FOOD COMPOSITION AND ADDITIVES

A Novel Photometric Method for Evaluation of the Oxidative Stability of Virgin Olive Oils

VASSILIS N. KAMVISSIS
Minerva S.A. Edible Oils Enterprises, Analytical Laboratory Department, 165 Tatoiou and Odisseos Str, 14452 Metemorfosis Attica, Factory: 32009 Schimatari Viotias, Greece
EMMANOUIL G. BARBOUNIS
N. Asteriadis S.A., Application Department, 56–58 Sp. Trikoupi St, Athens, 10022, Greece
NIKOLAOS C. MEGOULAS and MICHAEL A. KOUPPARIS
University of Athens, Laboratory of Analytical Chemistry, Department of Chemistry, Panepistimiopolis, Athens, 15771, Greece

The Oxitester method, a novel, simple, and fast photometric method for the evaluation of the antioxidant capacity of olive oils, was validated and compared to the official oil stability index (Rancimat) method. The Oxitester method appeared to be a good alternative to the Rancimat method with adequate correlation for a wide range of virgin olive oil samples, including extrissima virgin olive oils (correlation coefficient 0.88), and extra virgin olive oils of increased acidity (free fatty acids >0.45%, correlation coefficient 0.89). Other quality factors (flavor, free fatty acids content, specific absorbance at 270 and 232 nm, peroxide value, and content of oleic, linoleic, and linolenic acids) were also measured and correlated to the antioxidant capacity values of the Oxitester and Rancimat methods. The Oxitester method, in contrast to the Rancimat method, was indicative of the flavor characteristics of the olive oils and the content of linolenic acid.

Virgin olive oil (VOO), the major edible vegetable oil of Mediterranean countries, is totally obtained from the olive fruit (*Olea europaea* L.) solely by mechanical means (i.e., water washing, filtration, pressure, decantation, or centrifugation), without other further treatment (1). In recent years, as a result of the growing body of evidence of VOO beneficial health effects, the VOO-rich Mediterranean-style diet is highly promoted. In conjunction with its great gastronomic characteristics, VOO has been established as a widespread food product of great value (2, 3).

The quality of VOO is determined by flavor criteria, as stated by the International Olive Oil Council (IOOC), and by several physical and chemical parameters, as indicated by the European Community Codex (EC-2568/91 and its amendments). The highest grade is designated as extra-VOO, which corresponds to the oil that is produced in controlled mild temperature, with limited oxidative or other degradation (1, 4).

Apart from the above standards, which suggest or enforce limits for quality and authenticity criteria, the overall quality of VOO is further highly dependent on its stability over time (shelf life). The evaluation of VOO shelf life seems to be a rather complicated issue, as long as it is a multivariable dependent parameter. The stability of VOOs is mainly attributed to their characteristic fatty acid composition (low unsaturation), but in order to estimate the overall stability behavior, the determination of several stability factors, such as the free fatty acid content, the peroxide value, the specific absorbance, the organoleptic score, and the phenolic compounds, is required (5, 6).

The abundance of oleic acid (56–84%), a monounsaturated fatty acid (18:1, $n = 9$), is a unique feature of VOO among other vegetable oils. Linoleic acid, a diunsaturated fatty acid (18:2, $n = 6$), is present in concentrations between 3 and 21%, and the linolenic acid (18:3, $n = 3$) between 0.4 and 0.9% (7, 8). The main antioxidants of VOO are carotenes and phenolic compounds (polyphenols), including lipophilic and hydrophilic phenols (9). Although the lipophilic phenols, among which are tocopherols, can be found in other vegetables oils, the profile of VOO hydrophilic phenols is unique, with a well-established antiradical activity (10, 11).

Polyphenols is a broad term used in the natural products literature to define substances that possess a benzene ring bearing one or more hydroxy groups, including functional derivatives (12). Phenolic acids with the basic chemical structure of C_6-C_1 (benzoic acids) and C_6-C_3 (cinnamic acids), such as caffeic, vanillic, syringic, *p*-coumaric, *o*-coumaric, protocatechuic, sinapic, and *p*-hydroxybenzoic acid, were the first group of polyphenols observed in VOO (13–15). The prevalent phenols in VOO, however, are the secoiridoids that are characterized by the presence of either elenolic acid or elenonic acid derivatives in their molecular structure. These compounds (e.g., oleuropein, demethyl oleuropein,

Received November 30, 2007. Accepted by SG March 30, 2008.
Corresponding author's e-mail: vkamvi@minerva.com.gr

ligstroside) are derivatives of the secoiridoids glucosides of olive fruits. The breakdown products of the 2 major phenolic constituents of the olive fruit, oleuropein and ligstroside, form the majority of the phenolic fraction (16).

For the prediction of the overall olive oil resistance to oxidation under storage conditions, the oil stability index (OSI) method [commonly called Rancimat method, American Oil Chemists' Society (AOCS) Cd 12b-92] is the most widely used. In the OSI method, purified air is passed through the heated sample. The effluent air from the oil sample is then bubbled through a vessel containing deionized water, in which the conductivity is continuously monitored. The OSI is defined as the time, expressed in hours, that is needed to reach the maximum change of conductivity (due to volatile organic acids swept from the oxidizing oil). Other available methods are the Swift test (International Union of Pure and Applied Chemistry 2.506), the Active oxygen method (AOCS Cd 12-57), the Oven storage test for accelerated aging of oils (AOCS Cg 5-97), the Accelerated light exposure of edible oils (AOCS Cg 6-01), and several others of minor use (17, 18). Apart from the Rancimat method, none of the previous methods is supported by a commercially available instrument, and therefore a significant variance is observed among independent laboratories or analysts. Furthermore, a common drawback of the previous methods is the prolonged analysis time and the elevated temperature of the test (accelerated degradation), which may result in deviations from the ambient–shelf mechanism of autoxidation (19).

A different way to estimate the olive oil shelf life is the determination of individual classes of antioxidants. Most common of them is the spectrophotometric and the high-performance liquid chromatographic/diode array detection/mass spectrometric determination of phenolic compounds (20).

The Oxitester method was recently introduced by the CDR S.r.l., Production and Development of Cybernetic Systems (Ginestra Fiorentina, Florence, Italy) and it is supported by a commercially available instrument. The method is based on photometry and a redox reaction, in which the antioxidant (reductive) substances of VOO react with a colored free-radicals reagent, reducing its visible absorption. The reduction of absorption (at 505 nm) is correlated to the total polyphenols content of the sample, expressed as μg/g of gallic (or caffeic) acid. In contrast to the other available methods, the Oxitester method does not include any sample treatment and sophisticated procedure, and therefore it is a very fast and low-cost method, ideal for routine analysis.

This paper describes the evaluation of the ability of the Oxitester method to estimate the oxidative stability of VOO. The proposed method was validated and compared to the Rancimat method. The oxidative stability of several samples of Greek VOO were determined (both with the Oxitester and the Rancimat method), along with other stability factors (flavor, FFA content, specific absorbance at 270 and 232 nm, peroxide value, and oleic, linoleic, and linolenic acids). The correlation between the Oxitester and the Rancimat methods was investigated, as well as the correlation of the antioxidant capacity and the several stability factors.

Materials and Methods

Instrumentation and Reagents

The analytical system used to perform the Oxitester method is commercially called CDR-Oliveoxitester, and is provided by CDR S.r.l. (Ginestra Fiorentina). This compact instrument consists of a UV-Vis photometer of selectable dual wavelength, based on interferential filters and a solid-state detector. CDR-Oliveoxitester includes a central processing unit (MC 68331 microprocessor), 12 thermostated incubation positions (at 37°C), and 3 independent measurement cells (also thermostated at 37°C).

Gallic acid monohydrate standard material (puriss., ≥99%) was purchased from Riedel-de Haën (Seelze, Germany). Aqueous standard solutions were prepared daily.

The required oxidative reagent was exclusively provided by CDR S.r.l. (Part Nos. 300478 and 300475), in the form of prefilled cuvettes of single use. Prefilled cuvettes were stored in the refrigerator; they have a 1-year shelf life, do not require any special precaution, and are ready to be used. Apart from the prefilled cuvettes, no other reagent is required.

Extra olive oil samples were provided by local food industries or local producers from various areas of Greece.

Procedures

No pretreatment of the VOO samples (e.g., filtration or dilution) was required. A prefilled cuvette was placed in an incubation cell (37°C) for at least 15 min before measurement. Then the cuvette was placed in the measurement cell and the absorbance of the blank was determined. A 10 μL volume of the sample (VOO) was added, mixed well, and the cuvette was further incubated for 5 min, until the completion of polyphenols oxidation. Absorbance was measured at 505 nm.

Quantitation of antioxidants was performed by external standardization, using standard aqueous solutions of gallic acid in the range of 60–150 μg/g.

The FFA content, peroxide value, organoleptic assessment, specific absorbance, and contents of oleic, linoleic, and linolenic acids were determined according to the EC regulation 2568/91 (4). The OSI (Rancimat method) was determined according to AOCS Cd 12b-92 (17).

Results and Discussion

Comparison of the Oxitester and Rancimat Methods for Evaluation of the Antioxidant Capacity of Olive Oils

The antioxidant capacity of 66 VOO samples from several Greek areas was determined, using both the Oxitester and the Rancimat methods. The organoleptic characteristics [flavor (i.e., fruity, spicy, and bitter); musty, fusty, winey, and rancid defects], FFA content, peroxide value, specific absorbance at 270 (K_{270}) and 232 nm (K_{232}), and content of oleic and linoleic acids were also determined for each sample, since

Table 1. Comparison of the Rancimat and photometric Oxitester methods for evaluation of the antioxidant capacity of Greek extrissima virgin olive oil samples and correlation with other antioxidant factors

Oxitester					Organoleptic score[c]								
Gallic acid, μg/mL	Rancimat equivalence, h[a] *I*	Rancimat, h *II*	Relative difference, % $\frac{(I-II)\times 100}{I}$	FFA, %[b]	Defect	Fr.	Sp.	Bit.	Peroxide value, meq[d] O_2/kg	K_{270}, AU[e]	K_{232}, AU	Oleic acid, %	Linoleic acid, %
120.4	12.1	12.0	0.8	0.33	0.0	5.0	2.5	1.0	5.6	0.117	1.57	77.7	6.03
110.5	11.4	11.3	1.2	0.37	0.0	5.0	3.0	0.8	9.5	0.117	1.88	77.2	6.41
139.2	13.4	12.8	4.2	0.37	0.0	5.0	3.2	1.3	9.5	0.117	1.88	76.2	6.95
138.9	13.3	13.3	0.3	0.37	0.0	5.0	2.5	1.0	9.4	0.114	1.78	78.7	6.18
132.1	12.9	12.2	5.3	0.35	0.0	5.0	3.0	2.5	9.4	0.110	1.72	78.5	6.23
118.1	11.9	12.0	–0.4	0.37	0.0	5.5	3.5	1.0	8.4	0.110	1.69	78.4	6.29
103.9	11.0	11.6	–5.5	0.40	0.0	5.5	4.0	1.5	7.2	0.118	1.69	77.7	5.72
131.1	12.8	12.4	3.3	0.40	0.0	5.5	4.0	1.5	6.8	0.116	1.70	77.7	5.72
120.1	12.1	12.4	–2.6	0.39	0.0	5.5	3.5	1.0	6.8	0.110	1.66	75.9	6.41
139.8	13.4	14.8	–10.4	0.35	0.0	6.0	4.0	1.5	9.4	0.122	1.79	77.2	6.75
132.1	12.9	12.3	4.6	0.32	0.0	5.0	2.8	1.0	8.0	0.129	1.50	78.6	5.23
115.8	11.8	12.8	–8.6	0.33	0.0	4.5	3.2	0.8	8.3	0.124	1.70	78.3	6.03
103.9	11.0	10.8	1.7	0.29	0.0	4.5	2.8	0.8	11.7	0.143	1.92	78.49	5.14
98.8	10.6	10.5	1.4	0.45	0.0	4.5	2.6	0.7	8.4	0.127	1.76	77.3	6.12
98.2	10.6	10.6	0.1	0.45	0.0	5.0	2.8	0.8	8.8	0.127	1.83	78.3	5.55
100.7	10.8	11.1	–3.0	0.40	0.0	5.0	3.2	1.2	9.6	0.109	1.74	77.2	6.55
100.9	10.8	11.5	–6.6	0.40	0.0	5.0	3.2	1.2	9.2	0.109	1.75	76.4	6.44
95.5	10.4	9.7	7.0	0.42	0.0	5.0	2.8	0.6	9.3	0.130	1.87	76.4	6.55
93.6	10.3	10.5	–1.9	0.41	0.0	4.5	2.5	0.8	7.2	0.140	1.69	76.2	5.87
95.5	10.4	9.4	9.9	0.37	0.0	4.5	3.2	0.6	6.6	0.111	1.57	77.4	7.26
96.3	10.5	10.4	0.8	0.41	0.0	4.5	2.8	0.6	7.2	0.117	1.69	77.6	6.00
94.5	10.4	10.8	–4.2	0.39	0.0	4.5	2.8	0.8	10.6	0.100	1.51	78.3	6.29

[a] Calculated according to the linear regression relationship between the Oxitester and Rancimat values.
[b] FFA = Free fatty acids.
[c] Fr. = Fruity; Sp.= spicy; Bit. = bitter.
[d] meq = Milliequivalents.
[e] AU = Absorbance units.

Table 2. Comparison of the Rancimat and photometric Oxitester methods for evaluation of the antioxidant capacity of Greek nominal acidity virgin and extra virgin olive oil samples and correlation with other antioxidant factors

Oxitester					Organoleptic score[c]								
Gallic acid, μg/mL	Rancimat equivalence, h[a] *I*	Rancimat, h *II*	Relative difference, % $\frac{(I-II)\times 100}{I}$	FFA,%[b]	Defect	Fr.	Sp.	Bit.	Peroxide value, meq[d] O_2/kg	K_{270}, AU[e]	K_{232}, AU	Oleic acid, %	Linoleic acid, %
90.6	9.4	8.9	5.0	0.70[f]	0.3 M	4.0	1.8	0.4	8.6	0.124	1.67	77.1	5.80
100	10.2	9.2	9.1	0.51[g]	0.0	5.0	2.5	0.7	12.2	0.146	2.04	76.2	5.75
82.7	8.8	8.5	2.8	0.56[g]	0.0	4.0	3.5	0.6	8.4	0.114	1.62	77.4	7.02
68.1	7.6	6.2	18.2	0.59[f]	0.5 R + 0.5 M	2.5	1.0	0.4	8.3	0.110	1.93	74.6	8.92
118	11.6	11.6	–0.4	0.49[g]	0.0	4.2	2.5	0.5	10.8	0.147	1.88	77.03	5.95
135.1	13.0	12.6	2.4	0.48[g]	0.0	5.0	4.0	1.0	7.6	0.118	1.71	77.1	6.66
138	13.2	12.3	6.4	0.46[g]	0.0	5.0	3.0	1.0	6.6	0.127	1.66	78.8	5.31
118	11.6	11.5	0.5	0.47[g]	0.0	5.0	3.2	1.0	11.8	0.153	1.88	77.2	5.83
91.9	9.6	9.4	0.8	0.53[g]	0.0	4.0	2.5	0.7	10.5	0.127	1.89	77.5	6.10
100.2	10.2	10.8	–6.6	0.49[g]	0.0	5.0	2.5	0.8	9.0	0.130	1.80	78.1	5.53
118	11.6	11.6	–0.4	0.49[g]	0.0	4.2	2.5	0.5	10.8	0.147	1.88	77.03	5.95
91.5	9.5	10.2	–8.0	0.47[g]	0.0	4.0	0.8	1.8	7.8	0.145	1.87	78.0	6.39
83.5	8.9	10.0	–13.6	0.48[g]	0.0	3.7	2.3	0.4	12.0	0.140	1.98	77.57	5.65
109.6	11.0	12.4	–13.9	0.53[g]	0.0	4.5	2.9	0.8	9.6	0.145	1.85	78.44	5.56

[a] Calculated according to the linear regression relationship between the Oxitester and Rancimat values.
[b] FFA = Free fatty acids.
[c] Fr. = Fruity; Sp.= spicy; Bit. = bitter; R = rancid; M = musty.
[d] meq = Milliequivalents.
[e] AU = Absorbance units.
[f] Virgin olive oil of nominal acidity with flavor defects.
[g] Extra virgin olive oil of nominal acidity without flavor defects (FFA > 0.45%).

Table 3. Comparison of the Rancimat and photometric Oxitester methods for evaluation of the antioxidant capacity of Greek extra virgin olive oil samples with fruity ≤4.0, spicy <2.5, bitter <0.6, and virgin olive oil samples with any kind of flavor defect (rancid, musty, fusty, or winey flavor) and correlation to other antioxidant factors

Oxitester					Organoleptic score[c]								
Gallic acid, μg/mL	Rancimat, equivalence, h[a] *I*	Rancimat, h *II*	Relative difference, % $\frac{(I-II)\times 100}{I}$	FFA,%[b]	Defect	Fr.	Sp.	Bit.	Peroxide value, meq[d] O_2/kg	K_{270}, AU[e]	K_{232}, AU	Oleic acid, %	Linoleic acid, %
139.5	13.5	15.5	–15.1	0.24	0.0	4.0[f]	3.8	1.2	7.1	0.107	1.67	78.7	6.37
147.3	14.1	16.4	–16.6	0.27	0.0	4.0[f]	4.0	1.5	6.6	0.109	1.73	78.8	6.38
95.5	10.1	9.4	6.8	0.37	0.0	4.0[f]	3.2	0.6	6.6	0.111	1.57	77.4	7.26
100.5	10.5	11.1	–6.0	0.42	0.0	5.0	2.0[f]	0.8	6.4	0.119	1.73	77.5	6.06
102.9	10.7	11.5	–8.0	0.37	0.0	6.0	2.0[f]	0.5[f]	9.2	0.110	1.61	78.0	5.88
99.6	10.4	10.8	–3.9	0.38	0.0	5.0	2.0[f]	0.5[f]	11.4	0.110	1.78	78.2	5.78
99.9	10.4	10.5	–0.7	0.42	0.0	4.5	1.6[f]	0.6	8.7	0.140	1.69	78.5	5.19
101.3	10.5	11.3	–7.3	0.42	0.0	5.5	2.3[f]	0.5[f]	6.8	0.135	1.64	76.0	5.81
133.2	13.0	12.4	4.5	0.39	0.0	6.0	2.0[f]	0.5[f]	9.2	0.112	1.67	78.0	5.88
121.5	12.1	12.0	0.7	0.37	0.0	6.0	2.0[f]	0.5[f]	9.0	0.110	1.63	78.0	5.88
138.2	13.4	14.7	–10.0	0.37	0.0	4.0[f]	2.2[f]	0.7	6.0	0.132	1.75	76.4	6.70
137.9	13.3	13.5	–1.2	0.37	0.0	5.0	2.0[f]	0.8	7.4	0.108	1.72	78.1	7.02
99.0	10.4	10.8	–4.3	0.35	0.0	5.5	2.0[f]	1.0	6.8	0.133	1.60	78.4	5.05
97.9	10.3	11.0	–7.1	0.43	0.5 R[f]	4.0[f]	1.0[f]	0.5[f]	6.6	0.140	1.74	78.3	5.36
94.0	10.0	10.8	–8.3	0.35	0.0	5.5	2.0[f]	1.0	6.8	0.133	1.60	78.4	5.05
99.7	10.4	11.0	–5.7	0.43	0.5	4.0[f]	1.0[f]	0.5[f]	6.6	0.140	1.74	78.3	5.36
92.4	9.8	11.5	–16.8	0.40	0.0	4.0[f]	2.0[f]	0.5[f]	9.5	0.111	1.73	77.6	6.60
101.7	10.6	12.4	–17.4	0.41	0.0	4.0[f]	2.2[f]	0.6	9.1	0.111	1.73	77.6	6.60
99.1	10.4	11.7	–12.9	0.42	0.2 R[f]	5.0	2.8	0.6	10.1	0.136	1.82	77.4	5.77
89.3	9.6	8.8	8.4	0.31	0.7 R[f]	5.0	2.3[f]	0.8	11.1	0.148	1.91	78.7	5.34
91.2	9.8	9.0	7.7	0.51	0.5 R[f]	4.0[f]	2.7	0.8	10.8	0.139	1.91	77.4	6.05
86.6	9.4	10.5	–11.7	0.36	2.0 M[f]	5.0	2.5	0.9	9.5	0.100	1.68	77.9	6.72
65.0	7.7	12.5	–61.5	0.38	1.0 M[f]	5.0	2.2[f]	0.8	6.3	0.134	1.65	74.8	6.65
88.2	9.5	12.1	–27.1	0.39	0.8 M[f]	4.0[f]	2.5	1.0	5.7	0.139	1.44	77.0	5.73
82.2	9.1	10.6	–17.0	0.37	0.5 M[f]	5.5	2.6	0.8	8.4	0.120	1.56	78.0	5.80
85.2	9.3	11.9	–28.1	0.39	0.8 M[f]	4.8	3.0	1.1	10.1	0.111	1.49	76.9	6.12
67.2	7.9	11.1	–40.4	0.34	1.1 M[f]	5.4	2.4[f]	1.0	7.1	0.141	1.69	75.0	6.72
83.1	9.1	11.7	–28.1	0.39	0.7 M[f]	4.0[f]	2.5	0.7	10.0	0.144	1.90	78.0	5.10
84.4	9.2	10.4	–12.7	0.38	1.4 M[f]	4.8	2.3[f]	1.0	9.2	0.112	1.72	77.8	6.69
75.4	8.5	10.0	–17.1	0.42	1.2 M[f]	3.7[f]	2.2[f]	0.4[f]	12.0	0.151	1.98	77.5	5.61

[a] Calculated according to the linear regression relationship between the Oxitester and Rancimat values.

[b] FFA = Free fatty acids.

[c] Fr. = Fruity; Sp.= spicy; Bit. = bitter; R = rancid; M = musty.

[d] meq = Milliequivalents.

[e] AU = Absorbance units.

[f] Extra virgin olive oil samples with fruity ≤4.0, spicy <2.5, bitter <0.6, and virgin olive oil samples with any kind of flavor defect (rancid, musty, fusty, winey flavor).

Table 4. Influence of fortification of an extra VOO with reference defective samples on its antioxidant capacity as estimated by the Oxitester and Rancimat methods

	Antioxidant capacity	
Spiking reference[a]	Rancimat, h	Oxitester, μg/g
No fortification	10.2 (±0.5)	107.8 (±2.0)
Fusty	10.0 (±0.4)	82.8 (±1.8)
Winey	9.8 (±0.4)	80.2 (±1.3)
Rancid	8.5 (±0.3)	83.5 (±1.3)

[a] Fortification level = 10%.

they are considered to contribute to the antioxidant profile of VOO. Results are presented in Tables 1–3.

Samples were classified into 3 categories: (*1*) extrissima VOO [i.e., FFAs ≤0.45%; good flavor profile (i.e., fruity >4.0; spicy ≥2.5; bitter ≥0.6), and no organoleptic defect]; (*2*) extra VOO and VOO of nominal acidity, with or without flavor defect (i.e., FFAs >0.45%); and (*3*) VOO with any kind of flavor defect (i.e., fruity ≤4.0; spicy <2.5; bitter <0.6; rancid, musty, fusty, or winey flavor).

Extrissima VOO

A total of 22 samples belonged to this category. Results from the proposed Oxitester method (units: μg/mL gallic acid) showed good linear correlation to the Rancimat method (units, h; correlation coefficient >0.88, according to the least-squares method). Linear regression 1: *Rancimat*, h = 4.01 (±0.90) + 6.72 (±0.79) × 10^{-2} × *Oxitester*, μg/mL.

The relative difference between the 2 methods was within the range of –10 to 10%, and the mean relative difference was –0.13% (Table 1).

The other parameters of the antioxidant profile of the samples showed little or no correlation to the results of the Rancimat or Oxitester methods, probably because of the multiparametric dependence of the overall antioxidant capacity of the VOO.

Extra VOO and VOO of Nominal Acidity

Fourteen samples belonged to this category (i.e., FFA >0.45%). Results from the proposed Oxitester method showed good linear correlation to the Rancimat method (correlation coefficient >0.89). Linear regression 2: *Rancimat*, h = 2.2 (±1.2) + 8.0 (±1.2) × 10^{-2} × *Oxitester*, μg/mL.

The relative difference between the 2 methods ranged from –14 to 18%, and the mean relative difference was 0.16% (Table 2).

No statistic difference was observed (*t*-test) between the intercepts and slopes of linear regressions 1 and 2 (extrissima and nominal acidity olive oils, respectively). Therefore, a common linear regression for the extrissima and nominal acidity olive oils could be established (correlation coefficient >0.88).

Linear regression 3: *Rancimat*, h = 2.74 (±0.75) + 7.69 (±0.66) × 10^{-2} × *Oxitester*, μg/mL.

Flavor Defect VOO

Thirty samples belonged to this category. In general, the antioxidant capacity of VOO is considered to be influenced by its flavor profile. In contrast to the previous 2 extra VOO classes, the Oxitester and Rancimat methods showed poor correlation (Table 3). The relative difference between the 2 methods ranged from –61.5 to 8.4% and the mean relative difference was –11.5%. For the previous 2 categories, it was only –0.13 and 0.16%, respectively. Because the mean relative difference between the Oxitester and the Rancimat methods was significantly negative, a lower antioxidant estimation of the Oxitester than the Rancimat method was concluded. This conclusion implied that the Oxitester method was indicative of the flavor defect of the VOO, in contrast to the Rancimat method.

Especially for musty VOO (defect produced by yeast activity), the mean relative difference between the 2 methods was enhanced (nearly 2 times) and became equal to –20.3%.

In order to establish the dependence between the Oxitester method and the VOO degradation (due to fermentations), an extra VOO was spiked by reference defective samples of IOOC (fortification level, 10%). Results are presented in Table 4. For the rancid spiked sample, both methods determined a significant reduction of the antioxidant estimation (*t*-test), but for the winey and fusty spiked samples, only the Oxitester method showed a significant reduction.

Correlation of the Antioxidant Capacity to the Linolenic Acid Content

Linolenic acid is a triunsaturated fatty acid (18:3, n = 3), which is involved in the process of VOO oxidation. Unsaturated bonds in the carbon chain of the fatty acids are potential sites of oxidation, and thus, the concentration of linolenic acid plays a more significant role than the oleic (monounsaturated) and linoleic (diunsaturated) acids.

The antioxidant capacity of 54 samples of VOO (with varying linolenic acid content) was determined, both with the Rancimat and the Oxitester methods. The results revealed that the Oxitester method, in contrast to the Rancimat method, is

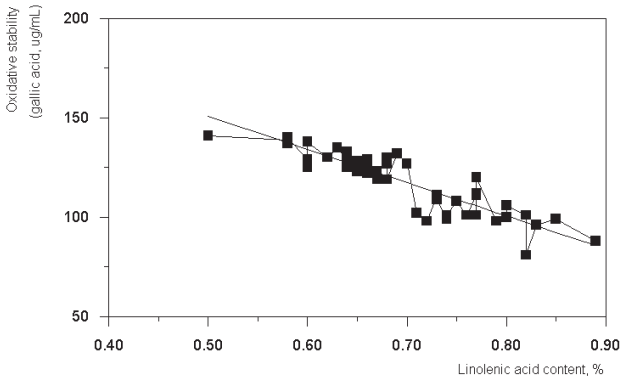

Figure 1. Linear correlation between the linolenic acid content (%) and the antioxidant capacity, as determined by the Oxitester method (gallic acid μg/g).

Table 5. Validation data for the photometric Oxitester method

Precision (RSD, %, n = 8)/antioxidant capacity	
High (90 μg/g)	1.6
Medium (115 μg/g)	1.3
Low (180 μg/g)	1.6
RSD, %, intraday ($n = 3 \times 6$)[a]	1.7
Linear regression (n = 5)	
Range, μg/g	10–900
Intercept (±SD)	–0.9 (±2)
Slope (±SD)	0.913 (±0.014)
Quantitation limit, μg/g	10
Recovery from spiked samples[b], %	100.5 (±1.5)
Estimated No. of samples/h by 1 analyst	70
Correlation coefficient (n = 5)	0.991

[a] RSD = Relative standard deviation; 3 working days, 6 determinations/day of a VOO, 115 μg/g antioxidant capacity.

[b] Five independent fortification levels within the range of 10–900 μg/g.

indicative of the linolenic acid content. Correlation coefficient among the linolenic acid content and the antioxidant capacity of the Oxitester method was 0.90 (Figure 1); the corresponding coefficient for the Rancimat method was only 0.16.

Validation of the Oxitester Method

Table 5 summarizes the validation data of the Oxitester method. Good linearity and precision were obtained, and the quantitation limit (expressed as gallic acid) was about 10 times lower than the nominal content of olive oils. The accuracy of the method was evaluated by recovery experiments. A VOO of low antioxidant capacity was fortified by a VOO of high antioxidant capacity, at 5 fortification levels. The recovery was in the range of 98–102%, and the mean recovery was 100.5 ± 1.5%.

Further study of the matrix effect on the determination of the antioxidant capacity was conducted by dilution experiments [determination of the equivalent to gallic acid content in several diluted solutions (n = 5) of an olive oil sample]. The correlation curves of the determined gallic acid content (in the diluted solutions) versus the dilution factor D ($V_{initial}/V_{final}$) were very linear ($r > 0.99$), with a slope equal to the antioxidant capacity of the olive oil sample and a statistically (proven by t-test) zero intercept. Similarly, the correlation curves of the determined gallic acid content (in the undiluted olive oil) versus D were very linear with statistically (proven by t-test) zero slope. These results confirmed the absence of any systematic (constant or proportional) determinate error from the matrix compounds.

Conclusions

The Oxitester method appeared to be a fast and reliable method for the evaluation of the antioxidant stability of VOO. The extrissima and nominal acidity olive oils showed good correlation with the official Rancimat method. Moreover, the Oxitester method appeared to be flavor-dependent; for flavor-defective samples, a lower antioxidant capacity was determined in comparison to the Rancimat method (especially for musty and winey VOO). This is possibly because according to the Oxitester method, the oxidation of VOO is conducted in mild ambient conditions by free radicals, simulating the natural autoxidation process. On the other hand, according to the Rancimat method, olive oils are oxidized at high temperature by a passing stream of purified air.

The Oxitester method was found to be indicative of the linolenic acid content, a significant degradation parameter, but for the Rancimat method no correlation was proved.

In comparison to the Rancimat method, the Oxitester method requires much shorter analysis time (about 20 min, whereas the Rancimat requires about 12 h). It is also a very simple sample treatment, and therefore of lower cost. All of the previous characteristics are essential for monitoring of the industrial production.

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