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Autochthonous and International Olive Cultivars in Malta: Morphometric characteristics and Physicochemical Analysis

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Abstract - *The olive has been cultivated since antiquity for its use as a table olive or for the production of olive oil. This study aims at obtaining chemical characterization of olive leaves and from locally grown Maltese and International varieties. Thirteen olive varieties of both oil and dual purpose were selected, two of which were of Maltese origin, known as Tal-Bidni and Tal-Malti. The maturity index at harvest and olive oil yield were recorded. Chemical analysis was carried out on all samples for polyphenols, chlorophyll content, anthocyanins, tint, colour intensity, and tonality using spectrophotometry. The findings from the olive leaf and fruit analysis indicated that the polyphenolic content had a similar trend in both fruit and leaf, and anthocyanin and chlorophyll content had different trends. PCA conducted on the olive leaf and fruit samples revealed that tint and tonality showed an inverse correlation with anthocyanin and polyphenolic content, and that chlorophyll content of leaves showed an inverse relationship with that of the fruit. Agglomerative hierarchical clustering (AHC) analysis revealed that the local variety Tal-Bidni is closely related to Bosana, Pendolino and Leccino, whilst the other local variety Tal-Malti is closely related to Frantoio and Ottobratica.*

Keywords: *Olives, Tal-Malti, Bidni, Leaves, Fruits, Polyphenols.*

I. INTRODUCTION

The Maltese Islands form an archipelago composed of a group of islands with a total area of 316 km^2 at the centre of the Mediterranean Sea. The larger two islands, Malta and Gozo, are inhabited by a total population of 493,559 [1], with a population density of 1,282 inhabitants per km^2. The islands were inhabited by settlers around 5900 BC [2]. Ever since, several rulers have set foot on the islands, bringing with them culture, traditions and new plants and trees. Apparently, the olive tree was introduced in Malta by the first Phoenician settlers [3]. However, carbonized remains found at Skorba prehistoric temple, the olive tree was found amongst other tree species [4], suggesting the introduction ot this tree earlier. The extension of olive cultivation along with olive oil production went on through Roman domination. This is evidenced by the presence of olive trapetums, at San Pawl Milqi, in the area of Burmarrad, showing extensive olive oil activity.

According to the Regional Statistics of 2019 published by the National Statistics Office, in 2016 [5] out of the total land under permanent crops of 1311 hectares in Malta, 12.4 per cent were dedicated to olive cultivation. With a total of 163 hectares under cultivation, these are distributed as 136 hectares (12.7%) in Malta and 27 hectares (11.2%) in Gozo and Comino. The land under olive cultivation is relatively fragmented with 93% of the parcels being less than one hectare.

Recently, the olive oil industry was revived, leading to the importation of a significant number of olive varieties, mainly from Spain and Italy. Trees are mainly cultivated here for olive oil production, even though some table varieties are also grown. In Malta Frantoio, Leccino, Carolea, Pendolino, Picholine, Coratina, Ogliarola, and Cipressino are cultivated for olive oil whereas Uova di Piccione and Bella di Spagna are cultivated as table olives. Despite the wide distribution of imported cultivars, there are still indigenous varieties that are being cultivated and their revival is being promoted.

This study is aimed at the determination of morphometric and physicochemical characterization of leaves and fruit from locally-grown Maltese and International olive varieties.

II. MATERIALS AND METHODS

All samples used in this study were obtained from the olive trees being cultivated under full organic certification at the Government Experimental Farm of Għammieri, within the limits of Marsa. The experimental olive grove consists of 205 trees and 20 varieties of both olive and oil types, that were planted in 2003. Thirteen samples of olive fruit and leaves were collected from each studied variety during the time of harvest. Both leaf and olive samples were labelled. The varieties included Bosana, Carolea, Cerasuola, Coratina, Frantoio, Leccino, Nocellara Messinese,

Ogliarola, Ottobratica, Pendolino, Picholine, Tal-Bidni and Tal-Malti, the last two being indigenous to the Maltese Islands.

A. Morphometric analysis

Morphometric analysis of fruit was carried out as follows. The length, diameter, length:diameter ratio and weight were measured for fruit and their respective stones. The fruit measurements were taken whilst intact, while the stone measurements were taken following crushing.

B. Sample preparation

Each fruit sample was crushed, separating the stone from the flesh. Fruit flesh or leaves were vortexed with 15ml of a methanol and water (80:20) solution and placed under ultrasonication for 15 minutes. 15ml hexane were then added and samples were vortexed again. The mixture resulted in two separate layers consisting of the polar and non-polar extract. The hexane layer was separated for the analysis of chlorophyll content.

C. Physicochemical characterisation

UV-Vis analysis was carried out on both leaves and olives. For chlorophyll content, 50 µl of hexane samples, were mixed with 950 µl of 70% acetone in reduced-volume quartz cuvettes and read on a UV-Vis spectrophotometer at 646 and 663 nm. For other physicochemical parameters, 10µl of hexane samples, were mixed with 990µl of 80% methanol in reduced-volume quartz cuvettes and read on a UV-Vis spectrophotometer (LIGHTWAVE II, WPA) at 280nm, 420nm, 520nm and 620nm [6].

D. Polyphenolic content

The methanol/water layer was kept for the analysis of the polyphenolic content using the Folin-Ciocalteu microplate method [7]. Briefly, standards of five different concentrations of tannic acid (Sigma-Aldrich, TA) were prepared in concentration of 60, 120, 240, 480 and 960 µg/ml. 5 µl sample (in triplicates) or TA standard solution and 5 µl distilled water were transferred to each well. Then 100 µl Folin-Ciocalteu reagent (Sigma-Aldrich, 10%) and 80 µl sodium carbonate (Sigma-Aldrich, 1M) were added successively. The plates were incubated at room temperature in the dark for 20 minutes. The absorbance was read at 630 nm on a microtiter plate reader (BioTek ELx800, Winooski, VT, USA).

E. Statistical Analysis

All determinations were carried out in triplicates. All parameters were analyzed by one-way ANOVA with the Bonferroni post hoc test to compare the means of data sets using Prism 5 version 5.01 (GraphPad Software, Inc.). The differences between the means of different samples were also compared in this way to pair the data sets together. Principal Component Analysis with the correlation matrix (Pearson) was carried out to determine any discrimination between varieties vis-à-vis morphometric and physicochemical parameters, using XLSTAT version 2014.4.04 (Addinsoft). Statistical significance was considered at $p<0.05$.

III. RESULTS AND DISCUSSION

Several studies were conducted by research groups taking into account either morphometric [8-13] or physicochemical [14-22] parameters. Few research groups considered both types within the same study [23, 24]. In this current study, morphometric and physicochemical analyses were conducted on thirteen olive varieties grown locally at the Government Experimental Farm in the Southern part of the main island. This study was aimed at providing information on two autochthonous varieties in relation to international varieties.

A. Morphometric analysis

The morphometric parameters investigated in this study include fruit parameters (weight, length, diameter and length to diameter ratio), flesh parameters (weight and thickness) and stone parameters (weight, length, diameter and flesh to stone ratio).

The thirteen olive varieties exhibited a wide range of fruit weights, between 0.62 and 6.30 g (Table 1). The Tal-Bidni variety had an average fruit weight of 0.79±0.04 g while the Tal-Malti had a weight of 2.40±0.14 g with a significant difference between them ($p<0.001$). The Tal-Bidni variety stands within the oil producing olives, whereas the Tal-Malti variety is at the borderline between oil-producing varieties and varieties suitable for oil production and as table olives. The Bosana variety fruit weight from this study was higher than that reported by Piga and co-workers [23] (3.20±0.17 g and 2.73 g, respectively). In two previous studies by Inglese and co-workers [8] and by Tous and co-workers [10], the Carolea fruit weight was reported to be 4.3±0.5 g and 4.4 g respectively, whereas in this present study this was 4.90±0.34 g, which goes in accordance with the weight of Carolea fruit reported by Farinelli and co-workers [9] (4.93 g). The latter study also reported that the fruit weights for the Frantoio and Leccino varieties were 2.32 g and 2.69 g. Tous and co-workers [10] also reported a fruit weight of 2.5 g for Leccino. Whereas the Frantoio fruit weight is in accordance with that of the present study (2.3±0.09 g), that of Leccino was different (1.70±0.12 g for this present study). With regards to the Picholine variety the fruit weight in this present study (3.20±0.21 g) was similar to that reported by Tous and co-workers [10] (3.8 g). According to Preziosi and Tini [25], the Coratina fruit weight was 2.4 g slightly higher than that obtained in the present study (2.10±0.12 g). Gucci and co-workers [11] compared fruit weights under irrigation and rain-fed systems for Leccino and Frantoio, which were 2.14 and 1.79 g for Leccino and 2.54 and 2.31 g for Frantoio. In this present study, the values for Leccino and Frantoio (1.70±0.12 and 2.30±0.09 g, respectively) were

similar to the rain-fed values. In fact, the olive trees are rain-fed and not irrigated. Results for this present study also go in accordance with the study by Giuffrè [13] who investigated a number of varieties amongst which Coratina, Frantoio, Leccino, Noccellara Messinese, Ottobratica, Pendolino and Picholine.

Table 1: Fruit Morphometric parameters

	Weight (g)	Length (mm)	Diameter (mm)	Length to Diameter
Bosana	3.20±0.17	22.0±0.3	17.0±0.23	1.30±0.025
Carolea	4.90±0.34	25.0±0.54	19.0±0.41	1.40±0.017
Cerasuola	2.60±0.12	20.0±0.3	16.0±0.38	1.30±0.023
Coratina	2.10±0.12	21.0±0.39	14.0±0.21	1.50±0.026
Frantoio	2.30±0.09	21.0±0.36	14.0±0.24	1.50±0.017
Leccino	1.70±0.12	22.0±0.26	14.0±0.26	1.60±0.027
Noccellara Messinese	6.30±0.32	27.0±0.36	20.0±0.27	1.40±0.017
Ogliarola	1.40±0.28	18.0±0.33	11.0±0.27	1.70±0.029
Ottobratica	0.62±0.04	15.0±0.49	9.4±0.29	1.50±0.04
Pendolino	1.10±0.04	17.0±0.3	10.0±0.13	1.70±0.029
Picholine	3.20±0.21	25.0±0.54	16.0±0.34	1.50±0.032
Tal-Bidni	0.79±0.04	22.0±3.1	9.9±0.2	2.20±0.27
Tal-Malti	2.40±0.14	20.0±0.39	16.0±0.25	1.30±0.015

*Twenty fruits for each sample were represented as mean ± standard error of the mean

The fruit length for the studied varieties ranged between 15.0 and 27.0 mm (Table 1). The Tal-Malti variety had an average fruit length of 20.0±0.39 mm and the Tal-Bidni with a fruit length of 22.0±3.1 mm, showing no statistically significant difference. The values obtained by Giuffrè [13] for Coratina, Frantoio, Leccino, Noccellara Messinese, Ottobratica, Pendolino and Picholine were similar to those of this present study. Though the fruit lengths for Carolea, Coratina, Frantoio, Leccino, Nocellara Messinese, Ottobratica in this present study were generally smaller than those of Magotra [12] (20.66 – 31.34 mm), there was a similar trend for both studies.

The fruit diameter varied between 9.4 and 20.0 mm for all the varieties (Table 1). The Tal-Bidni variety had a small diameter (9.9±0.2 mm) and the Tal-Malti had a moderate diameter (16.0±0.25 mm) ($p<0.001$). The values obtained by Giuffrè [13] for the diameter of Coratina, Frantoio, Leccino, Noccellara Messinese, Ottobratica, Pendolino and Picholine varieties were similar to those of this present study. In the study by Magotra [12], fruit diameters for Carolea, Coratina, Frantoio, Leccino, Nocellara Messinese, Ottobratica were similar to those in this present study with the exception of the Ottobratica fruit diameter which were 14.25 and 9.4±0.29 mm, respectively.

Fruit length to diameter ratio represents the shape of the olive fruit. A spherical fruit would exhibit a ratio of 1, whereas a ratio greater than 1 would result in an oval-shaped fruit. All thirteen varieties had an oval shape with ratios ranging between 1.30 and 2.20 (Table 1). Tal-Bidni had the largest ratio (2.20±0.27) as compared to all varieties including Tal-Malti (1.30±0.015) which was at the other end of the range ($p<0.001$). For Carolea, Leccino and Picholine, Tous and co-workers [10] reported ratio values of 1.31, 1.33 and 1.51, which in part concur with those of the present study for these three varieties (1.40±0.017, 1.60±0.027 and 1.50±0.032, respectively). The fruit length to diameter ratios for Coratina, Frantoio, Leccino, Noccellara Messinese, Ottobratica, Pendolino and Picholine obtained by Giuffrè [13] were similar to those of this present study. In another study by Magotra [12], fruit length to diameter ratios for the six common species (1.42-1.58) were similar to those in this present study (1.40-1.60).

The phenotypic characterisation of olives, as with many other fruit types, is also based on flesh and stone morphometric characteristics. The internal characteristics of the olive fruit may differ from the external characteristics.

The flesh thickness of the thirteen varieties varied between 1.8 and 5.7 mm. The Tal-Bidni ranked at the lower end (2.2±0.11 mm) with Pendolino, Ottobratica and Ogliarola, while the Tal-Malti was grouped with the higher end (4.3±0.12 mm) together with Picholine and Bosana ($p<0.001$). Although the values obtained by Giuffrè [13] for the flesh thickness of Coratina, Frantoio, Leccino, Noccellara Messinese, Ottobratica, Pendolino and Picholine varieties were similar in trend to those of this present study, there were some varietal differences. The value obtained in this present study were higher than those obtained by Giuffrè [13], with apparent significant differences for Coratina, Frantoio, Leccino and Ottobratica.

Flesh weight ranged between 0.42 and 5.6 g. Tal-Bidni variety had a similar weight to Ottobratica and Pendolino (0.54±0.044 g) and Tal-Malti was closely related to that of Frantoio and Cerasuola (1.9±0.13 g). The flesh weights of the two autochthonous varieties were significantly different from each other ($p<0.001$). In the study by Magotra [12], flesh weights varied significantly for Carolea, Coratina, Frantoio, Leccino, Nocellara Messinese, Ottobratica (2.77 – 7.15 g) with those in this present study (0.42 – 5.6 g).

Stone weight varied significantly between varieties ($p<0.001$ for Ottobratica vs Noccellera Messinese) from 0.2 to 0.72 g (Table 2). The stone weight of Tal-Malti was approximately twice that of Tal-Bidni (0.48±0.018 g and 0.25±0.0063 g, respectively, $p<0.001$). The Tal-Bidni variety had a weight similar to Ottobratica, Ogliarola and Pendolino, whereas the stone weight of Tal-Malti variety was similar to that of Frantoio, Leccino and Coratina. The stone weight values obtained by Giuffrè [13] for Coratina,

Frantoio, Leccino, Noccellara Messinese, Ottobratica, Pendolino and Picholine varieties were similar to those in this present study. The stone weights for Coratina, Frantoio, Leccino and Nocellara Messinese, in the study by Magotra [12], were very similar. Those for Carolea and Ottobratica were lower for this present study as compared to the study by Magotra [12] (0.54±0.02 and 0.75 g, and 0.2±0.012 and 0.48 g, respectively).

Table 2: Fruit Stone Morphometric parameters

	Weight (g)	Length (mm)	Diameter (mm)
Bosana	0.39±0.016	14.0±0.28	7.20±0.12
Carolea	0.54±0.02	16.0±0.35	7.90±0.16
Cerasuola	0.42±0.028	14.0±0.28	8.30±0.16
Coratina	0.51±0.017	16.0±0.29	7.70±0.093
Frantoio	0.45±0.024	15.0±0.26	7.50±0.075
Leccino	0.46±0.022	15.0±0.34	7.10±0.1
Noccellara Messinese	0.72±0.039	17.0±0.24	8.30±0.097
Ogliarola	0.22±0.01	12.0±0.22	6.50±0.48
Ottobratica	0.2±0.012	12.0±0.44	5.80±0.11
Pendolino	0.24±0.013	13.0±0.26	6.50±0.1
Picholine	0.42±0.021	18.0±0.35	7.10±0.15
Tal-Bidni	0.25±0.0063	14.0±0.17	5.40±0.072
Tal-Malti	0.48±0.018	14.0±0.34	7.20±0.075

*Twenty fruits for each sample were represented as mean ± standard error of the mean

The overall difference in stone length was that of 6 mm, i.e. from 12.0 to 18.0 mm (Table 2). The stone length for both Tal-Bidni and Tal-Malti were similar in size (14.0±0.17 and 14.0±0.34 mm, respectively) and hence not significantly different from each other. These are also mid-range between Ogliarola and Picholine. In general, stone lengths in this present study were smaller (12.0 – 17.0 mm) than those in the study by Magotra [12] (15.55 – 19.28 mm) for Carolea, Coratina, Frantoio, Leccino, Nocellara Messinese and Ottobratica.

The stone diameter ranged between 5.4 and 8.3 mm with Tal-Bidni variety on the lower end of the range (Table 2). The Tal-Malti variety had a stone diameter of 7.20±0.075 mm, which is relatively on the upper side of the range. In fact, these two varieties had a significantly different diameter ($p<0.001$). In general, stone diameters in this present study were smaller (5.8 – 8.3 mm) than those in the study by Magotra [12] (6.44 – 11.15 mm) for Carolea, Coratina, Frantoio, Leccino, Nocellara Messinese and Ottobratica.

The flesh to stone ratios for the varieties varied between 2.2 and 8.5. Tal-Bidni ranked at the lower end (2.2±0.19) while Tal-Malti exhibited a value of 4±0.22, which is midway within the range of values but not significantly different from each other. For Carolea, Leccino and Picholine, Tous and co-workers [10] reported ratio values of 5.3, 3.3 and 6.5, which in part concur with those of the present study for these three varieties (8.5±0.75, 3.0±0.39 and 7.0±0.67, respectively). The flesh to stone ratios obtained by Giuffrè [13] for Coratina, Frantoio, Leccino, Noccellara Messinese, Ottobratica, Pendolino and Picholine varieties were similar in trend to those of this present study. However, there are some varietal differences. The values obtained for this present study were higher than those obtained by Giuffrè [13], almost doubled for Frantoio, Nocellara Messinese and Picholine. Flesh to Stone ratios in this present study (2.4 – 8.5) varied than those in the study by Magotra [12] (5.81 – 8.29) for Carolea, Coratina, Frantoio, Leccino, Nocellara Messinese and Ottobratica.

In table 3, weight, length, diameter and length:diameter ratio showed a positive correlation for the fruit and stones as shown in table 3 ($r=0.861$, 0.882, 0.851 and 0.661, respectively). This shows that morphometrically, the fruit and the stone inside, exhibit uniform dimensions, regardless the variety of the olive.

Varietal grouping was based on agglomerative hierarchical clustering (AHC). This was preferred over principal component analysis, as results from several studies related to phenotypic and genotypic characterisation of olives are presented in the AHC format.

Table 3: Pearson Correlations between fruit and stone parameters

Variables*	r values
Fr Wt vs St Wt	0.861
Fr Lt vs St Lt	0.882
Fr Wd vs St Wd	0.851
Fr Lt:Wt vs St Lt:Wd	0.661

*Fr denotes fruit; St denotes stone

Figure 1 shows the dendrogram obtained for the morphometric characteristics in this present study. The thirteen varieties were grouped into three classes at a node index of 0.9691. Tal-Malti was grouped with Bosana, Carolea, Cerasuola and Noccellara Messinese whereas Tal-Bidni was placed in a class of its own. The other varieties, Coratina, Frantoio, Leccino, Ogliarola, Ottobratica, Pendolino and Picholine, were all grouped within the same class. In their study, Muzzalupo and co-workers [26] concluded that genetically Coratina and Carolea were grouped in the same class, which was different from that of

Frantoio. This present study shows that Coratina and Frantoio are in a different class to that of Carolea. The AHC analysis conducted by Giuffrè [13] on morphometric characteristics, showed a relatively different clustering in spite that most characteristics were similar in both studies except for the fruit length to diameter ratio (r=0.144). As indicated in the study by Caruso and co-workers [27], genetically Cerasuola different from Nocellara Messinese and Ogliarola, which is in accordance with this present study showing morphometric differences between the three varieties at the node index of 0.9891. In this present study, Carolea and Leccino originate from different lineages, which goes in accordance with that by Zaher and coworkers [28].

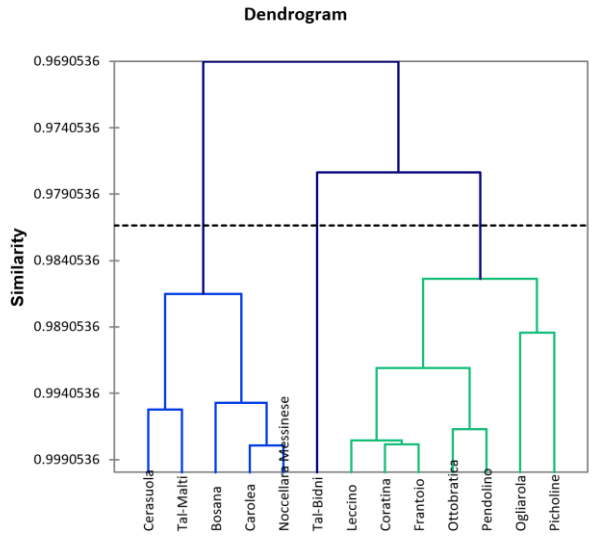

Fig 1. The dendrogram for the morphometric characteristics of the thirteen varieties.

B. Physicochemical characterisation

The analysis of physicochemical factors for the thirteen varieties was conducted on leaves and fruit. Several studies investigated the determination of metabolite content in the olive oil rather than the fruit. However, in this present discussion, results of previous research related to both fruit and olive oil will be mentioned. Some studies relate the transfer of chlorophyll and other pigments from fruit to oil stating that chlorophylls, xanthophylls and carotenoids are all transferred from fruit to olive oil [29] with some losses during the extraction process [30].

Colour intensity (CI) is determined by the content and structure of the anthocyanins present in a matrix and is defined as the sum of the absorbances at 420, 520 and 620 nm [31, 32]. The range for the colour intensity for the leaves of the thirteen varieties varied between 6.00 and 13.70 AU. The two Maltese autochthonous varieties Tal-Bidni and Tal-Malti, exhibited a colour intensity of 7.7±0.802 and 6.73±0.895 AU, showing no statistical difference. The fruit exhibited weaker colour intensities than the leaves, ranging between 0.059 AU in Carolea and 0.665 AU in Bosana. Although the Maltese varieties exhibited different values, i.e 0.529±0.05 and 0.129±0.03 (for Tal-Bidni and Tal-Malti, respectively), the difference was not considered statistically significant. Pearson correlation between the leaf and fruit colour intensity, indicated a weak positive correlation between the two (r=0.576) irrespective of the olive.

Tonality (T) represents the ratio of absorbances at 420 and 520 nm, and referred to the hue of a matrix [33]. The tonality ratio for the leaves ranged between 3.52 and 16.32. Tal-Malti and Tal-Bidni varieties had values of 4.3±1.01 and 6.17±1.87 showing no statistical difference. Fruit tonality ratios varied between 1.34 and 6.71. None of the varieties exhibited a significantly different tonality ratio. The values for Tal-Malti and Tal-Bidni were 3.29±1.26 and 1.48±0.04, respectively. There was no correlation between the leaf and fruit tonality ratio (r=-0.050).

The anthocyanins (Anth) are the red coloured flavonoids present in several matrices of vegetal origin, which progress with fruit ripening, for example in olives [34]. The anthocyanin content in the leaves of all varieties ranged between 140 and 676 mg/kg. Within this range, the two Maltese varieties, Tal-Bidni and Tal-Malti, contained 316±104 and 350±98.5 mg of anthocyanins per kg of leaves, being not statistically different from each other. The anthocyanin content of the fruit ranged between 1.22 and 9.83 mg/kg. For this parameter, the Maltese indigenous varieties exhibited a significant difference ($p<0.001$) with values of 9.83±0.929 and 1.49±0.502 mg/kg, for Tal-Bidni and Tal-Malti, respectively. In fact, Tal-Bidni had the highest anthocyanin content amongst the thirteen varieties. There was no correlation between the leaf and fruit anthocyanin content (r=0.280).

The polyphenolic (PP) content indicates the presence of flavonoid and non-flavonoid metabolites with potential antioxidant activities [35]. The polyphenolic content in leaves ranged between 0.606 and 7.62 mg/g. Tal-Bidni and Tal-Malti contained 4.3±0.101 and 0.606±0.002 mg/g of polyphenols ($p<0.001$). In fruit, the polyphenolic content varied between 0.486 and 5.87 mg/g. The Maltese varieties exhibited statistically different values ($p<0.001$), with Tal-Bidni and Tal-Malti having 3.24±0.1 and 0.647±0.09 mg/g, respectively. There was a strong correlation between the polyphenolic content of the leaves and fruit for the olive varieties (r=0.958).

Table 4: Physicochemical Parameters exhibited by the leaves of the varieties

	CI (Abs)	T	Anth (mg/kg)	PP (mg/g)	ChA (%)	ChB (%)	T-Ch (%)
Bosana	13.7± 1.1	9.71± 2.07	388± 119	5.95± 0.136	54.3± 3.77	18.7± 2.92	73.0± 6.4
Carolea	6.78± 0.203	7.98± 0.96	205± 26.5	1.30ii ±0.03	79.2± 3.00	30.0± 2.89	109.0 ±5.8
Cerasuola	6.53± 0.393	16.3± 7.85	146± 59.5	1.56± 0.04	77.9± 2.79	35.9± 2.15	114.0 ±4.0
Coratina	6.13± 1.39	15.2± 9.49	229± 132	1.29± 0.02	55.9± 2.92	21.2± 1.49	77.1± 4.3
Frantoio	12.1± 2.07	3.52± 0.47	676± 172	7.38± 0.185	62.6± 6.06	26.5± 6.31	89.0± 12.3
Leccino	7.39± 1.18	7.94± 1.65	247± 87.2	0.794 ±0.02	76.3± 3.68	30.2± 0.79	106.0 ±3.30
Nocellara Messinese	6.00± 0.35	11.2± 2.2	140.0 ±21.1	0.204 ±0.01	55.7± 2.04	23.2± 3.17	78.8± 5.2
Ogliarola	6.39± 0.75	6.50± 1.89	263± 67.9	0.432 ±0.01	53.9± 2.17	28.4± 4.43	82.2± 6.6
Ottobratica	8.62± 1.99	5.66± 2.43	467± 183	3.59± 0.05	44.7± 2.2	18±2.85	62.7± 5.0
Pendolino	7.27± 1.28	7.91± 3.21	279± 98.2	7.62± 0.20	72.8± 0.658	26±1.57	98.7± 2.2
Picholine	6.71± 0.757	10.3± 4.26	199± 60.9	0.885 ±0.01	60.5± 0.687	25.9± 2.95	86.3± 3.4
Tal-Bidni	7.70± 0.80	6.17± 1.87	316± 104	4.30± 0.10	72.6± 3.84	23.5± 1.34	96.1± 5.2
Tal-Malti	6.73± 0.895	4.30± 1.01	350± 98.5	0.61± 0.002	20.2± 1.25	9.72± 0.554	29.9± 1.8

Table 5: Physicochemical Parameters exhibited by the fruit of the varieties

	CI (Abs)	T	Anth (mg/kg)	PP (mg/g)	ChA (%)	ChB (%)	T-Ch (%)
Bosana	0.665 ±0.05	1.34± 0.01	9.14± 0.64	3.62± 0.07	1.07± 0.12	1.48± 0.24	2.57± 0.33
Carolea	0.059 ±0.02	4.60± 1.52	1.79± 1.09	1.43± 0.196	8.63± 2.48	8.01± 4.14	16.6± 6.6
Cerasuola	0.169 ±0.0152	3.67± 0.591	1.38± 0.27	1.11± 0.135	3.8±0.115	2.93± 0.134	6.73± 0.233
Coratina	0.161 ±0.03	2.97± 0.21	1.78± 0.34	1.24± 0.101	19.8± 0.24	7.29± 0.37	27.1± 0.58
Frantoio	0.353 ±0.02	2.90± 0.133	3.35± 0.132	5.87± 0.584	3.03± 1.29	2.95± 2.11	5.97± 3.39
Leccino	0.193 ±0.04	2.53± 0.09	1.97± 0.472	0.564 ±0.06	6.07± 0.437	4.49± 0.664	10.5± 1.07
Nocellara Messinese	0.126 ±0.03	2.68± 0.332	1.22± 0.402	0.31± 0.009	0.767 ±0.15	0.737 ±0.23	1.47± 0.376
Ogliarola	0.56± 0.386	6.71± 3.12	2.27± 0.878	0.486 ±0.05	3.1±0.833	2.67± 1.39	5.77± 2.22
Ottobratica	0.161 ±0.02	3.46± 0.279	1.66± 0.275	4.23± 0.62	13.8± 1.79	9.54± 2.99	23.3± 4.76
Pendolino	0.355 ±0.01	1.97± 0.06	4.42± 0.177	4.90± 0.386	9.27± 0.088	3.68± 0.187	12.9± 0.273
Picholine	0.15± 0.033	3.43± 0.685	1.24± 0.412	0.811 ±0.04	3.47± 0.186	1.79± 0.296	5.20± 0.458
Tal-Bidni	0.529 ±0.05	1.48± 0.04	9.83± 0.929	3.24± 0.099	0.967 ±0.41	1.45± 0.665	2.43± 1.05
Tal-Malti	0.129 ±0.03	3.29± 1.26	1.49± 0.502	0.647 ±0.09	9.57± 0.348	4.47± 0.69	14.0± 1.07

Table 6: Pearson Correlations between leaf and fruit physicochemical parameters

Parameters*	r value
CI_L vs CI_F	0.576
T_L vs T_F	-0.050
$Anth_L$ vs $Anth_F$	0.280
PP_L vs PP_F	0.958
$T\text{-}Ch_L$ vs $T\text{-}Ch_F$	-0.224
ChA_L vs ChA_F	-0.243
ChB_L vs ChB_F	-0.141

*F denotes fruit; L denotes leaves

Comparing the fruit polyphenolic content from this study to that of respective varietal oils from the study by Rotondi and co-workers [24], both study are in agreement that the Leccino variety exhibited the lowest polyphenolic content (0.379 and 0.564±0.064 mg/g), whereas the two studies report that Frantoio fruit (5.87±0.584 mg/g) and the Coratina olive oil (0.542 mg/g) as the highest yield of polyphenols when comparing varieties. Moreover, Aguilera and co-workers [16] also reported higher phenolic content in Frantoio oils (0.635-0.426 mg/g) as compared to Leccino oils (0.472-0.718 mg/g). Sivakumar and co-workers [17] reported that with HPLC-MS, Coratina fruit exhibited higher phenolics than Carolea. This was not the case with this present study (1.24±0.101 and 1.43±0.196 mg/g for Coratina and Carolea fruit, respectively). Piscopo and co-workers [22] reported polyphenolic values of 0.317 and 0.286 mg/g for Carolea and Ottobratica oils which was not the case in this present study. According to Favati and co-workers [21], the polyphenolic content of Coratina, Ogliarola and Leccino oils were 0.0456, 0.0382 and 0.0352 % w/v. In this present study, the polyphenolic content in the fruit of the three varieties showed a similar trend (0.124, 0.0486 and 0.0564 % w/w, respectively).

Chlorophyll, and other related pigments, impart colour to olives and olive oil. Chlorophyll determination has been used to determine the authenticity of olive oil from specific olive varieties [36]. Int this present study, the chlorophyll content in leaves ranged between 29.9 and 114.0 % (w/w). According to Aparicio-Ruiz and co-workers [18], the

chlorophyll a/b ratios for Frantoio and Coratina were 3.81 and 3.92 whereas for the present study for these same varieties, the values were 1.03 and 2.72, respectively. An increase in the chlorophyll a to b ratio is expected when the tree is during its productive phase with less demands on nitrogen under high lighting conditions [37]. In another study [20], Leccino, Frantoio, Ottobratica and Ogliarola oils contained 21.44, 26.64, 6.57 and 3.14 ppm total chlorophyll with a different order of chlorophyll content in the fruit of these four varieties in this present study. Sinelli and co-workers [19] also obtained higher total chlorophyll content for Frantoio than Leccino (6.20±2.53 and 4.37±1.74 ppm, respectively).

Principal component analysis for the physicochemical parameters showed varietal differences. Two latent factors had an eigenvalue greater than 1, which together explained 62.30 % of the total variance (Fig. 2a). The factor loadings demonstrated the different groups of variables (Fig. 2b). Factor 1, displayed on the horizontal axis, weighed heavily on flavonoid and non-flavonoid polyphenolic content. Tal-Bidni, Bosana, Frantoio and Pendolino exhibited superior polyphenolic and anthocyanin content than the other varieties including Tal-Malti. On the other hand, F2, displayed on the vertical axis, weighed heavily on the chlorophyll content. Tal-Malti, Coratina, Frantoio and Ottobratica exhibited a high chlorophyll content in fruit but low chlorophyll content in leaves, as opposed to the other varieties including Tal-Bidni. Similar observations were made by Giansante and co-workers [15], indicating that Leccino, Frantoio and Bosano oils did not show physicochemical similarities. The Cerasuola variety exhibited different characteristics from the rest, which goes in accordance with the findings of D'Imperio and co-workers [38]. In this same study, Nocellara messinese, Carolea and Ottobratica were grouped together chemometrically. In this present study only the first two showed similar characteristics. Whereas Coratina and Leccino shared similar chemometric characteristics in the study by Esti and co-workers [14], this was not observed in this present study. In another study, Leccino and Frantoio were distinctively different from each other chemometrically [39]. This goes in accordance with this present study.

VI. CONCLUSIONS

This was the first study to demonstrate the morphometric and physiochemical properties of two indigenous autochthonous varieties of the Maltese Islands. This study lays the foundation for further research on these two and potentially more varieties that pertain to these islands.

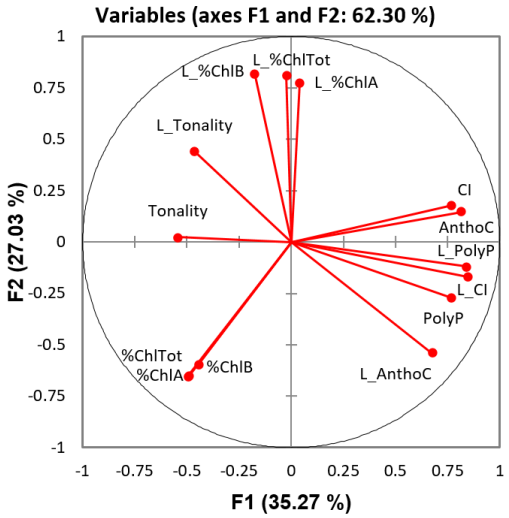

Fig. 2a Variables plot for the individual leaf and fruit parameters.

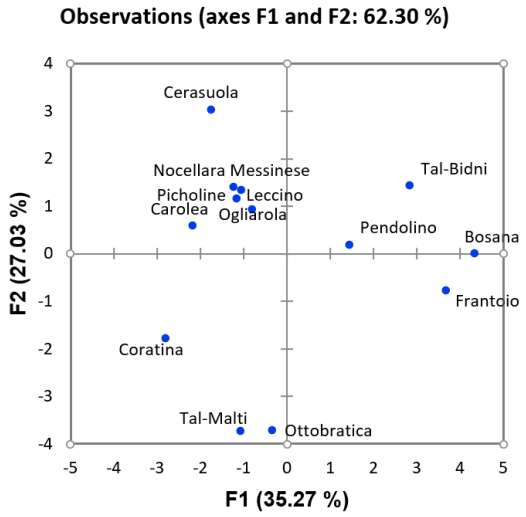

Fig. 2b Observations plot for the varieties with respect to their physicochemical analysis (PCA)

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