

# Correlation of Predictor Variables to Squalene Content in Olive Fruits Using Multivariate Statistical Analysis

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## ABSTRACT

The present study was designed to examine the correlation of some predictor variables including pH, organic matter (OM), lime (L), phosphorus (P), potassium (K) and total salt (TS) content of soils of olive orchards to squalene content of olive fruits. Multiple linear regression analysis was used to model the relationship between explanatory variables (pH, OM, L, P, K and TS) and responses variable (squalene content) by fitting a linear equation to observed data. The olive fruits (Kilis Yaglik cv.) were sampled from ten-different locations. The soil characteristics of the sampling sites were determined. Oil extraction was carried out in similar industrial extraction conditions using an oleodisor system. Accordingly, pH ( $p=0.596$ ), OM ( $p=0.359$ ), L ( $p=0.490$ ), P ( $p=0.127$ ), K ( $p=0.265$ ) and TS ( $p=0.572$ ) were not individually statistical significant but their interaction was significant ( $p=0.006$ ). Standardizing the olive oil quality is not easy at all due to complex and variable environmental conditions. Along with the results herein, the factors themselves were not significant but their complex interaction was significant.

**Keywords:** Squalene, Olive oil, Soil characteristics, Kilis Yaglik cv., Multivariate statistical analysis.

## INTRODUCTION

*Olea europaea* L. (olive), which its fruit and oil have a major agricultural importance in Turkey, is of significant crop especially in Mediterranean countries. The oil obtained from olive fruits have been used not only nutrient and as a flavor enhancer in Mediterranean dishes, but also a medicine because of having essential roles for human health. The pharmaceutical value of olive oil is related to its biochemical content. Mainly four chemical sources: polyphenols, carotenoids, mono-unsaturated fatty acids and squalene are functional bioactives in olive oil particularly for pharmaceutical affects.<sup>1</sup> Squalene is a polyunsaturated triterpene widely prevalent in living being, especially among wheat germ, rice bran, shark liver oil and olives.<sup>2</sup> Squalene in olive oil is in general considerably high as compared to other oils.<sup>3,4</sup> Because of its potential pharmaceutical

impacts researchers have been interested in production and biochemistry of squalene in recent years. It has various favourable effects as a nutrient, preventive and therapeutic medicine.<sup>5,6,7</sup> A number of various studies indicate that squalene has important pharmaceutical activity like prevention cardiovascular diseases, strengthen the immune system, interference many cancer types, improving antitumor action of chemotherapeutic agents, protection the skin by scavenging singlet oxygen generated by UV light, anti-aging, reducing blood cholesterol, inhibition the development of tumors.<sup>5-8,9,10</sup> The olive oil contains about 0.2–0.7 % squalene. Humans absorbs 60 % of squalene from food and the average intake of squalene is 30 mg/day.<sup>11</sup> Plant growth may be positively or adversely influenced in growing area by a num-

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ber of factors. In this study, variation in the squalene was examined with soil characteristics. Also, the size of variation by soil conditions where the sampling done, and evaluations were compared based on the regression and linear trend analysis.

## MATERIAL AND METHODS

### Analysis of soil characteristics

Total dissolved salts by Saturation Extract Method, calcium carbonate ( $\text{CaCO}_3$ ) contents by Scheibler Calcimeter, organic matter by a modification of the Walkley and Black method, soil potassium content by flame photometer were determined for 10 locations.<sup>12</sup>

### Oil extraction and squalene analysis

The olive fruits were harvested from the same aged trees and cultivar (Kilis Yaglik cv.) in mid-December. The oils were extracted from olive fruits with n-hexane for four hours using a Soxhlet Extraction Apparatus (Thermal). The squalene analyses were conducted according to the official method COI/T.20/Doc.no.24 2001. Gas chromatography with flame ionization detector (GC-FID) analyses of squalene methyl esters was carried out on a Shimadzu gas chromatography (GC-2010 series) equipped with an Supelco SP 2380 fused silica capillary column (100 m, 0.25 mm i.d., 0.2  $\mu\text{m}$  film thickness).

### Statistical analysis

SPSS statistical program was used to determine statistical significance levels by employing the independent

one-way ANOVA followed by Duncan multiple range test and the differences between individual averages were considered to be statistically important at  $p < 0.05$ . The results were expressed as mean.

## RESULTS

Squalene content of olive oils and soil characteristics from 10 locations have been shown Table 1. Squalene contents varied at different locations. Accordingly, the highest squalene was obtained from locations 4 and 9 (Table 2). According to the regression coefficients, soil characteristics, in order of priorities, total salt, pH, organic matter, phosphorus and potassium have influenced the squalene content of oil. While total salt and pH has a greater effect on squalene content, other factors have been less effective. Along with the results herein, the factors themselves were not significant but their complex interaction was significant. Accordingly, pH ( $p=0.596$ ), OM ( $p=0.359$ ), L ( $p=0.490$ ), P ( $p=0.127$ ), K ( $p=0.265$ ) and TS ( $p=0.572$ ) were not individually statistical significant but their interaction was significant ( $p=0.006$ ) (Table 3). According to the results of the regression analysis, the mathematical expression of the effect of soil properties on the squalene content is shown below:

$$\text{Squalene content (\%)} = (1.554) + (-0.106 \times \text{pH}) + (-0.003 \times \text{Lime}) + (1.821 \times \text{Total Salt}) + (0.027 \times \text{P}) + (0.001 \times \text{K}) + (-0.024 \times \text{Organic Matter})$$

**Table 1: Squalene content of olive oil and soil characteristics in ten location**

Location	Squalene %	pH	Lime (%) $\text{CaCO}_3$	T. Salt %	P (%) $\text{P}_2\text{O}_5$	K (kg da <sup>-1</sup> ) $\text{K}_2\text{O}$	O. Matter %
1	0.80	7.89	16.88	0.016	1.77	88.75	2.66
2	0.75	7.79	30.00	0.032	2.40	75.00	3.59
3	0.69	7.87	45.56	0.002	0.70	48.58	2.48
4	1.04	7.68	11.56	0.031	3.61	92.00	0.62
5	0.89	7.69	11.48	0.033	2.52	105.60	3.16
6	0.79	7.83	23.31	0.032	5.04	67.50	2.48
7	0.88	7.87	22.91	0.037	4.29	22.50	2.66
8	0.95	8.20	4.55	0.037	5.61	95.28	1.44
9	0.97	8.03	16.67	0.023	7.28	80.75	4.77
10	0.72	7.67	22.91	0.025	1.95	40.00	2.11

**Table 2: Squalene content in olive oils from different locations**

Locations	1	2	3	4	5	6	7	8	9	10
Squalene(%)	0.8 b-e	0.75 c-e	0.69 e	1.04 a	0.89 a-c	0.79 b-e	0.88 a-d	0.95 ab	0.97 a	0.72 de

**Table 3: Coefficients for squalene content in olive oil.**

	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
(Constant)	1,554	1.536		1.011	0.322
pH	-0.106	0.197	-0.100	-0.537	0.596
Lime	-0.003	0.004	-0.214	-0.701	0.490
Total salt	1.821	3.178	0.117	0.573	0.572
P	0.027	0.017	0.395	1.585	0.127
K	0.001	0.001	0.242	1.142	0.265
Organic Matter	-0.024	0.025	-0.157	-0.935	0.359

**Table 4: Correlations between Squalene content and soil characteristics.**

	Squalene	pH	Lime	T. Salt	P	K	O. Matter
Squalene	1	0.124	- 0.667**	0.472**	0.510**	0.412*	- 0.19
pH	0.124	1	- 0.147	- 0.021	0.543**	0.037	0.185
Lime	- 0.667**	- 0.147	1	- 0.599**	- 0.557**	- 0.578**	0.24
Total Salt	0.472**	- 0.021	- 0.599**	1	0.454*	0.09	- 0.153
P	0.510**	0.543**	- 0.557**	0.454*	1	0.089	0.153
K	0.412*	0.037	- 0.578**	0.09	0.089	1	- 0.025
O. Matter	- 0.19	0.185	0.24	- 0.153	0.153	- 0.025	1

\*. Correlation is significant at the 0.05 level (2-tailed).

\*\*. Correlation is significant at the 0.01 level (2-tailed).

In comparison with correlation of squalene content and soil characteristics at different locations, the contents of squalene determined the highest correlation with lime and followed by phosphorus, total salt and potassium as soil characteristics, respectively. Factors have similar relationships among each other (Table 4).

## DISCUSSIONS

The chemical and physical properties of the soil influence plant growth, development and subsequently main primary and secondary metabolite production, secretion and accumulation. It is worth to note that the produced metabolites transport among the organs of the plants is also significantly affected by soil properties. The profile of the oils was influenced by the different ecological and topographical conditions, supplying additional information about the climatic conditions and its impact on the oil quality. Many agronomical and soil factors effect composition of olive oil, such as the climate, cultivar, degree of maturation and harvest time, irrigation, the place of cultivation, crop season, the production process and type.<sup>13,14,15,16,17</sup>

## CONCLUSION

Fatty acids, phenolic compounds and other minor compounds indicate the quality of olive oil. Standardizing the olive oil quality is not easy at all due to complex and variable environmental conditions. Ecological factors influence the amount of squalene in olive oil. For olive quality and squalene suitable edaphic factors may be chosen.

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## CONFLICT OF INTEREST

The author declares that they have no conflict of interest.

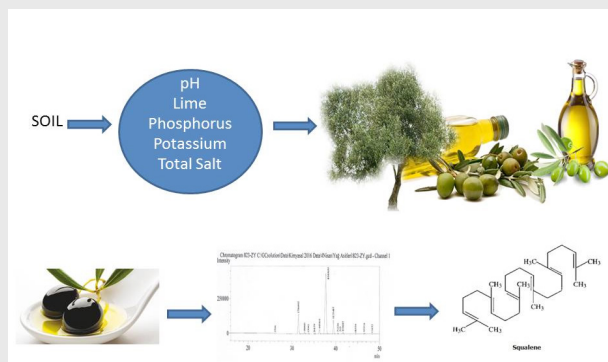
## ABBREVIATION USED

OM: Organic Matter; L: Lime; P: Phosphorus; K: Potassium; TS: Total Salt; GC-FID: Gas Chromatography with Flame Ionization Detector; m: meter; mm: millimeter;  $\mu$ m: micrometer; p: significance value.

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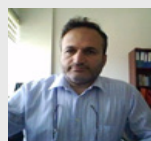
## PICTORIAL ABSTRACT



## SUMMARY

- Influence of soil characteristics including pH, organic matter (OM), lime (L), phosphorus (P), potassium (K) and total salt (TS) on squalene content of olive fruits have been determined
- Oil has extracted from Kilis Yagli cv. olive fruits sampled from different location
- Squalene content (%) =  $(1.554) + (-0.106 \times \text{pH}) + (-0.003 \times \text{L}) + (1.821 \times \text{Total Salt}) + (0.027 \times \text{P}) + (0.001 \times \text{K}) + (-0.024 \times \text{OM})$
- The factors themselves were not significant but their complex interaction was significant.

## ABOUT AUTHORS



**Dr. Hakan Çetinkaya:** He is assistant professor at the Agriculture Faculty, Horticulture Department. His research area of interest is plant physiology, horticulture, fruits, olive and pistachio. Also he has researches on secondary metabolites of plants in abiotic stress conditions.

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