбоновых кислот, содержание щавелевой и яблочной кислоты было наибольшим – 3616,41 и 3343,03 мг / кг, соответственно. Пальмитиновая и линолевая кислоты преобладали среди жирных кислот, (мг / кг) 2343,49 и 1963,60 соответственно. Среди фенольных кислот, в наибольшем количестве содержалась бензойная кислота (488,43 мг / кг).

**Ключевые слова:** миндаль, листья, газовая хроматография, органические кислоты

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### ВИВЧЕННЯ ОРГАНІЧНИХ КИСЛОТ В ЛИСТЯХ МИГДАЛЮ

Ленчик Л.В., Баят С., Кисличенко В.С.

Методом газової хроматографії з мас спектрометричним детектором був вивчений якісний склад і кількісний вміст органічних кислот в листях мигдалю, заготовлених в богарних садах Ірану. При хроматографування використовували хроматографічну колонку - INNOWAX, діаметром 0,25 мм, завдовжки 30 м. Швидкість газу-носія (гелій): 1,2 мл / хв; температура нагрівача - 250 ° С. Температура термостата програмувалася від 50 до 250 ° С зі швидкістю 4 ° С / хв. Встановлено наявність і визначено кількісний зміст 32 органічних кислот. Всього сировина містила 1,80% органічних кислот, у тому числі (%) – 0,75 жирних; 0,95 ді- і три карбонових; 0,10 фенолкарбонових. Серед карбонових кислот, вміст щавлевої і яблучної кислоти було найбільшим - 3616,41 і 3343,03 мг / кг, відповідно. Пальмітинова і ліолева кислоти переважали серед жирних кислот, (мг / кг) 2343,49 і 1963,60 відповідно. Серед фенольних кислот, в найбільшій кількості містилася бензойна кислота (488,43 мг / кг).

**Ключові слова:** мигдаль, листя, газова хроматографія, органічні кислоти.