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Free and total sterols in olive oils. Effects of neutralization

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RESUMEN

Esteroles libres y totales en aceites de oliva. Efectos de la neutralización

Se ha realizado una determinación directa cuali-cuantitativa por CGL de los esteroles libres y totales, para investigar las variaciones de la relación entre ellos en diferentes categorías de aceite de oliva y estudiar la posibilidad del uso de esta relación como parámetro para la evaluación de la calidad del aceite. Dicha relación se calculó sobre un conjunto de 13 muestras de aceites de oliva virgen y 28 muestras de aceites vírgenes corrientes y lampantes. Para estudiar los efectos de la neutralización sobre los esteroles libres y totales, se determinaron sus contenidos y relación entre ellos tras la neutralización de los aceites vírgenes corrientes y lampantes. También se han analizado los componentes individuales de los esteroles libres y totales, especialmente los más representativos.

El examen total de los valores de la relación esteroles libres x 100 / esteroles totales realizado sobre una población de 41 muestras de aceites naturales y 28 de aceites neutralizados indicó que, cuando esta relación supera el 70%, puede excluirse la presencia de aceites neutralizados en aceites vírgenes extra.

PALABRAS-CLAVE: Aceite de oliva - Calidad (evaluación) - Esteroles - Neutralización (efecto de).

SUMMARY

Free and total sterols in olive oils. Effects of neutralization

In order to further investigate the variations of the ratio of free to total sterols in different categories of olive oil and to test the possibility to use this ratio as a parameter for olive oil quality evaluation, a direct GLC quali-quantitative determination of either free or total sterols was made, and their ratio calculated, over a set 13 samples of extra virgin oils and over a set of 28 current virgin and *lampante* oils. In order to study the effects of neutralization on free and total sterols, their amounts and ratio were also determined after the neutralization of the current virgin and *lampante* oils. The single components of free and total sterols were also analyzed, especially the most represented ones.

The overall examination of the free x 100 / total sterols' ratio values found over all the samples of 41 natural oils and 28 neutralized oils indicated that, when this ratio exceeds 70%, it can exclude the presence of neutralized oils in extra virgin ones.

KEY-WORDS: Neutralization (effect of) - Olive oil - Quality (evaluation) - Sterols.

1. INTRODUCTION

It is now quite accepted by technologists, doctors, nutritionists and, above all, by final consumers, that virgin olive oils represent the best food, within the category of all vegetable and animal alimentary fats.

Actually, within this wide class of oils, there are different chemical and organolectic characteristics and commercial values as related to the different categories of extra virgin, virgin, current virgin and *lampante* oils. Despite this fact, the EC, Regulations no. 2568/91, no. 183/93 and no. 656/95 have established only differences in such chemical parameters as acidity percentage, so that less valuable oils (current virgin ones or *lampante* virgin edible ones, for example) could be commercially revalued simply eliminating free fatty acids by neutralization or by distillation at relatively low temperatures (deacidification + deodorization).

The same EC Regulations assign to the different categories of virgin olive oils the corresponding limits for total sterols and for their quali-quantitative composition. However, no indications are provided on the state of sterols themselves, if free or esterified to fatty acids. Actually, all alimentary fats contain both free and esterified sterols, as extensively reported in recent literature.

For example, as far as virgin olive oils are concerned, Kochhar (1983) reports data determined over a single sample and finds a free / esterified sterols' ratio of 2.2. Mariani *et al.* (1989) analyze, *via* direct GLC, 19 samples of virgin and *lampante* oils from a Southern Italy region and find total sterol amounts of 2000-2700 mg/kg with a higher quantity of stigmasterol within free sterols. Grob *et al.* (1990) determine total and free campesterol, stigmasterol and β-sitosterol and find free β-sitosterol amounts ranging from 71 to 92 % of total β-sitosterol in virgin olive oils and from 41 to 85 % in *lampante* oils and in the corresponding rectified ones. Mariani *et al.* (1991) find much lower amounts of free sterols in rectified olive oils than in virgin ones. Serani and Piacenti (1992), analyzing a set of edible vegetable oils including two raw olive oils and the related rectified ones, observe again a reduction in free sterols after refining. Caponio *et al.* (1995, 1996) analyze, via combined TLC and GLC, current virgin, *lampante* and the corresponding neutralized olive oils finding, in virgin oils, a ratio of free to esterified sterols greater than 2.0 (with the highest value of 4.3). In the neutralized oils the same ratio lowers below 2 (ranging from 0.9 to 1.8), the decrease of free sterols during neutralization being equal to almost 50%. Mariani and Venturini (1996) determine, via direct GLC, the changes in free / esterified sterols ratio after 18-month storage. They observe a

significant lowering of free sterol amounts and a subsequent increase in esterified ones.

In the present report, in order to further investigate the variations in the ratio of free to total sterols in different categories of olive oil and to test the possibility to use this ratio as a parameter for olive oil quality evaluation, a direct GLC quali-quantitative determination of both free and total sterols was made, and their ratio calculated, over a set of 13 samples of extra virgin oils and over a set of 28 current virgin and *lampante* oils. In order to study the effects of neutralization on free and total sterols, their amounts and ratio were also determined after the neutralization of the current virgin and *lampante* oils. The single components of free and total sterol fractions were also analyzed, especially the most represented ones.

2. MATERIALS AND METHODS

Samples. Forty-one samples of surely natural virgin olive oils, directly drawn at the oil-mills, were analyzed. Out of them, 13 belonged to the extra virgin category and 28 to the current virgin and *lampante* ones.

Acidity percentage determination was made following EC Regulation no. 2568/91, enclosure II (1991).

Neutralization. Samples having acidity percentage values higher than 1.00 were neutralized using the following conditions (similar to that of the discontinuous system in oil industries): oil temperature 40°C; 5 % NaOH water solution; repeated washing with water to reach exact neutrality of washing water.

Total sterol determination was made following C 72 N.G.D. (1987) methodology. Oils were first saponified with methanolic KOH, the unsaponifiable fraction was recovered by diethyl ether and the sterol fraction was separated by TLC. Sterols were then transformed in trimethylsilylethers and analyzed *via* GLC by a 25 m long, 0.25 mm internal diameter fused silica capillary column SPB-5™ (Supelco), film 0.25 μ, with 5α-cholestan 3β-ol (Sigma Aldrich) as internal standard.

Free sterols were determined by TLC separation, using 5α-cholestan 3β-ol (Sigma Aldrich) as internal standard, followed by GLC quali-quantitative evaluation. 1.00 g of oil was supplemented with 0.50 mg of 5α-cholestan 3β-ol and underwent TLC on G 60 silicagel plates (1.0 mm thickness) in elution mixture composed of petroleum ether (b.p. 40-60 °C): diethyl ether: formic acid (70:30:1.5 v:v:v). The most polar band containing free sterols was recovered and subjected to the same treatments as for total sterols.

3. RESULTS AND DISCUSSION

A total number of forty-one samples of surely natural olive oil was analyzed. After the acidity percentage determination, 13 out of the 41 samples resulted to be extra virgin oils, with acidity values below 1%. The remnant 28 samples were found to be current virgin and *lampante* oils, with acidity values ranging from 2.70 to 11.80 %. These 28 oils were neutralized in the laboratory and were analyzed both before and after neutralization.

The data obtained from the analysis of sterol contents in the 13 extra virgin oil samples, reported in Tab. I, showed total sterol contents always greater than 1000 mg/kg (that is the lowest limit established by current laws) and ranging from 1151 to 1793 mg/kg (1418 mg/kg on average). In particular, it is evident that free sterols, ranging from 939 to 1394 mg/kg (1126 mg/kg on average), represented the major component in these oils, with respect to the esterified sterols, leading to a free x 100 / total sterols'ratio value of 79% on average and, in any case, greater than 70% in 93 % of the samples examined.

Total sterols were found to be higher than 1000 mg/kg also in the 28 samples of oils belonging to less valuable commercial categories (current virgin and *lampante* oils) with values varying from 1219 to 2961 mg/kg (1901 mg/kg on average) (Tab. II). As far as free sterols are concerned, they were the major component of total sterols, with values ranging from 863 up to 2546 mg/kg (1448 mg/kg on average) and with a free x 100 / total sterols' ratio comprised between 65.4 and 86.0 % (76. 1% on average).

In any case, considering the totality of the 41 natural samples, free sterols were always 65% higher (77.1% on average) than total sterols.

These values dropped significantly after the neutralization of current virgin and *lampante* oils. The neutralized oils (Tab. III), with a very little residual acidity (0.07-0.29%) showed, in fact, total sterols varying from 629 to 1331 mg/kg (mean value 1020 mg/kg). These values were lower than those observed in the same samples before deacidification, with a 52% loss in total sterols on average. Free sterols showed an even stronger decrease with values ranging, after neutralization, from 353 to 791 mg/kg (563 mg/kg as mean value) and with a 62% average loss.

The best evaluation of the loss in sterols was expressed by the free x 100 / total sterols' ratio that was found much lower than before neutralization, with values ranging from 44.8 to 63.1% and an average value of 55.2%. In particular, while in current virgin and in *lampante* oils this parameter was always greater than 65%, in the same oils, after neutralization, it was always less or equal to 63%. The ratio was not related to free nor to residual acidity and indicated that the highest losses affected free sterols instead of esterified ones.

The overall examination of the free x 100 / total sterols' ratio values found over 41 samples of natural oils and 28 neutralized oils revealed that when this

Table I
Sterol contents in extra virgin oils

Sample n.	Acidity (%)	Total sterols (mg/kg)	Free sterols (mg/kg)	Free/total sterols (%)
1	0.92	1618	1134	70.1
2	0.85	1390	939	67.6
3	0.83	1362	989	72.6
4	0.76	1226	1141	93.0
5	0.85	1151	969	84.2
6	0.75	1548	1144	73.9
7	0.81	1388	1050	75.6
8	0.78	1793	1394	77.7
9	0.92	1302	1147	88.1
10	0.70	1491	1144	76.7
11	0.61	1166	1049	80.0
12	0.43	1793	1394	77.7
13	0.58	1203	1147	95.3
mean value	0.75	1418	1126	79.4

Table II
Sterol contents in current virgin and *lampante* oils

Sample n.	Acidity (%)	Total sterols (mg/kg)	Free sterols (mg/kg)	Free/total sterols (%)
1	2.70	1469	1189	80.9
2	2.80	2961	2546	86.0
3	4.00	2287	1607	70.3
4	4.00	1830	1361	74.4
5	4.10	1712	1408	82.2
6	4.40	1831	1199	65.4
7	4.50	1764	1342	76.1
8	4.60	2039	1395	68.4
9	4.80	1381	1027	74.4
10	5.00	1570	1164	74.1
11	5.10	1272	1048	82.4
12	5.20	1620	1279	78.9
13	5.30	1596	1211	75.9
14	5.40	2277	1833	80.5
15	5.50	1383	1150	83.1
16	5.70	2398	1773	73.9
17	5.90	2198	1637	74.5
18	6.10	2186	1710	78.2
19	6.20	2290	1590	69.4
20	6.40	2292	1756	76.6
21	6.50	2187	1835	83.9
22	7.60	1716	1350	78.7
23	6.70	2409	1786	74.1
24	7.40	1755	1261	71.8
25	8.30	1807	1381	76.4
26	10.40	1912	1416	74.1
27	11.00	1874	1423	75.9
28	11.80	1219	863	70.8
mean value	5.98	1901	1448	76.1

Table III
Sterol contents in neutralized oils

Sample n.	Acidity (%)	Total sterols (mg/kg)	Free sterols (mg/kg)	Free/total sterols (%)
1	0.07	904	508	56.2
2	0.09	1326	791	59.6
3	0.14	1080	650	60.2
4	0.26	1104	610	55.2
5	0.25	954	602	63.1
6	0.16	894	484	54.1
7	0.25	986	584	59.2
8	0.16	1331	700	52.6
9	0.26	969	562	58.0
10	0.29	909	493	54.2
11	0.24	629	353	56.1
12	0.26	919	536	58.3
13	0.15	843	510	60.5
14	0.08	1100	639	58.1
15	0.05	736	436	59.2
16	0.17	1257	671	53.4
17	0.25	1307	734	56.2
18	0.15	1250	743	59.4
19	0.08	1080	549	50.8
20	0.25	1040	496	47.7
21	0.16	842	446	53.0
22	0.07	1268	779	61.4
23	0.18	1276	607	47.6
24	0.26	965	490	50.8
25	0.14	802	454	56.6
26	0.20	1035	498	48.1
27	0.17	943	473	50.1
28	0.21	804	360	44.8
mean value	0.18	1020	563	55.2

ratio reaches values higher than 70%, it enables to exclude the presence of neutralized oils in extra virgin ones. However, for minor values it might not permit the detection of neutralized oils, especially in virgin ones. In any case, also considering the other analytical data, this parameter could represent an additional tool to express a judgement about the quality of an oil.

Also the composition of total and free sterols, especially the most represented sterols, was determined for extra virgin oil samples (Tab. IV) and for current virgin and *lampante* oil samples both before (Tab. V) and after neutralization (Tab. VI).

The data obtained in the extra virgin oil samples (Tab. IV) did not show any significant difference in composition between free and total sterol fractions. In current virgin and *lampante* oils (Tab. V), focussing the attention to campesterol and stigmasterol, we could

Table IV

Percent composition of total and free sterol fraction in extra virgin olive oils (the most represented sterols)

sample n.	campesterol		stigmasterol		chlerosterol		β-sitosterol + sitostanol		Δ^5 avenasterol		$\Delta^{5,24}$ stigma-stadienol		Δ^7 stigma-stenol		Δ^7 avenasterol		appearing β- sitosterol	
	total	*free*	total	*free*	total	*free*	total	*free*	total	*free*	total	*free*	total	*free*	total	*free*	total	*free*
1	3.5	*4.1*	2.5	*2.2*	0.8	*0.8*	85.4	*85.2*	6.5	*6.0*	0.6	*0.8*	0.2	*0.3*	0.5	*0.6*	93.3	*92.8*
2	3.3	*3.7*	2.7	*2.5*	0.9	*0.6*	86.1	*86.4*	5.1	*5.2*	0.9	*0.8*	0.3	*0.3*	0.7	*0.5*	93.0	*93.0*
3	3.8	*3.6*	2.4	*2.4*	1.0	*0.8*	85.2	*85.5*	6.1	*6.1*	0.7	*0.9*	0.2	*0.3*	0.6	*0.4*	93.0	*93.3*
4	3.6	*3.7*	2.4	*2.6*	1.0	*1.1*	85.7	*85.5*	5.2	*5.4*	1.0	*1.0*	0.6	*0.2*	0.6	*0.5*	92.9	*93.0*
5	3.5	*3.2*	2.2	*2.4*	1.0	*0.9*	85.3	*86.5*	6.5	*5.6*	0.8	*0.5*	0.3	*0.4*	0.4	*0.5*	93.6	*93.5*
6	3.4	*3.4*	2.0	*2.1*	1.0	*0.8*	86.0	*86.0*	6.3	*6.1*	0.6	*0.8*	0.2	*0.2*	0.5	*0.6*	93.9	*93.7*
7	3.7	*3.7*	2.2	*2.2*	0.6	*0.8*	86.9	*86.4*	5.1	*5.3*	0.7	*0.8*	0.2	*0.3*	0.6	*0.5*	93.3	*93.3*
8	3.8	*4.1*	2.2	*2.3*	1.0	*1.0*	85.2	*85.8*	6.2	*5.5*	0.6	*0.6*	0.3	*0.2*	0.7	*0.5*	93.0	*92.9*
9	3.8	*3.4*	2.1	*2.5*	0.9	*1.2*	85.7	*85.4*	5.9	*5.8*	0.7	*0.9*	0.3	*0.2*	0.6	*0.6*	93.2	*93.3*
10	3.4	*3.8*	1.8	*2.0*	0.7	*0.7*	86.0	*85.9*	6.2	*5.5*	0.9	*0.9*	0.4	*0.4*	0.6	*0.8*	93.8	*93.0*
11	3.8	*3.4*	2.0	*2.4*	0.7	*0.7*	84.9	*85.9*	6.8	*6.2*	0.8	*0.6*	0.3	*0.2*	0.7	*0.6*	93.2	*93.4*
12	3.2	*2.7*	2.2	*1.9*	0.9	*0.6*	85.7	*86.1*	5.9	*6.9*	0.9	*1.0*	0.4	*0.2*	0.8	*0.6*	93.4	*94.6*
13	3.1	*3.2*	2.5	*2.5*	0.8	*0.6*	85.2	*85.0*	6.6	*6.6*	0.7	*1.0*	0.5	*0.4*	6.0	*0.7*	93.3	*93.2*
mean value	3.5	*3.5*	2.2	*2.3*	0.9	*0.8*	85.6	*85.8*	6.0	*5.9*	0.8	*0.8*	0.3	*0.3*	1.0	*0.6*	93.3	*93.3*

Table V

Percent composition of total and free sterol fraction in current virgin and in *lampante* olive oils (the most represented sterols)

sample n.	campesterol		stigmasterol		chlerosterol		β-sitosterol + sitostanol		Δ^5 avenasterol		$\Delta^{5,24}$ stigma-stadienol		Δ^7 stigma-stenol		Δ^7 avenasterol		appearing β- sitosterol	
	total	*free*	total	*free*	total	*free*	total	*free*	total	*free*	total	*free*	total	*free*	total	*free*	total	*free*
1	3.1	*3.2*	1.9	*2.1*	0.7	*0.7*	89.9	*89.7*	4.0	*3.7*	0.4	*0.6*	tr	*tr*	tr	*tr*	95.0	*94.7*
2	3.5	*3.6*	2.2	*3.1*	1.0	*0.9*	85.2	*85.6*	7.0	*5.9*	0.5	*0.4*	0.2	*0.1*	0.4	*0.4*	93.7	*92.8*
3	3.7	*3.4*	1.6	*2.2*	0.7	*0.8*	86.5	*86.8*	6.3	*6.3*	0.5	*0.4*	0.1	*tr*	0.6	*0.1*	94.0	*94.3*
4	3.4	*3.3*	2.5	*3.2*	0.4	*0.6*	85.3	*85.5*	7.2	*6.6*	0.7	*0.5*	0.2	*tr*	0.3	*0.3*	93.6	*93.2*
5	3.4	*3.1*	2.7	*2.7*	0.6	*0.6*	88.3	*89.1*	4.6	*4.5*	0.4	*tr*	tr	*tr*	tr	*tr*	93.9	*94.2*
6	3.6	*3.5*	2.8	*3.3*	0.7	*0.7*	83.8	*84.2*	7.9	*7.4*	0.6	*0.4*	0.2	*tr*	0.4	*0.5*	93.0	*92.7*
7	4.0	*3.6*	2.6	*3.1*	0.5	*0.6*	85.0	*84.8*	6.5	*6.6*	0.7	*1.0*	0.2	*0.3*	0.5	*tr*	92.7	*93.0*
8	3.0	*2.6*	1.2	*2.2*	0.8	*0.7*	87.1	*86.8*	6.8	*6.9*	0.6	*0.4*	tr	*0.1*	0.5	*0.3*	95.3	*94.8*
9	3.7	*3.5*	1.7	*2.3*	0.6	*0.7*	90.0	*88.8*	3.3	*3.4*	0.7	*0.3*	tr	*tr*	tr	*tr*	94.6	*94.2*
10	3.1	*2.9*	2.8	*3.2*	0.7	*0.6*	87.5	*86.5*	4.9	*5.6*	0.6	*0.9*	0.1	*0.3*	0.3	*tr*	93.7	*93.6*
11	3.2	*3.1*	2.5	*2.8*	0.6	*0.6*	86.3	*86.9*	6.3	*6.4*	0.6	*0.2*	0.2	*tr*	0.3	*tr*	93.8	*94.0*
12	2.8	*2.7*	1.6	*2.2*	0.6	*0.7*	89.8	*89.7*	4.5	*4.5*	0.3	*0.2*	0.2	*tr*	0.2	*tr*	95.2	*95.1*
13	2.8	*2.6*	2.1	*2.3*	0.8	*0.8*	89.9	*90.3*	3.9	*3.4*	0.3	*tr*	0.2	*tr*	tr	*tr*	94.9	*95.1*
14	3.5	*3.4*	2.8	*2.5*	0.9	*0.7*	84.4	*85.2*	6.8	*5.8*	0.8	*0.7*	0.5	*0.4*	0.3	*0.3*	92.9	*92.4*
15	2.8	*2.9*	1.2	*1.2*	0.6	*0.6*	86.4	*87.2*	8.4	*8.1*	0.6	*tr*	tr	*tr*	tr	*tr*	96.0	*95.9*
16	3.3	*3.3*	2.4	*2.5*	0.7	*0.9*	85.3	*85.2*	6.6	*6.7*	0.8	*0.7*	0.4	*0.3*	0.5	*0.4*	93.4	*93.5*
17	3.6	*3.2*	2.6	*2.4*	0.8	*0.7*	84.3	*85.4*	7.5	*6.8*	0.5	*0.6*	0.2	*0.5*	0.5	*0.2*	93.1	*93.5*
18	3.6	*3.3*	2.3	*2.8*	0.6	*0.5*	86.6	*89.9*	6.5	*3.5*	0.4	*tr*	tr	*tr*	tr	*tr*	94.1	*93.9*
19	2.9	*2.1*	2.5	*3.2*	0.8	*0.8*	85.0	*86.6*	7.4	*5.4*	0.7	*0.9*	0.2	*tr*	0.5	*tr*	93.9	*93.7*
20	3.4	*3.4*	2.4	*3.3*	0.9	*1.0*	85.2	*85.0*	6.7	*6.7*	0.6	*0.4*	0.2	*0.1*	0.6	*0.1*	93.4	*93.1*
21	3.3	*3.1*	1.5	*1.6*	0.8	*0.6*	89.1	*88.8*	4.8	*5.5*	0.5	*0.2*	tr	*0.2*	tr	*tr*	95.2	*95.1*
22	3.5	*3.6*	2.1	*2.2*	0.7	*0.7*	88.3	*88.2*	5.4	*5.3*	tr	*tr*	tr	*tr*	tr	*tr*	94.4	*94.2*
23	3.8	*3.8*	2.2	*2.9*	0.6	*0.6*	85.4	*85.7*	6.6	*6.5*	0.7	*tr*	0.2	*tr*	0.5	*0.5*	93.3	*92.8*
24	3.5	*3.6*	1.8	*2.3*	0.5	*0.6*	88.2	*88.4*	5.4	*4.8*	0.4	*0.3*	0.1	*tr*	0.1	*tr*	94.5	*94.1*
25	3.2	*3.3*	1.8	*2.2*	0.6	*0.7*	88.8	*88.6*	4.7	*4.8*	0.6	*0.2*	0.1	*0.2*	0.2	*tr*	94.7	*94.3*
26	3.3	*3.0*	1.9	*2.3*	0.5	*0.6*	88.9	*89.5*	4.2	*3.9*	0.6	*0.5*	0.5	*0.2*	0.1	*tr*	94.2	*94.5*
27	3.4	*3.4*	3.1	*3.4*	0.6	*0.7*	85.2	*85.5*	6.7	*6.8*	0.5	*tr*	0.3	*0.2*	0.2	*tr*	93.0	*93.0*
28	3.2	*2.9*	3.1	*2.3*	0.5	*0.5*	83.1	*83.7*	9.1	*9.6*	0.7	*0.2*	0.3	*0.4*	tr	*0.4*	93.4	*94.0*
mean value	3.3	*3.2*	2.2	*2.6*	0.7	*0.7*	86.7	*87.1*	6.1	*5.8*	0.6	*0.5*	0.2	*0.3*	0.4	*0.3*	94.0	*93.9*

Table VI
Percent composition of total and free sterol fraction in neutralized olive oils (the most represented sterols)

sample n.	campesterol		stigmasterol		chlerosterol		β-sitosterol + sitostanol		Δ^5 avenasterol		$\Delta^{5,24}$ stigma-stadienol		Δ^7 stigma-stenol		Δ^7 avenasterol		appearing β- sitosterol	
	total	*free*	total	*free*	total	*free*	total	*free*	total	*free*	total	*free*	total	*free*	total	*free*	total	*free*
1	3.2	*2.9*	1.6	*2.1*	0.7	*0.7*	88.6	*89.9*	4.6	*4.0*	0.7	*0.4*	0.2	*tr*	0.4	*tr*	94.6	*95.0*
2	2.9	*3.0*	1.9	*2.2*	0.8	*1.1*	85.7	*85.6*	7.0	*7.2*	0.6	*0.5*	0.3	*0.1*	0.8	*0.3*	94.1	*94.4*
3	3.4	*3.2*	1.1	*2.8*	0.7	*0.8*	87.2	*86.1*	6.9	*6.4*	0.5	*0.6*	0.2	*tr*	tr	*0.1*	95.3	*93.9*
4	3.9	*3.9*	2.4	*3.1*	0.4	*0.2*	86.3	*86.9*	5.1	*5.9*	1.2	*tr*	0.3	*tr*	0.4	*tr*	93.0	*93.0*
5	3.4	*3.3*	1.7	*2.2*	0.7	*0.5*	87.9	*88.1*	5.1	*5.1*	0.6	*0.6*	0.2	*0.2*	0.4	*tr*	94.3	*94.3*
6	3.8	*4.0*	2.7	*3.0*	0.4	*0.4*	86.2	*85.3*	6.2	*7.3*	0.7	*tr*	tr	*tr*	tr	*tr*	93.5	*93.0*
7	4.0	*4.2*	2.7	*2.3*	0.4	*0.6*	89.0	*87.3*	3.8	*5.5*	tr	*tr*	tr	*0.1*	tr	*tr*	93.2	*93.4*
8	3.7	*3.4*	2.0	*1.8*	0.8	*0.8*	85.8	*86.1*	6.7	*7.3*	0.7	*0.6*	0.2	*tr*	tr	*tr*	94.1	*94.8*
9	3.5	*3.4*	2.5	*2.2*	0.8	*0.7*	88.6	*89.4*	4.1	*3.5*	0.5	*0.6*	tr	*0.1*	tr	*0.1*	94.0	*94.2*
10	3.1	*3.7*	2.2	*2.3*	0.7	*0.6*	87.3	*87.6*	5.4	*5.1*	0.7	*0.7*	0.2	*tr*	0.4	*tr*	94.1	*94.0*
11	3.2	*2.8*	2.2	*2.3*	0.7	*1.9*	86.7	*86.2*	6.1	*6.2*	0.5	*0.6*	0.3	*tr*	0.3	*tr*	94.0	*94.9*
12	2.6	*2.6*	1.4	*1.8*	0.8	*0.8*	89.3	*89.4*	5.1	*5.8*	0.4	*0.6*	tr	*tr*	0.4	*tr*	95.6	*95.6*
13	2.9	*3.1*	1.8	*2.5*	0.7	*0.6*	88.9	*87.9*	4.5	*4.8*	0.6	*0.6*	0.2	*0.5*	0.4	*tr*	94.7	*93.9*
14	3.5	*3.2*	2.0	*2.4*	0.7	*1.0*	85.7	*86.1*	6.5	*6.7*	0.7	*0.3*	0.2	*0.1*	0.7	*0.2*	93.6	*94.1*
15	3.2	*3.4*	1.2	*1.4*	0.6	*0.7*	86.5	*87.1*	7.7	*7.4*	0.6	*tr*	0.2	*tr*	tr	*tr*	95.4	*95.2*
16	3.7	*3.6*	2.4	*2.5*	0.9	*0.6*	85.6	*84.7*	6.2	*7.2*	0.8	*1.0*	tr	*tr*	0.4	*0.4*	93.5	*93.5*
17	3.5	*3.5*	3.0	*2.6*	1.0	*1.0*	84.3	*84.5*	6.9	*7.0*	0.6	*0.4*	tr	*0.2*	0.7	*0.8*	92.8	*92.9*
18	3.5	*3.3*	2.0	*2.9*	0.6	*0.8*	86.9	*87.2*	6.2	*5.8*	0.5	*tr*	0.3	*tr*	tr	*tr*	94.2	*93.8*
19	3.2	*2.8*	2.7	*2.0*	0.9	*0.9*	84.9	*85.7*	6.9	*6.9*	0.6	*0.8*	0.3	*0.3*	0.5	*0.6*	93.3	*94.3*
20	3.0	*2.8*	2.7	*2.7*	1.0	*0.9*	85.3	*86.1*	6.6	*6.5*	0.5	*0.4*	0.3	*0.2*	0.6	*0.4*	93.4	*93.9*
21	3.4	*3.1*	1.5	*1.7*	0.7	*0.7*	89.1	*89.3*	4.6	*4.9*	0.4	*0.3*	tr	*tr*	03	*tr*	94.8	*95.2*
22	3.2	*2.9*	2.4	*2.8*	0.6	*0.7*	89.5	*88.4*	4.2	*4.4*	tr	*0.8*	0.1	*tr*	tr	*tr*	94.3	*94.3*
23	3.6	*3.7*	1.6	*3.0*	0.4	*0.4*	87.7	*89.2*	5.2	*3.7*	0.9	*tr*	0.2	*tr*	0.4	*tr*	94.2	*93.3*
24	3.7	*3.4*	1.6	*2.4*	0.6	*0.8*	89.4	*89.2*	3.7	*3.7*	0.5	*0.5*	0.3	*tr*	0.2	*tr*	94.2	*94.2*
25	3.3	*2.7*	1.8	*2.4*	0.7	*1.0*	89.2	*88.9*	4.4	*4.0*	0.4	*0.5*	tr	*0.5*	0.2	*tr*	94.7	*94.4*
26	3.3	*2.9*	1.7	*2.5*	0.7	*0.8*	89.4	*88.7*	4.1	*4.3*	0.5	*0.8*	0.3	*tr*	tr	*tr*	94.7	*94.6*
27	3.3	*3.1*	2.8	*2.5*	0.4	*0.5*	86.6	*87.0*	5.6	*5.5*	0.6	*0.9*	0.3	*0.2*	0.4	*0.3*	93.2	*93.9*
28	3.3	*3.2*	2.5	*2.9*	0.3	*0.4*	84.7	*85.6*	7.8	*6.4*	0.6	*1.1*	0.3	*tr*	0.5	*0.4*	93.4	*93.5*
mean value	3.4	*3.3*	2.1	*2.4*	0.7	*0.7*	87.2	*87.3*	5.6	*5.7*	0.6	*0.6*	0.2	*0.2*	0.4	*0.4*	94.1	*94.1*

only find very slight differences in the composition of free sterols as compared to total ones. Campesterol was comprised between 2.8 and 4.0%, with an average of 3.34%, into the total sterol fraction and ranged from 2.1 to 3.8%, with an average of 3.19%, into the free sterol fraction; stigmasterol ranged from 1.2 to 3.1% (2.2% on average) in total fraction and from 1.2 to 3.4% (2.56 % on average) in free fraction.

Sterol compositions of current virgin and *lampante* oils, after neutralization, are reported in Tab. VI. As for campesterol and stigmasterol, slight differences in composition between free and total fractions were observed. Total campesterol ranged from 2.6 to 4.0% (3.4% on average) and free campesterol from 2.7 to 4.2% (3.3% on average). Total stigmasterol ranged from 1.2 to 3.0% (2.07% on average) and

free stigmasterol from 1.4 to 3.1% (2.40% on average).

Thus both free and total campesterol and stigmasterol did not show any significant variations after neutralization, hence indicating that this process affects indistinctly these kinds of sterols.

Some differences were found in Δ^7-avenasterol and in $\Delta^{5,24}$-stigmastadienol amounts which resulted lower in current virgin, *lampante* and neutralized oils than in extra virgin ones. Infact, Δ^7-avenasterol showed mean values of 0.3% (free) and 0.1% (total) both in current virgin and in *lampante* and neutralized oils while they ranged from 1.0 % (free) to 0.6% (total) in extra virgin oils; $\Delta^{5,24}$-stigmastadienol showed mean values of 0.5 and 0.4% (free and total, respectively) in current virgin and *lampante* oils, 0.6 and 0.5 % (free and total) in neutralized oils and 0.8% both for free and for total fraction in extra virgin oils.

4. CONCLUSIONS

Over all the 41 samples belonging to the different commercial categories of extra virgin, current virgin and *lampante* oils, the ratio free x 100 / total sterols was shown to be greater than 65%. In extra virgin oils the same ratio was above 70% in 93% of the samples being analyzed.

The neutralized oils showed a 50% loss in total sterols and 60% loss in free sterols. As a consequence, in these oils the ratio free x 100 / total sterols was always comprised between 44.8 and 63.1%.

Based on these results, this ratio could represent a further tool for the evaluation of extra virgin oil quality and somehow a proof of their genuinity. It would be thus desirable to determine the free x 100 / total sterols' ratio as a quality index for extra virgin oils able to exclude, for values over 70%, the presence of neutralized oils. This is a quite interesting result because, despite the provisions included in the EC Regulation no. 2568/91 and in the following ones, it could still be possible to assign the extra virgin class to both virgin, current virgin and *lampante* oils if previously neutralized, deodorized and appropriately mixed with extra virgin ones.

No differences, however, were detected in the composition of free sterol fraction with respect to the total one, except for a slightly lower percentage of free campesterol balanced by slightly higher stigmasterol values both in current virgin and in *lampante* and neutralized oils. No differences in sterol fraction composition were found between virgin and neutralized oils.

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