Preliminary observations on veiled olive oil turbidity with regards to wax content

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This work is a preliminary contribution towards understanding the nature of the material responsible for the physicochemical status of veiled olive oil (described as suspension–dispersion system). Twenty four olive oil samples with different cloudy appearances were grouped into three classes according to increasing turbidity, both by means of a visual evaluation and by spectrophotometric optical density (absorbance at 630 nm). Chemical analyses were then performed, i.e. free acidity (FA), peroxide value (PV), UV absorption (UVa), moisture (MO), impurity, fatty acid composition (Fac), sterols and waxes concentration. The oil samples were very similar, i.e. no significant or minimal differences in FA, PV, UVa, Fac and sterols, whereas large and significant differences were found both for MO and waxes (both total and specific esters) among the three turbidity classes. Impurities were not detected in any samples. An additive effect of waxes along with other minor constituents in the establishment of the physicochemical state of the veiled VOO was hypothesized.

Osservazioni preliminari sulla torbiditá di oli di oliva con riferimento al loro contenuto in cere

Il presente lavoro è un contributo preliminare alla conoscenza della natura del materiale responsabile dello stato fisico–chimico degli oli extra vergini di oliva torbidi (anche detti oli velati), già descritto come un sistema eterogeneo in cui coesistono una fase dispersa ed una in sospensione. A questo scopo 24 campioni di olio extra vergine di oliva con differente torbidità, sono stati raggruppati in tre classi con torbidità crescente per mezzo sia di una analisi visiva, che della loro densità ottica misurata spettrofotometricamente (assorbanza a 630 nm). Successivamente, sui campioni sono state effettuate le seguenti analisi chimiche: acidità libera (FA), numero di perossidi (PV), assorbimento UV (UVa), contenuto in acqua (MO), impurità, composizione in acidi grassi (Fac), contenuto in steroli e cere. I risultati analitici hanno evidenziato differenze minime o non significative tra le tre classi di torbidità per FA, PV, UVa, Fac e steroli, mentre differenze significative sono state riscontrate sia nel contenuto in acqua che in quello in cere (sia totali che nei singoli esteri). Le impurità non sono state rilevate in nessun campione. Sulla base di tali risultati è stato ipotizzato un effetto additivo della frazione cerosa insieme ad altri composti minori, nel determinare lo stato fisico–chimico degli oli torbidi.

INTRODUCTION

Virgin olive oil (VOO) extraction process is achieved by physical methods only: crushing of olive drupes, malaxation (mixing) of the resulting pastes, separation and clarification of the oil phase by means of centrifugation. Right after production the fresh olive oil is turbid and it exhibits a cloudy appearance (veiled VOO) [1]. This condition can persist for several months before a spontaneous separation into a two separate–phase system. In the current industrial practice, the oil is left in tanks for a relatively long period for sedimentation and easy filtration [1]. However, veiled olive oil can be bottled and sold without further treatments as some consumers prefer its flavor and consider it to be of higher nutritional value.

The physicochemical state of veiled olive oil was characterized as suspension–dispersion system [2]. Several studies in literature deal with the stability of veiled VOO, although the experimental results are rather conflicting [2–11]. On the contrary information concerning the chemical characterization of the suspended–dispersed phases is rather poor and indicates the presence of solids derived from the olive fruit, sugars, enzymes, protein, phospholipids and vegetative water [1, 2, 12], whereas the presence of waxes is not reported. These substances are present on the external cuticle of the olive fruit and leaves to form a surface hydrophobic layer [13, 14].

Wax esters occurring in vegetable oils consist of high–molecular–mass alcohols linked to fatty acids by ester bonds. The length and structure of the alcoholic moiety is variable; generally long chain aliphatic alcohols are present in VOO resulting in aliphatic waxes, i.e. C36, C38, C40, C42, C44, and C46 [1, 15–17]. Wax analysis is of major interest in VOO quality control because it is a parameter generally used to detect the presence of olive–pomace oil as the wax content differs among the various commercial categories of olive oil [17].

Some evidence is present in literature on the influence of wax content on the turbidity of some vegetable oils [18–22], while Ranalli et al. [23, 24] showed indirectly that high turbid VOO often corresponds to a higher wax concentration. This work is a preliminary contribution towards understanding the nature of the material responsible for the physico–chemical status of veiled olive oil with regards to wax content.

MATERIALS AND METHODS

Experimental procedure

All the olive oil samples used for the experiment were collected from an industrial company (Carapelli Firenze SpA, Florence, Italy) and consisted of blends of VOO from different Mediterranean productions, i.e. Spain, Greece and Italy; the exact composition of each sample with respect to the origin production area, was unknown at the time of the experiment.

A previously selected set of 24 unfiltered VOO samples with different cloudy appearances was used for the assessment of turbidity. For this purpose the samples were examined both by means of a visual evaluation (two assessors) and, on the basis of a previous experience, by spectrophotometric absorbance at a wave length of 630 nm (Table I).

Table I – Spectrophotometric absorbance (630 nm) of the oil samples used for the experiment

Turbidity Class	Absorbance#
class1	0.06±0.02
class2	1.13±0.21
class3	2.00±0.14

#Data are means ± standard deviations of eight independent samples per class

In this way the samples were grouped into three classes with increasing turbidity, i.e. class 1, class 2 and class 3 respectively. Then, some chemical parameters, i.e. free acidity, peroxide value, UV absorption (K_{232} and K_{270}), moisture, impurity, fatty acid composition, sterols and wax concentration, were measured on all the oil samples.

Because of the small sample size, i.e. 8 oil samples per group, statistical significance of the investigated differences was evaluated through Kruskal Wallis–one way ANOVA by rank nonparametric method [25, 26].

Chemical analyses

All the considered chemical parameters, i.e. free acidity, peroxide value, UV absorption (K_{232} and K_{270}), fatty acid composition, sterols and waxes were measured following the analytical methods described in Regulations EEC/2568/91 of the European Union Commission [17]. Moisture and impurity were determined accor-

Table II – Standard quality parameters of the oil samples corresponding to the three turbidity classes

Parameter#	Turbidity class	Median	Rank sum	Sum rank variance	p
Free acidity (%)	class1	0.49	69.00	5.24	0.07
	class2	0.50	133.50		
	class3	0.49	97.50		
Peroxide value ($meqO_2*kg^{-1}$)	class1	9.54	101.00	2.21	0.33
	class2	9.60	120.50		
	class3	8.18	78.50		
K_{232}	class1	1.88	87.00	3.52	0.17
	class2	1.98	130.50		
	class3	1.79	82.50		
K_{270}	class1	0.13	118.50	2.72	0.26
	class2	0.13	107.50		
	class3	0.12	74.00		

#Data are median of eight independent samples per class.

ding to the AOCS methods Ca 2c–25(97) and Ca 3a–46(97), respectively [27]. Each analysis was performed in duplicate.

RESULTS AND DISCUSSION

Results

The oil samples used for the turbidity assessment were selected and classified only on the basis of their visual aspect and their absorbance values (Table I). Therefore, the chemicals analyses were performed only after the turbidity scale assignment.

As shown in Table II, where the results of standard quality parameters are reported separately for the three turbidity classes (median of concentrations), all the VOO samples used in the experiment felt into the category of extra virgin olive oil according to the EC regulation. Only slight variations can be observed in these quality parameters between the three different classes and the observed differences were not statistically significant at $p<0.05$. Hence, the basic quality of these oils seems not to be related to their visual appearance as was expected.

Similar considerations can be drawn for fatty acids composition and sterols on the basis of the results shown in Table III and Table IV respectively. The former showed significant differences among the turbidity classes for some fatty acids only, with the greatest difference less than 4%. Likewise, the sterol composition showed relevant and significant differences for β–sitosterol only whereas no significant differences were found in the total sterols concentration. Despite this high chemical similarity of the VOO samples across the classes of turbidity, large differences were found both in moisture and wax concentration (Table V). If we could easily expect such a result for the first of these two parameters, i.e. the moisture content which is probably the major factor influencing the cloudy appearance of the oil (in all the samples impurities were not detected), otherwise it is not so for wax concentration that showed a clear differentiation across the turbidity classes.

As reported in Table V a more marked distinction exists between class 1 and class 2 of the turbidity scale with a mean difference of about $67 mg*kg^{-1}$, as compared with the difference between class 2 and class 3 (about $37 mg*kg^{-1}$). There was also a greater variability within class 2, in accordance with the more marked variability in the optical density shown in Table I, as compared to the other two classes. Probably, this condition could be attributed to the difficulty of the assessors in the visual evaluation of these VOO samples, as they often showed an intermediate veiled appearance making their sorting more complex as a function of increasing turbidity.

As reported in Table VI significant differences were found among the three turbidity classes for almost all esters. Also, the individual wax constituents did not register substantial percent differences among the turbidity classes, with the greatest differences less than 3% (C36 ester).

Discussion

It is known that the turbidity of some seed oils before refining (winterization), e.g. sunflower oil, is related to the high concentration of waxes. Turbidity is caused by crystallization of waxes due to their low solubility in oil and generally it is facilitated by temperature decrease [18, 19, 28–30]. A visual observation used for the turbidity assessment of the oils during a storage period of one month evidenced some critical points.

Table III – Fatty acid composition (%) of the oil samples corresponding to the three turbidity classes

Fatty acids (%)[#]	Turbidity class	Median	Rank sum	Sum rank variance	p
Myristic	class1	0.01	86.00	1.00	0.61
	class2	0.01	101.50		
	class3	0.01	112.50		
Palmitic	class1	12.12	118.00	6.34	0.04
	class2	12.53	123.00		
	class3	10.83	59.00		
Palmitoleic	class1	1.02	123.00	4.75	0.09
	class2	1.14	112.00		
	class3	0.75	65.00		
Heptadecanoic	class1	0.05	108.50	2.14	0.34
	class2	0.04	77.00		
	class3	0.04	114.50		
9–Heptadecenoic	class1	0.08	108.00	0.25	0.88
	class2	0.08	95.00		
	class3	0.08	97.00		
Stearic	class1	2.58	81.50	1.55	0.46
	class2	2.59	102.00		
	class3	2.62	116.50		
Oleic	class1	73.66	81.00	6.97	0.03
	class2	72.76	76.00		
	class3	76.81	143.00		
Linoleic	class1	9.44	115.00	5.81	0.05
	class2	9.31	124.00		
	class3	7.54	61.00		
Linolenic	class1	0.59	77.00	4.20	0.12
	class2	0.60	90.50		
	class3	0.61	132.50		
Arachidic	class1	0.43	72.50	5.00	0.08
	class2	0.43	93.00		
	class3	0.45	134.50		
11–Eicosenoic	class1	0.29	90.00	1.91	0.39
	class2	0.27	87.50		
	class3	0.30	122.50		
Behenic	class1	0.12	62.00	8.69	0.01
	class2	0.12	93.50		
	class3	0.14	144.50		
Lignoceric	class1	0.05	69.50	7.81	0.02
	class2	0.05	86.00		
	class3	0.06	114.50		

[#]Data are median of eight independent samples per class

Table IV – Total sterols content and relative sterols composition (%) of the oil samples corresponding to the three turbidity classes

Sterols (%)#	Turbidity class	Median	Rank sum	Sum rank variance	p
24–Methylenecholesterol	class1	0.17	51.00	11.87	0.00
	class2	0.20	101.50		
	class3	0.22	147.50		
Campesterol	class1	3.22	122.00	1.83	0.40
	class2	3.17	89.50		
	class3	3.17	88.50		
Campestanol	class1	0.26	100.00	2.28	0.32
	class2	0.25	79.00		
	class3	0.26	121.00		
Stigmasterol	class1	0.80	123.00	2.11	0.35
	class2	0.76	83.50		
	class3	0.77	93.50		
Δ^7–Campesterol	class1	0.01	80.00	4.03	0.13
	class2	0.01	116.00		
	class3	0.01	104.00		
$\Delta^{5,23}$–Stigmastadienol	class1	nd	nd	nd	nd
	class2	nd	nd		
	class3	nd	nd		
Clerosterol	class1	1.02	98.50	1.78	0.41
	class2	0.99	82.00		
	class3	1.02	119.50		
β–Sitosterol	class1	82.48	139.00	8.97	0.01
	class2	81.76	106.00		
	class3	80.22	55.00		
Δ–Sitostanol	class1	0.74	103.50	1.63	0.44
	class2	0.70	80.50		
	class3	0.73	116.00		
Δ^5–Avenasterol	class1	9.89	62.00	8.54	0.01
	class2	10.58	94.00		
	class3	12.05	144.00		
$\Delta^{5,24}$–Stigmastadienol	class1	0.70	90.00	0.75	069
	class2	0.71	113.50		
	class3	0.70	96.50		
Δ^7–Stigmastenol	class1	0.20	84.50	2.35	0.31
	class2	0.23	124.50		
	class3	0.21	91.00		
Δ^7–Avenasterol	class1	0.43	83.00	4.62	0.10
	class2	0.53	135.00		
	class3	0.43	82.00		
Total sterols (mg*kg^{-1})	class1	1547.15	120.00	3.31	0.19
	class2	1548.04	109.00		
	class3	1452.00	71.00		
Apparent β–Sitosterol	class1	94.72	104.00	0.11	0.95
	class2	94.72	101.00		
	class3	94.72	95.00		

#Data are median of eight independent samples per class

Table V – Moisture and total waxes concentration of the oil samples corresponding to the three turbidity classes

Parameter[#]	Turbidity class	Median	Rank sum	Sum rank variance	p
Moisture (%)	class1	0.10	36.50	19.35	<0.01
	class2	0.16	103.00		
	class3	0.22	160.50		
Total waxes (mg*kg^{-1})	class1	134.56	36.00	19.86	<0.01
	class2	201.72	102.00		
	class3	238.71	162.00		

[#]Data are median of eight independent samples per class

Table VI – Specific wax esters concentration of the oil samples corresponding to the three turbidity classes

Wax esters (mg*kg^{-1})[#]	Turbidity class	Median	Rank sum	Sum rank variance	p
C36	class1	42.81	36.00	15.77	<0.01
	class2	70.98	123.00		
	class3	78.62	141.00		
C38	class1	36.72	36.00	19.86	<0.01
	class2	58.65	102.00		
	class3	70.29	162.00		
C40	class1	18.91	36.00	19.28	<0.01
	class2	27.81	104.00		
	class3	33.91	160.00		
C42	class1	17.88	45.00	16.62	<0.01
	class2	21.85	95.00		
	class3	28.68	160.00		
C44	class1	11.92	43.00	14.72	<0.01
	class2	14.10	106.00		
	class3	19.30	151.00		
C46	class1	5.84	65.00	5.95	0.05
	class2	6.70	101.00		
	class3	7.76	134.00		

[#]Data are median of eight independent samples per class

– temperature oscillation due to the day–night alternation results in a regular change of the cloudy appearance of the samples corresponding to class 3 of the turbidity scale (higher turbidity at a lower temperature and less turbidity at higher temperature); such behavior was recorded only to a minor extent for class 2, whereas it was not observed for class 1;
– oil samples corresponding to class 3 showed a more rapid clarification with separation of the different phases, i.e. a semi–solid residue that settles at the bottom of the bottles in few days, compared to the other two classes.

Considering that, as mentioned before, waxes tend to crystallize and cause turbidity when the oil is cooled, these last observations support the possible interaction between wax concentration and the veiled appearance of the oil.

In conclusion we can hypothesize that along with moisture, i.e. surely the main factor affecting the turbidity of the oil, waxes exert an additive effect along with other minor constituents to determine the physicochemical state of the veiled VOO. This hypothesis is in agreement with the findings of other researchers who showed that waxes crystallization could be affected both by the presence of surface–active agents, e.g. phospholipids [31, 32], and by fatty acid composition [21].

For the first of these two parameters we can only suppose there is a different phospholipids content in the oil samples, as this parameter was not considered at the time of the experiment. However, Koidis et al. [12] showed that unfiltered olive oils were characterized by higher phospholipid content with respect to the filtered ones.

Concerning the fatty acid composition Adhvaryu et al. [19] reported that wax appearance temperature increases with higher oleic content; in other words at the same temperature, crystallization takes place to a major extent in correspondence with the higher oleic acid content. This is in accordance with what is reported in Table III where, although no significant differences were found in the overall fatty acid composition among the three different turbidity classes, a slightly higher content of oleic acid was found in correspondence of increasing turbidity.

CONCLUSIONS

From the results of this preliminary work, it would be seen that there is an additive effect of waxes determining the physicochemical state of the veiled olive oil along with other minor constituents. Recently, veiled olive oils have become more popular among consumers who habitually consider this type of oil more natural and less processed. So, future investigations should be performed to confirm these preliminary results and to better understand the nature of the physicochemical state of the veiled olive oil with regard to the possible interaction of minor constituents, e.g. waxes and phospholipids, to determine the stability of the suspension–dispersion system. Also, as it is widely known that the extraction process affects the amount and nature of minor compounds occurring in olive oil, the role of the extraction conditions should be considered.

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