Variation in the pigment content and phenolic composition of virgin olive oil from the olive cultivar *Roghiani* produced in Libya

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The aim of this work was to determine the pigments content, colour characteristics, unsaponifiable matter as well as phenolic compounds of virgin olive oils (VOO) from the olive cultivar *'Roghiani'*, harvested in five different regions of northern Libya. The colour of virgin olive oils was evaluated by determining chlorophyll and carotenoids content, transparency values, and colour characteristics using CIE and CIE *L*a*b** systems. In addition to the total phenols, content of single phenolic compounds were investigated, too. Obtained results indicated that the oil from Gharyan production region was quite different in relation to the oils from other harvest regions. Namely, this oil had not only the highest content of carotenoids, 9.15±0.21 mg/kg and high level of total chlorophylls, 37.7±1.89 mg/kg, but also a maximum content of phenols, 266.5±31.53 mgGAE/kg. Moreover, this is the first report on pigment investigation and colour characterisation in VOO from the *'Roghiani'* cultivar produced in Libya in different harvest regions.

Keywords: Virgin olive oil; Carotenoids; Chlorophylls; Colour characteristics, Phenolic compounds

1. INTRODUCTION

Growing olive trees (*Olea europaea* L.) is a widespread activity throughout the Mediterranean Basin, as well as in other regions, and is an important issue for the economic activity of the producing areas. Virgin olive oil (VOO) is produced from fresh and healthy olive fruits by first pressing or by other physical processes under low thermal conditions that do not lead to alteration in the oil which has not undergone any treatment other than washing, decantation, centrifugation or filtration [1, 2].

Libya tends to be one of the biggest olive oil producers in North Africa. During the 2008/09 and 2017/18 period, the average quantity of olive oil manufacturing in Libya was between 15.000 tons/year and 18.000 tons/year, i.e., up to 0.5% of the world's olive oil production [3]. According to the latest information, there are about 8.000.000 olive trees in Libya today. The most important cultivars are Endory, Roghiani, Rasli, and Hammudi. Individual manufacturers mostly produce olive oil in this country. According to the information from the year 2010, there are 250 individual oil producers. Anyway, Libya has excellent natural conditions for the development of olives and to enhance olive oil production. In Libya, the consumption of olive oil is a part of the cultural heritage, and a significant number of individual producers traditionally manufacture olive oil using a cold-pressing process. Producers use some of the oil for their use, a part is sold, and the lower quality oil is delivered to the only refinery in the country for refining [4].

Consumption of virgin olive oil is highly recommended due to its great health ben-

efits known since antiquity [1]. Among others, pigments are important bioactive microconstituents of VOOs. During the oil extraction, mass partitioning phenomena occurs that determines the pigment distribution of the olive paste between the solid (pomace) and the liquid phases (oil and wastewater). The lipophilic nature of chloroplast pigments determines their affinity for the oily phase, and the more hydrophilic nature of anthocyanins determines their retention in the pomace and wastewater. Many studies confirmed the antioxidative and antiradical properties of the chlorophylls and carotenoids [5]. Moreover, pigments' bioactivity is associated with healthy features (skin ailment, eye disorders, cancer, cardiovascular diseases) [6]. The study focusing on the characterisation of the pigment fraction of nine-single Spanish VOOs was carried out by Gandul-Rojas and Minguez-Mosquera [7].

Additionally, some studies have focused on the pigment composition of monovarietal virgin olive oils from various Italian [8, 9], and Greek olive varieties [10]. Accordingly, it would be important to increase studies on the characterisation of the pigments of virgin olive oil produced from different olive cultivars grown in different countries and in different olive growing areas to improve the database to standardise the characteristics that make any VOO unique and typical for its geographical and genetic origin. Nowadays, the extra-virgin olive oil traceability is also of great importance. Traceability consists of documented proof of the identity of a product, including many analytical characteristics, too.

Besides health benefits, the chloroplast pigments, chlorophylls, and carotenoids are mainly responsible for the colour of VOO, so they are important parameters of sensory quality [11, 12]. Although the colour of the oil is not considered as a quality parameter by the official regulations, it is one of the major attributes that affect consumers perception. Namely, consumers often judge food according to their external appearance, so they can reject any oil based on the appreciation of its colour, even though the other quality parameters of oil are appropriate [6].

Melgosa et al. [13] confirmed that the colour is related to the other chemical and physical properties of virgin olive oils. Since 2004, they have suggested that producers of virgin olive oils should pay increasing attention to the precise colour specifications of their products. Consequently, a colorimetric characterisation of virgin olive oil would be useful in the quality control of this product.

The olive flesh components are transferred to the oil, which mainly consists of saponifiable and a variety of unsaponifiable, non-glyceride compounds that add up to 0.5%-2% of the olive oil [14]. The nutritional value and health benefits of VOO are ascribed to many valuable minor components of unsaponifiable fraction, including aliphatic and triterpene alcohols, sterols, squalene, tocopherols, pigments such as chlorophylls, carotenoids, and others [6, 15]. The composition of bioactive compounds in VOO is affected by many agronomical and technological factors: cultivar, climatic conditions, ripeness of the olives at harvesting, fruit quality, processing system and storage [9, 14, 16].

There is an increasing interest in the phenolic compounds in olive oil due to their health-promoting properties, such as antioxidant activity and decreasing the risk of coronary and cardiovascular system diseases, diabetes, cell aging, and certain types of cancer [17, 18]. Phenolic compounds of VOO belong to different classes. The main phenolic alcohols are the hydroxytyrosol and tyrosol. Other bioactive compounds in olive oil are oleuropein, squalene, lignanes, caffeic acid, phytosterols, vanillic acid, flavonoids, syringic acid, rutin, protocatechuic acid, luteolin and p-hydroxyphenyl acetic acid [15, 19].

Accordingly, the pigment profile, colour parameters, as well as, the phenolic compound characterisation of VOO are very relevant to their commercialisation and increase the product value.

Since all the above-mentioned characteristics of oil depend on cultivar and growing areas the aim of this study was concerned with assessing the total chlorophylls and carotenoids content, colour parameters, unsaponifiable matter content, and to analyse the phenolic profile of virgin olive oils from the '*Roghiani*' cultivar, originated from five different olive-growing regions throughout northern Libya. Pattern recognition technique, such as Principal Component Analysis (PCA) was applied to the experimental data (used as descriptors) to characterise and differentiate among the observed samples of virgin olive oil produced in Libya. To the best of our knowledge, until now there is no published data on the pigment content and colour measurement of the olive oils processed in Libya from the '*Roghiani*' olive cultivar.

2. EXPERIMENTAL PART

2.1. PLANT MATERIAL

The investigations were carried out on olive oils from fruits of the *Roghiani* (*Olea europea* L.) cultivar harvested from five different geographic regions in Libya. Olive fruit samples were hand-picked and collected at the beginning of January 2015. The cultivar grows in the north of Libya in the following regions: Gharyan -32° 10′ N, 13° 01′ E (also known as Garyan), Tarhuna -32° 26′ N, 96 +13° 10′ E, Msallata -32° 35′ N, 14° 2′ E, Tripoli -32° 53′ N, 13° 10′ E and Q. B. Ghashir -32° 40′ N, 13° 10′ E (Fig. 1). The mean rain precipitation registered in these regions was about 383 mm/year, with a mean temperature of approximately 27°C.

Figure 1 - Geographical distribution of VOOs from Libya

2.2. EXTRACTION OF VOO

Olive fruit samples were collected from regions described above and processed within three days after harvesting. Fruits were washed, milled and olive pastes were malaxed with a mixer for 40 min at 35-40°C. After separation at 3000 rpm by centrifugal separation process (Rapanelli, Foligno, Italy), extracted olive oil samples were decanted and filtered through a filter press. VOO samples were stored in the refrigerator at 8°C in dark glass bottles until further analysis. Samples then were tempered at room temperature for 24 h before analysis.

2.3. METHODS

Chlorophylls

The content of total chlorophyll was estimated according to the spectrophotometric method described by Pokorny et al. [20]. The absorbancy of pure oil samples was measured in a cuvette of 10 mm for a path length at the wavelengths of 630, 670 and 710 nm against air. The content of the total chlorophyll pigments was calculated using the formula:

$$Ch\ (mg/kg) = 345.3 \times [A_{630} - (A_{670} + A_{710})/2] \times 10$$

Carotenoids

The content of total carotenoids was determined by the British Standard Method [21] measuring the absorbance of VOO dissolved in cyclohexane. Weigh 0.5-1 g of oil into a 10 ml flask and made up to the mark with cyclohexane. The absorbance of the solution was then measured at a wavelength of 445 nm. The content of total carotenoid pigments was calculated using the formula:

$$Carotenoids\ (mg/kg) = 383 \times A_{445}/d \times c$$

where:
A_{445}: absorbance measured at 445 nm;
d: the width of the cuvette (cm);
c: the concentration of oil in the solution.
This method considers β-carotene as a carotenoid equivalent.

Total pigments

The content of total pigments was expressed as the sum of chlorophyll and carotenoid contents.

Transparency

The transparency, i.e., relative transmittance of the radiation (%) of oil was measured at 455 nm in 1 cm glass cuvettes in relation to carbon tetrachloride (transparency of CCl_4 was 100%). For edible oils, transparency is correlated with the amount of pigments, primarily carotenoids, which absorb a specific range of electromagnetic radiation (light) at a wavelength of 445 nm. The rule is that the lower the transparency of the oil, the higher the total pigment content and vice versa.
All pigment contents and transparency measurements were conducted using UV/VIS spectrophotometer (model T80+, PG Instruments Limited, London).

Unsaponifiable matter

The content of unsaponifiable matter was determined by the standard method using hexane extraction [22]. After hydrolysis of the oil with KOH solution (1 mol/L concentration), the components that are insoluble in aqueous alkali, the so-called unsaponifiable matter, were extracted three times with organic solvent-hexane. The collected extract was then washed with the ethanol-aqueous solution (10 vol. %) to neutral reaction to remove residual soaps. After evaporation of the hexane and drying of the residue to constant weight, the content of the unsaponifiable matter in g/kg was calculated.

Colour measurements

The colour parameters of the uniform colour space CIELAB and CIE Y-xy colour coordinates [23] were determined using calibrated Minolta Chroma Meter CR-400 (Minolta Co., Ltd., Osaka, Japan) at D-65 light, standard angle of 2° and a head opening of 8 mm. The CIELAB parameters (*L*, a*, b**) were determined by using the software, that automatically displayed the CIE colour values (*L*, a*, b**). The *L** value defines the lightness as the property according to which each colour can be considered as an equivalent of a (member) position of the greyscale, between black and white, within the range of 0-100. The *a** and *b** values represent the chromaticity scalar coordinates, which in turn represent opponent red-green and blue-yellow scales.

Phenolic content

The content of total phenolic compounds (TPC) was estimated according to the Folin-Ciocalteu spectrophotometric method described by Haiyan et al. [24].

Oil was dissolved in hexane and extracted with methanol (three times). The sample was left to stand overnight. The methanolic extract was washed with hexane, and an aliquot (1 mL) was transferred to a volumetric flask (10 mL), to which the Folin–Ciocalteu reagent (0.5 mL) was added. The solution was shaken and left to stand for 3 min before the addition of a saturated solution of sodium carbonate and dilution with water. After 1h, the absorbance at 725nm was measured against a reagent blank on a UV/VIS spectrophotometer model T80+ (PG Instruments Limited, London). Calibration was performed using gallic acid ($y = 124.3x + 0.08$; $R^2 = 0.999$ in the range 0 to 100 µg/10 mL). The TPC was calculated according to the formula:

$$TPC\ (mg/kg) = c \times V/6 \times m$$

where:
c: the content of total phenolic compounds as GAE equivalent (µg/10mL)
V: the volume of the reaction mixture, 10 mL
m: the mass of the sample (g).
TPC was expressed as gallic acid equivalent (GAE) in mg/kg of oil [25].

Determination of single polyphenols

Phenols from the olive oil samples were extracted on the modified procedure by Gouvinhas *et al.* [26] and determined on Agilent Technologies 1200 HPLC-DAD coupled with Agilent Technologies 6410A ESI-QqQ-MS/MS. Approximately 800 µL of the sample was transferred to the 4 mL vial, the mass was accurately measured, and the sample was diluted with 400 µL of hexane. The mixture was extracted with 80% methanol (600 µL) with vigorous shaking on the vortex. After centrifugation (10 min at 2500 rpm), the aqueous methanol layer was transferred to a normal 2 mL vessel, and the oil layer was extracted twice more with 80% methanol. The combined extracts were supplemented with 80% methanol to 2 mL, filtered through a 0.45 µm regenerated cellulose membrane filter and analysed on LC-DAD-MS/MS.
The content of the selected secondary biomolecules was determined by the LC-DAD-MS/MS method, according to the customized procedure by Orčić et al. [27]. A 5µL sample was injected. The separation was performed by the Zorbax Eclipse XDB-C18 column (50 mm × 4.6 mm, 1.8 µm) thermostated at 50°C. The components were eluted with a mobile phase based on 0.05% aqueous solution of formic acid (A) and methanol (B) at a flow rate of 1.0 mL/min, in gradient mode: 0 min, 30% B, 6 min. 70% B, 9 min. 100% B, 12 min. 100% B, re-equilibrium time 3 min. For identification purposes of possible identity validation, the UV/VIS signal was observed in the range of 190-700nm. Effluents passed MS/MS detector without flow sharing. The ion source parameters were: the pressure of the nebuliser 50psi, temperature and flow of drying gas (N_2) 350°C and 10 L/min, the voltage on the capillary 4000V, negative polarity. The compounds were monitored in a dynamic SRM (selected reactions monitoring) mode, with the optimised parameters given in Table I.
By sequential dilution of 1:1, a series of commercial standard solutions were prepared in a concentration range of 1.53 ng/ml to 25.0 µg/ml. The concentration of the analyte was determined by the external standard method, using calibration curves in a narrower band-adjusted concentration in the sample. For the quantification, points were taken that directly surround the readings in the samples. In most cases, the dependency was not linear but square. For all the compounds, peak areas were determined using Agilent MassHunter Workstation Software – Qualitative Analysis (ver. B.03.01). Calibration curves were plotted and samples concentrations calculated using the OriginLabs Origin Pro (ver. 8.0) software.

Table I - Optimized SRM parameters adjusted on commercial standard

Compounds	*V* fragmentor (voltage fragmentor) [V]	*m/z* precursor	*V*col (collision energy)[V]	*m/z* product
Tyrosol	120	137	13	106
Hydroksytyrosol	130	153	11	123
Oleuropein	180	539	19	275
p-hydroksybenzoic acid	80	137	10	93
Protocatechic acid	105	153	9	109
p-coumaric acid	90	163	9	119
Vanillic acid	100	167	15	108
Caffeic acid	100	179	10	135
Quinic acid	150	191	20	85
Ferulic acid	90	193	11	134
Apigenin	130	269	25	117
Naringenin	130	271	16	151
Luteolin	135	285	25	133
Chrysoeriol	125	299	20	284

Chemicals

All used chemicals were of the finest analytical grade (Merck Corporation, Darmstadt, Germany).

Folin–Ciocalteu reagent and gallic acid were purchased from Institute Mol Ltd. Reference standards for the determination of phenols were obtained from Sigma-Aldrich.

2.4. STATISTICAL ANALYSIS

All determinations were carried out in triplicates, and values are expressed as a mean of three measurements ± standard deviation. Statistical analysis was performed using the software SPSS Statistica 20 (IBM Corporation, Armonk, New York, U.S). To test the mean differences, a variance analysis (one-way ANOVA) was performed, and then the Duncan test at the significance level $p < 0.05$. The degree of a linear relationship between two variables was measured using Pearson's correlation coefficient (r). The Principal Component Analysis (PCA) was used to discover the possible correlations among measured parameters, and to classify objects.

3. RESULTS AND DISCUSSION

3.1. CONTENT OF PIGMENTS, OIL TRANSPARENCY, AND UNSAPONIFIABLE MATTER

Pigments are responsible for the colour of oils and are important components directly related to the sensory and nutritive quality of VOO. The content of chlorophylls and carotenoids along with the oil transparency values, as well as unsaponifiable matter content, of investigated VOO produced in Libya from cultivar *"Roghiani"* are presented in Table II.

Based on results (Tab. II), it can be seen that the total chlorophylls content in analysed samples varied from only 1.63±0.39 mg/kg in oil from Q. B. Ghashir region to 38.1±0.55 mg/kg in oil from Tripoli region. The mean values of the chlorophylls content were different for most of the samples (except Gharyan and Tripoli region), statistically significant at $p<0.05$ level. On the other hand, the total carotenoid pigments content in oil samples was in a narrow range from 1.45±0.07 to 9.15±0.21 mg/kg (all samples were statistically different at $p < 0.05$ level). In terms of the pigments profile, it can be said that all investigated VOO samples had much more chlorophyll than carotenoids. If the content of the total pigment is considered, only two oil samples had values higher than 40 mg/kg (oil from Gharyan and Tripoli region, 46.85±6.16 and 41.80±0.28 mg/kg, respectively). The lowest amount of total pigments, only 3.08±0.24 mg/kg, was determined in oil from the Q. B. Ghashir region.

Data found in literature also considered the differences in the content of the pigment in extra virgin olive oils based on different growing regions and countries, which are influenced by many factors [8, 12, 28]. It was confirmed that the chlorophylls and carotenoids content of oil decrease with the increased maturity of the olive fruits [29]. By Giuffrida et al. [8] organic impurities, especially green leaves present in the bulk of olive fruits before pressing, contribute to the content of the increased pigment in the oil. Criado et al. [30] published values for the pigment content in 30 samples of extra virgin olive oils from Catalonia (Spain). They found the chlorophyll content of 2.28 to 4.73 mg/kg and carotenoids content of 3.89 to 5.15 mg/kg. Sinelli et al. [31] analysed 82 samples of Italian VOO manufactured from 3 different cultivars. The total carotenoid content ranged between 1.78 and 11.79 mg/kg for the *Casaliva* cultivar, 2.41-9.14 for the *Leccino* and 3.70-11.71 for the *Frantoio* cultivar. Respectively, the chlorophyll content of these cultivars was 1.98-13.97, 1.90-8.80, and 3.85-13.66 mg/kg. In olive oil from Tunisia, Borchani et al. [32] reported a very low level of chlorophylls, 1.88 mg/kg, while the carotenoids content was 19.10 mg/kg. Also, Arslan et al. [29] published results for the olive oil made in Turkey that had chlorophyll content from 7.33 to 8.83 mg/kg and carotenoids content of 7-14 mg/kg. Lazerrini and Domenic [9] established that the pigments' content in EVOO could be well distinguished depending on anomalous climate conditions. Total carotenoids in olive oil may range between 1 and 20 mg/kg, but usually, values do not exceed 10 mg/kg [19]. From this point of view, the values of carotenoid contents in oils from investigated production regions of Libya, are in accordance with the average values according to literature data. However, if one considers the influence of different factors on the pigment content of the oil, it can be said that their differences in the oil samples could be

Table II - Content of pigments, values of transparency and unsaponifiable matters of virgin olive oils

Oil from region	Chlorophylls (mg/kg)	Carotenoids (mg/kg)	Total pigments (mg/kg)	Transparency of oils (%)	Unsaponifiable matters (g/kg)
Gharyan	37.7±1.89^{d}	9.15±0.21^{e}	46.85±6.16^{d}	20.95±0.07^{a}	8.05±0.07^{d}
Tarhuna	18.2±0.15^{c}	3.35±0.07^{c}	21.55±0.11^{c}	28.00±0.14^{b}	7.25±0.03^{c}
Msallata	8.3±0.00^{b}	1.78±0.00^{b}	10.08±0.00^{b}	34.25±0.07^{d}	4.42±0.02^{a}
Tripoli	38.1±0.55^{d}	3.70±0.00^{d}	41.80±0.28^{d}	33.30±0.14^{c}	4.41±0.05^{a}
Q. B. Ghashir	1.63±0.39^{a}	1.45±0.07^{a}	3.08±0.24^{a}	47.30±0.14^{e}	5.79±0.03^{b}

*Different letters in columns indicate that there is significant difference at $p < 0.05$

the result of a possible effect of the harvest region. Perhaps, the geographical origin modified the maturity pattern due to the different climatic conditions and the maturity degree of the olives could be different.

The colour of VOO ranges from green to yellow due to the prevalence of chlorophyll or carotenoids, respectively. The green colour of early-harvested olive oil, which is particularly intense in oil from some cultivars, is very appealing to many consumers. Chlorophyll and carotenoids influence not only the colour of oils but also contribute to their oxidative stability and health effect. It is believed that a positive effect on health is achieved through the antioxidant and antimutagenic properties of chlorophylls [8]. Furthermore, carotenoids have an important biological function, too: they reduce cancer risk; are effective in the prevention of coronary and heart diseases; prevent the degenerative pathology of eyes; have provitamin action; participate in cell functions, immune system, and UV protection [5]. Due to the aforementioned health effects, the higher content of carotenoids is a desirable oil characteristic. A statistically insignificant linear correlation (r = 0.774) between the chlorophylls and carotenoids content was found in this study.

Various pigment contents affected the transparency of oil from the minimum of 20.95±0.07% found in the Gharyan VOO sample, to the 47.30±0.14%, found in lightest and most transparent oil from Q.B. Ghashir region. Dimic and Romanic [33] reported that VOOs originating from the Mediterranean region had a transparency of 2.1-7.7%. However, the same authors also published the value of 25.2% for extra virgin olive oil from Montenegro. A negative correlation (r = -0.770) between transparency and total pigment contents of investigated oils was determined but statistically insignificant.

The use of a very simple analytical method, such as the determination of oil transparency, has proven to be very useful in our previous investigations in determining the colour stability of virgin olive oil at moderate temperature testing. Namely, by reducing the carotenoids and chlorophylls content at 63±2°C over 28 days, the VOO transparency increased linearly from 2.55 to 3.55 times. The transparency of oils was inversely proportional to the content of pigments [34].

It is well known that a variety of unsaponifiable compounds, as well as their content in oil, are important for the oxidative stability and unique flavour and taste of virgin olive oil [14, 35, 36]. In our investigations, unsaponifiable matter in VOO were present in low concentrations, in all cases <10 g/kg (that is <1%). In this range, the highest content of unsaponifiable matter, 8.05±0.07 g/kg, was found in the oil from the Gharyan region, while in oils from Msallata and Tripoli regions the content was less than half and statistically different (at p <0.05), 4.42±0.02 and 4.41±0.05 g/kg, respectively.

Content of unsaponifiable matter in investigated VOO samples is, probably, the cultivar characteristic because two of the most important factors that influence olive oil quality are the type of cultivar and the olive ripening stage [37].

3.2. COLOUR MEASUREMENT USING CIE AND CIE *L*a*b** SYSTEM

Results for the colour measurements of VOO samples are shown in Table III.

The lightness (L^*) of oil samples, using the CIE *L*a*b** system, was fairly uniform. L^* values were in the range of 22.37±0.04%, in oil from Gharyan region to 23.81±0.21% from the Q. B. Ghashir region. The a^* value was between -1.20±0.005 (sample from Q. B. Ghashir region) to -0.25±0.08 (sample from Gharyan region), and most of the sample means were statistically different ($p < 0.05$). The b^* value was between 7.94±0.50 (Gharyan region) to 8.83±0.92 (Tripoli region), but these values were not statistically significant. All oil samples from Libya (except oil from Tarhana region) had a colour formed between the green and yellow hues, where the dominant wavelength was in the greenish-yellowish range, by the CIE *x, y* chromacity diagram. Comparing the obtained results amongst the samples, it can be said that oil from the Q.B. Ghashir region was the greenest in colour with the highest value of the green hue. This was not expected since this sample had the lowest content of the total pigments (Tab. II).

The value of the coordinates of CIE *L*a*b** of vegetable

Table III - Results of color measurements using CIE *L*a*b** and CIE system

Oil from region	CIE $L^*a^*b^*$ system			CIE system		
	L^* (%) lightness	a^*	b^*	Y (%) glossiness	λ[1] (nm)	Color purity (%)
Gharyan	22.37±0.04[a]	-0.25±0.08[c]	7.94±0.50[a]	3.60±0.01[a]	574.6±1.15[a]	23.33±0.2[a]
Tarhuna	23.06±0.30[b]	0.23±0.07[d]	8.08±0.81[a]	3.81±0.08[b]	588.0±0.00[b]	39.68±0.7[d]
Msallata	23.76±0.36[bc]	-0.91±0.04[b]	8.54±0.16[a]	4.02±0.11c	572.3±1.15[a]	23.73±0.2[b]
Tripoli	23.31±0.09[b]	-0.97±0.12[b]	8.83±0.92[a]	3.89±0.02[b]	571.6±0.57[a]	23.73±0.2[b]
Q. B. Ghashir	23.81±0.21[c]	-1.20±0.005[a]	8.39±0.38[a]	4.04±0.06[c]	572.3±0.57[a]	25.00±0.3[c]

*Different letters in columns indicate that there is significant difference at $p < 0.05$

[1]dominant wavelenght of color

oils such as palm, soy, sunflower, olive and corn oils were from 63.4 to 69.5% for *L**-lightness, from 3.8 to 4.4 for *a** value, and from 9.2 to 10.4 for *b** value [38]. Extra virgin olive oil from Tunisia had different values for the same parameters: *b** = 30; *a** = -1 and *L** = 25 [32]. Arslan et al. [29] have reported the colour values for oils from Turkey as: *L** = 66.49-79.94%; *b** = 16.69-54.30, while extra virgin oils from Catalonia (Spain) had the following values: 87-88.1 (*L**); -2.98 to -4.14 (*a**); 75.9-92.4 (*b**) [12]. Recently results published by Sevim et al. [39] indicated that the deficit irrigation treatments significantly affected cv. Memecik olive fruit and oil quality such as *L*, a*, b**, and reducing total phenol content ($P < 0.05$), among other chemical compositions.

Instrumental measurements of colour characteristics using the CIE system (Tab. III), have confirmed that the glossiness of oils, Y-value, was fairly uniform (with the evident statistically significant differences in certain samples means), about 3 to 4%, while the dominant wavelength was in the range of about 571 to 575 nm, and covers the greenish-yellowish colour range, except the oil from Tarhana (a statistically significant difference was observed for all other samples). VOO from Tarhana region had the dominant wavelength of 588 nm and yellowish-orange colour. Colour purity (saturation) had low values (20-25%), except in oil from the Tarhana region (39.68±0.7%), the statistically significant differences in means were observed.

The PCA of the presented data of virgin olive oil explained that the first two components accounted for 88.80% of the total variance (62.12 and 26.68%, respectively) in the twelve variables system (chemical analysis data and colour parameters). Considering the map of the PCA performed on the data, Y (which contributed 14.1% of total variance, based on correlations), *L** (14.1%) and transparency of oils (12.0%) exhibited the largest positive scores according to first principal component, while the most negative influence according to the first principal component was observed by carotenoids content (11.5%), unsaponifiable matter content (10.1%) and *a** colour coordinate (10.1%). The positive contribution to the second principal component calculation was observed for: chlorophylls (12.4% of the total variance, based on correlations) and total pigments (11.9%), while colour purity 25.5%) and λ (22.5%) expressed the most negative effect to the second principal component.

The points shown in the PCA graphics, which are geometrically close to each other indicate the similarity of patterns that represent these points. The orientation of the vector describing the variable in factor space indicates an increasing trend of these variables, and the length of the vector is proportional to the square of the correlation values between the fitting value for the variable and the variable itself. The angles between corresponding variables indicate the degree of their correlations (small angles corresponding to high correlations). Based on the correlation analysis, carotenoids content was negatively correlated to *L** and Y (statistically significant at $p < 0.05$ level), which could be seen from the Figure 2, where the vector of carotenoids content is oppositely directed to *L** and Y vectors. The total pigments and chlorophylls content were also negatively correlated to *L** and Y, but statistically insignificant. According to the results presented in Figure 2, more transparency of oils, Y, and *L** was observed in samples taken from Msallata and Q.B. Ghashir. Samples from Tarhuna are characterised by increased unsaponifiable matters, *a**, λ, and colour purity. The higher chlorophylls content, total pigments content, and carotenoids content were observed in samples taken from Gharyan. Sample of virgin oil is characterised by the augmented *b**. According to results of correlation analysis and PCA study, the carotenoids contents and the colour coordinates *L** and Y could be probably used in future as a potential variable in authentication investigation of the geographical origin of olive samples produced in Libya. These considerations also indicate the impact of harvest region on the pigment and unsaponifiable matter content, as well as colour characteristics of VOO. However, authentication of VOO from this point of view is a quite difficult issue that will need more studies along several harvest years. Our presented results are only preliminary results and further studies are needed.

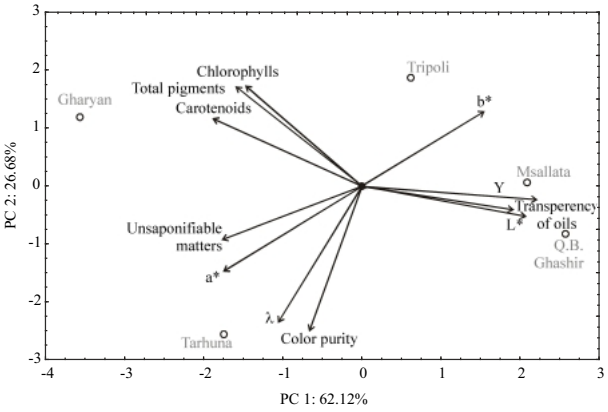

Figure 2 - PCA ordination of variables based on component correlations

3.3. PHENOLIC CONTENTS AND COMPOSITION

The content of phenolic compounds is very important in assessing the quality of olive oils. The results from our previous work on the total phenols content and their fractionation [40] have been included to support our findings. Namely, phenolic compounds affect not only the colour but also the sensory and nutritive quality, as well as the shelf life of the oil. Also, Becerra-Herrera et al. [38] as potential discriminating features among Spanish extra virgin olive oils with protected designation of origin, apply precisely characterisation

and evaluation of phenolic profiles and colour.

As can be seen in Table IV, the TPC in oil samples ranged from 56±11.49 to 238.3±16.26 mg GAE/kg. There is a significant difference between Gharyan VOO and VOO from other regions, while no significant difference ($p \leq 0.05$) was found between VOO from Tarhuna, Msallata, and Q. B. Ghashir regions.

Many research studies have been published in regard to the TPC. The most effective factors on the amount of phenolic compounds include variety, growing region, genetic base, crop year, agricultural techniques, the maturity of the fruit, processing (particularly milling and malaxation) and storage conditions [41]. Temime et al. [42] in Tunisian Chetoui VOO confirmed total phenol concentrations (as caffeic acid) from 250 to 600 mg/kg, but Borchani et al. [32] found much lower content, only 53.33±0.55 mg/kg, in extra virgin olive oil from Tunisia, too. On the other hand, some Sicilian VOOs showed total phenolic content of 180 mg/kg (as gallic acid) [43]. Quantitative determination of the total phenolic compounds in olive oil manufactured in Pakistan, performed according to the Folin-Ciocalteu colorimetric assay, was 157±10 mg/kg (as GAE), while oils manufactured from fruits of wild olives collected from three different locations in Pakistan had significantly lower contents of phenols, 30-52 mg/kg [44]. Najafi et al. [45] reported that phenolic content of virgin olive oils from *Leccino* and *Frantoio* cultivars grown in Qom (Iran) ranged from 321.14 mg/kg to about 250 mg/kg, respectively. In Spanish extra virgin olive oils with protected designation of origin Becerra-Herrera et al. [38] found TPC in a range from 62.81±6.844 to 180.9±5.948 mg/kg. On literature data, low phenolic content of common olive oil ranges from 10 to 70 mg/kg, while high phenolic content of VOO ranges from 150 to 400 mg/kg [14].

The fractionation of biophenols analysed by HPLC also presented in Table IV. The tyrosol and hydroxytyrosol (phenol alcohols) were the main phenolic compounds and ranged from 4.36±0.33 to 11.4±1.50 and 0.33±0.01 to 10.90±1.20 mg/kg, respectively. The statistical analysis showed significant differences ($p \leq 0.05$) of tyrosol and hydroxytyrosol content in Gharyan VOO compared to oils from all other regions.

Other phenolic acids were present in very low concentrations in investigated VOO samples, in all cases <0.1 mg/kg, except vanillic acid in Msallata VOO (0.18 ± 0.03 mg/kg) and quinic acid in Tarhuna VOO (0.13±0.02 mg/kg). The concentration of caffeic acid was <0.07 mg/kg in Gharyan, Tripoli, and Q. B. Ghashir VOO. Chrysoeriol level ranged from 0.07±0.01 to 0.15±0.01 mg/kg, whereby Q. B. Ghashir possessed the highest level, and the lowest level was in Gharyan VOO. Naringenin level ranged from 0.18±0.02 to 0.36±0.01 mg/kg. Apigenin level ranged from 0.34±0.02 to 0.70±0.02 mg/kg (some sample means were statistically different, at p <0.05 level). Q. B. Ghashir VOO was dominant, while the lowest level of apigenin was found in Tarhuna VOO. These results are consistent with reports by other authors for olive oils [46]. In their studies, Becerra-Herrera et al. [38] stated that phenolic profiles and colour could be used for the detection of fraud and control of the geographic authentication of extra virgin olive oils, as well as, the olive cultivars from different regions. However, it should be highlighted that in investigated VOO samples from cultivar *Roghiani* no secoiridoids derivatives have been detected.

The explanation of significant differences in the phenolic profile and their contents in our paper may be influenced by the type of cultivar *Roghiani* and the ripening stage of olive fruits in different regions. Probably, the climate and environmental factors were, in some degree, different in investigated harvesting regions. Based on literature data, limited water availability, as water deficit tends to generate a stress situation that induces the production of phenolics [47]. Furthermore,

Table IV - Total phenols content and their fractionation

Compounds	Gharyan	Tarhuna	Msallata	Tripoli	Q. B. Ghashir
Tyrosol	11.4 ± 1.50^{a}	4.83 ± 0.30^{b}	4.36 ± 0.33^{b}	5.76 ± 0.64^{b}	8.60 ± 0.71^{c}
Hydroxytyrosol	10.9 ± 1.20^{a}	0.36 ± 0.02^{b}	0.33 ± 0.01^{b}	1.08 ± 0.14^{c}	10.7 ± 0.50^{a}
p-coumaric acid	0.03 ± 0.01^{a}	0.21 ± 0.01^{b}	0.48 ± 0.02^{c}	0.28 ± 0.03^{b}	0.09 ± 0.00^{a}
Vanillic acid	<0.1	<0.1	0.18 ± 0.03	<0.1	<0.1
Caffeic acid	<0.07	0.07 ± 0.00	0.09 ± 0.02	<0.07	<0.07
Ferulic acid	<0.02	0.04 ± 0.01	0.09 ± 0.01	0.06 ± 0.00	<0.02
Luteolin	0.90 ± 0.11^{a}	0.91 ± 0.05^{a}	0.73 ± 0.03^{a}	0.86 ± 0.08^{a}	1.38 ± 0.07^{b}
p-hydroxybenzoic acid	<0.03	<0.03	0.08 ± 0.01	<0.03	<0.03
Protocatechic acid	<0.01	<0.01	0.04 ± 0.00	<0.01	<0.01
Quinic acid	<0.1	0.13 ± 0.02	<0.1	<0.1	<0.1
Naringenin	0.18 ± 0.02^{a}	0.21 ± 0.01^{a}	0.35 ± 0.01^{b}	0.25 ± 0.03^{c}	0.36 ± 0.01^{b}
Apigenin	0.40 ± 0.05^{ab}	0.34 ± 0.02^{a}	0.44 ± 0.03^{b}	0.48 ± 0.05^{b}	0.70 ± 0.02^{c}
Chrysoeriol	0.07 ± 0.01^{a}	0.08 ± 0.01^{ab}	0.08 ± 0.00^{a}	0.10 ± 0.01^{b}	0.15 ± 0.01^{c}
TPC§	238.3 ± 16.26^{a}	144.2 ± 19.69^{b}	139.4 ± 7.70^{b}	56.0 ± 11.49^{c}	136.8 ± 0.07^{b}

Values are means ± standard deviations (n = 3). Different letters in the same row indicate significantly different values ($p \leq 0.05$)
§ - Total phenolic content (mgGAE/kg)

since olive fruit samples were hand-picked and collected at the same time, and the other conditions of oil extraction were the same, differences in phenol contents and profile indicate a fruit ripeness effect. Vekiari et al. [47] observed significant differences in the total polyphenol content and the contents of tyrosol, hydroxytyrosol, 3,4-DHPEA-EDA, p-HPEA-EDA, and 3,4-DHPEA-EA in olive oils produced from two different varieties cultivated in Southern Greece during different stages of maturation. Throughout the ripening of the fruit or during processing, several metabolic processes take places, such as chemical and enzymatic reactions resulting in the formation of free phenols and the subsequent variations in the profiles of several compounds [48].

4. CONCLUSIONS

The virgin olive oils obtained from the 'Roghiani' cultivar from different harvest regions of northern Libya show a variation in their pigment and unsaponifiable contents, colour characteristics, and phenolic compounds. The content of total chlorophylls, in a range of 1.63±0.39 to 38.1±0.55 mg/kg and total carotenoids, 1.45±0.07 to 9.15±0.21 mg/kg are within the data published in literature. The total phenolics content in oils had a wide range, from 30.7±0.28 to 266.5±31.53 mg/kg. Among biophenolic fraction, tyrosol and hydroxytyrosol were the main phenolic compounds and ranged from 4.36±0.33 to 11.4±1.50 and 0.33±0.01 to 10.90±1.20 mg/kg, respectively.
Obtained results indicated that the oil from Gharyan production region was quite different from the other Libyans' VOOs. Namely, this oil not only had the highest content of carotenoids, 9.15±0.21 mg/kg, but also a maximum content of TPC, 266.5±31.53 mgGAE/kg, and high level of total chlorophylls, 37.7±1.89 mg/kg. Based on these characteristics, it can be concluded that this oil had the best nutritive value.
However, it should be noted that current knowledge of the nutritive bio components composition and colour quality of the Libyan *Roghiani* cultivar virgin olive oil is still incomplete and no consistent database compiling its properties is available, so further studies would be necessary.

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