Validation of the Oxygen Radical Absorbance Capacity (ORAC) Parameter as a New Index of Quality and Stability of Virgin Olive Oil

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ABSTRACT: The induction period (IP), determined using accelerated methods such as the Rancimat test, is a parameter that has been used to predict the shelf life of virgin olive oil. The oxygen radical absorbance capacity (ORAC) has recently been proposed as a quality index of virgin olive oil because it measures the efficiency of phenolic compounds in the protection against peroxyl radicals. This study aims to investigate relationships between the ORAC and IP values and proposes ORAC as a new parameter of virgin olive oil stability. The concentrations of phenolics, *o*-diphenols, tocopherol, β-carotene, lutein, and ORAC and IP values were determined in 33 virgin olive oils. Regression analyses showed that both ORAC and IP values correlate with total phenols and *o*-diphenols with highly significant indices, whereas the correlations of both ORAC and IP with tocopherols, β-carotene, and lutein were not significant. The ORAC values correlate with the IP values with low but significant indices ($R = 0.42$; $P < 0.02$). The results confirm the key role of phenolic compounds in accounting for the shelf life of virgin olive oil and suggest that the ORAC parameter may be used as a new index of quality and stability.

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KEY WORDS: β-Carotene, extra virgin olive oils, induction period, lipoperoxidation, oxygen radical absorbance capacity, phenolic compounds, tocopherol.

There is great interest in the components that are responsible for the beneficial health effects of the Mediterranean diet. These effects are correlated in particular with a lower incidence of coronary heart disease and cancer (1,2). Positive effects of this diet are related to its high intake of fruits, vegetables, grains, and legumes; the relatively low intake of meat; and the use of extra virgin olive oil in seasonings. Unlike other vegetable oils that people consume, virgin olive oil, the traditional oil of the Mediterranean area, is obtained directly from olives using only a mechanical extraction process. It is then consumed directly, without any further treatment. The health benefits of virgin olive oil are correlated with both its characteristic FA composition (3) and its rich content of natural antioxidants, mainly phenols and tocopherols. The most concentrated phenolic compounds of virgin olive oil include phenolic alcohols, acids, and aglycon derivatives of oleuropein, demethyloleuropein, and ligstroside (4–6). The phenolic concentration of virgin olive oil can vary from 50 to 800 mg/kg according to numerous factors such as fruit ripeness, climate, soil, cultivar, and processing conditions (5,7,8). The phenolic concentration has also been correlated with the shelf life of virgin olive oil (9,10). Indeed, antioxidants can react as free radical scavengers in the oil, and their concentrations are associated with stability of the oil (11,12).

Over the last 30 yr, a great deal of attention has been focused on methods that can evaluate the efficiency of the whole pool of antioxidant molecules in protecting against radical-induced oxidation (6,13–15). The oxygen radical absorbance capacity (ORAC) method allows us to make this sort of evaluation, and it is now one of the most widely used methods for fruit juices, vegetables, and pure compounds (14,16). The method also evaluates the antioxidant capacity of biological fluids in subjects fed with foods very rich in antioxidants (17). We have recently applied this method to evaluate the antioxidant activity of the virgin olive oil phenolic fraction (9,10,18) and proposed the ORAC value as a parameter of oil quality and stability. The use of the ORAC method to evaluate stability is quite new since, until now, the resistance of oils to oxidative rancidity was generally measured using accelerated methods, such as the Rancimat test (8).

In this paper, we analyzed for phenolics, *o*-diphenolics, tocopherols, β-carotene, and lutein in 33 virgin olive oils and determined both the ORAC and the induction period (IP), the latter evaluated by the Rancimat test. All possible correlations among these parameters were studied and discussed in terms of their possible significance for the quality, stability, and health benefits of virgin olive oil.

MATERIALS AND METHODS

Chemicals. Porphyridium cruentum β-phycoerythrin (B-PE) was obtained from Boehringer (Mannhein, Germany). 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was obtained from Polyscience (Warrington, PA). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was obtained from Aldrich Chemical Co. (Milwaukee, WI). All other analytical grade chemicals were from Sigma (Milan, Italy).

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TABLE 1
Characteristics of the Extra Virgin Olive Oils Used in This Study[a]

Country[b]	Region	Acidity (% as oleic acid)	Peroxide number (meq O_2/kg)	K_{232} (nm)	K_{270} (nm)
Italy	The Marches	0.36	5.68	1.53	0.14
	n = 11	1.12 *M*–0.17 *m*	18.2 *M*–1.4 *m*	1.98 *M*–1.31 *m*	0.20 *M*–0.11 *m*
	Apulia	0.41	6.7	1.85	0.18
	n = 5	0.50 *M*–0.22 *m*	10.2 *M*–4.2 *m*	1.98 *M*–1.65 *m*	0.22 *M*–0.13 *m*
	Umbria	0.23	10.9	1.73	0.14
	n = 9	0.22 *M*–0.25 *m*	13.5 *M*–8.3 *m*	1.76 *M*–1.69 *m*	0.16 *M*–0.12 *m*
	Venetia	0.50	8.95	1.56	0.12
	n = 4	0.77 *M*–0.30 *m*	11.0 *M*–5.0 *m*	1.81 *M*–1.36 *m*	0.13 *M*–0.11 *m*
Spain	*n* = 4	0.37	13.5	1.95	0.14
		0.56 *M*–0.23 *m*	18.5 *M*–6.9 *m*	2.39 *M*–1.72 *m*	0.17 *M*–0.11 *m*

[a]The reported value is the average of the values of each group of oils. *n* = number of oils; *M* = maximum value, *m* = minimum value found in the group.
[b]Spanish oils were major brand oils; Italian oils were from small local oil mills.

Sample preparation. In this study we used virgin olive oil produced in the 1999/2000 and 2000/2001 seasons in different Italian and Spanish regions. We selected olives (*Olea europea*) of different cultivars. Olives were picked and different mixtures were obtained. Tables 1 and 2 report the average characteristics of our virgin olive oils.

To perform the ORAC assay, the extraction of phenolics was performed as follows: 5 g of extra virgin olive oil was diluted (1:1 wt/vol) with 5 mL of 80% methanol in H_2O (4), vortexed 2 min at room temperature, and then centrifuged 10 min at 5000 × *g*. The supernatant was drawn off, and the extraction was repeated once more. The two supernatants were combined, and the final volume was recorded. The phenolic extract was diluted with 0.075 M Na-K phosphate buffer pH 7.0 (1:20 vol/vol), then used for the assays.

HPLC analysis of phenolic compounds. Methanolic extracts were analyzed for their phenolic composition by the HPLC technique as reported previously (5).

ORAC assay. The method of Cao *et al.* (19), slightly modified by us (18), was applied. The reaction mixture for the assay was prepared in glass cuvettes as follows: 1600 µL of 0.04 µM B-PE in 0.075 M Na-K phosphate buffer pH 7.0, 200 µL of diluted phenolic extract, or 200 µL of 50 µM Trolox. The mixture was kept 10 min at 37°C in the dark, and the reaction was started with the addition of 200 µL of 40 mM AAPH. The fluorescence decay was measured at 37°C every 5 min at 565 nm emission and 540 nm excitation, using a PerkinElmer (Norwalk, CT) LS-5 spectrofluorimeter. The ORAC value refers to the net area under the curve of B-PE decay in the presence of oil phenolic extract or Trolox, minus the blank. The activity of the sample was expressed by µmol of Trolox Equivalents (TE)/g of oil, with the following equation:

$$\text{ORAC (µmol TE/g)} = kah\,[(S_{\text{sample}} - S_{\text{blank}})/(S_{\text{Trolox}} - S_{\text{blank}})] \quad [1]$$

where *k* is the final dilution of the methanolic extract; *a* is the ratio between the volume (liters) of the methanolic extract and grams of oil; *h* is the final concentration of Trolox expressed as µmol/L; and *S* is the area under the curve of B-PE in the presence of sample, Trolox, or buffer solution.

Rancimat test. The Rancimat apparatus (Metrohm Ltd., Herisau, Switzerland) was used to evaluate accelerated oxidation at high temperature, i.e., 120°C with an air flow of 20 L/h (11,12,20,21). Results were expressed as IP, in hours.

TABLE 2
Phenols, *o*-Diphenols, and Liposoluble Antioxidants in the Extra Virgin Olive Oils[a,b]

Country	Region	β-Carotene (mg/kg)	α-Tocopherol (mg/kg)	Lutein (mg/kg)	Phenols (mg/kg)	*o*-Diphenols (mg/kg)
Italy	The Marches	3.0	145.2	1.2	255	87
		3.6 *M*–1.4 *m*	214.2 *M*–76.5 *m*	1.7 *M*–0.9 *m*	471 *M*–65 *m*	246 *M*–11.6 *m*
	Apulia	2.9	139.1	0.4	329	94.6
		4.2 *M*–1.7 *m*	174.2 *M*–106.6 *m*	1.1 *M*–0.3 *m*	527 *M*–171 *m*	179 *M*–57 *m*
	Umbria	8.3*	164.7**	1.8	340	94.3
		8.9 *M*–7.5 *m*	187.5 *M*–137.2 *m*	1.9 *M*–1.7 *m*	415 *M*–292 *m*	130.2 *M*–85.3 *m*
	Venetia	3.1	112.2	1.2	139*	42*
		4.0 *M*–2.6 *m*	124.7 *M*–97.3 *m*	1.4 *M*–1.1 *m*	186 *M*–88 *m*	62 *M*–15 *m*
Spain		3.2	137.7	1.2	269	89.9
		4.0 *M*–2.5 *m*	187.1 *M*–87.7 *m*	2.2 *M*–0.6 *m*	382 *M*–99 *m*	164.7 *M*–26.7 *m*

[a]The reported value is the average of the values of each group. *M* = maximum value; *m* = minimum value in the group of oils.
[b]*Significantly different from all other average values by ANOVA ($P < 0.05$). **Significantly different from the Venetian oil only, but not vs. the other oils.

Other assays. Total phenols and *o*-diphenols were evaluated on the methanolic extract as reported (4,22,23) using 3,4-dihydroxyphenylethanol as standard. Tocopherols, carotenoids, and lutein were tested using HPLC (4,12). Free acidity, peroxide number, absorbance at 232 and 270 nm (K_{232} and K_{270}) were assayed according to the Official EC Methods of Analysis of Oils and Fats (24).

Statistics. Statistical analysis was carried out using the ANOVA with the statistical significance level fixed at $P < 0.05$.

RESULTS

Table 1 shows several commercial parameters of the virgin olive oils used in this study. Oils are grouped according to the area where they were made. Each parameter is presented as an average value, together with the maximal and minimal values found in the group of oils. The indices of Table 1 are completely within the limits required by the European Community for distribution of virgin olive oils.

Table 2 shows the concentration of tocopherols, carotenes, luteins, total phenols, and *o*-diphenols in our groups of oils. Tocopherol and lutein values are quite similar among all the oils, although the Umbrian oil has a tocopherol value higher than that of the Venetian oil and the β-carotene content was significantly higher than in oils from other regions. The extraction in 80% methanol, obtained as reported in the Materials and Methods section, removes more than 95% of the hydrophilic phenols (4), whose typical composition is shown by the HPLC chromatogram reported in Figure 1.

The total phenols and *o*-diphenols were assayed by means of the colorimetric method, and results are reported in Table 2. Significant differences were observed for the phenolic and *o*-diphenolic concentrations, with the Venetian oil showing the lowest values (Table 2).

The IP as well as the ORAC values were determined for all the oils, and a linear regression analysis of the other parameters was performed.

Figure 2 shows that there is a significant correlation between ORAC and total phenols ($R = 0.825$; $P < 0.001$). Figure 3 shows that ORAC and *o*-diphenolic compounds also have highly statistically significant correlation coefficients ($R = 0.795$; $P < 0.001$).

Figure 4 shows the correlation between the IP and phenolic (Fig. 3A) and *o*-diphenolic (Fig. 3B) compounds. Correlation indices were highly significant for both phenolics ($R = 0.787$, $P < 0.001$) and *o*-diphenolics ($R = 0.705$, $P < 0.001$).

Figure 5 shows the regression analysis between ORAC and IP values. The correlation coefficients are low but still significant ($R = 0.42$; $P < 0.02$; 95% confidence interval = 0.42 ± 0.32).

We found no statistically significant correlation between tocopherol, carotene, lutein, and ORAC or IP values (data not shown).

DISCUSSION

In the edible-oil industry, the evaluation of an oil's shelf life is a key step in assessing its quality. The main causes of oil alteration are lipoperoxidative reactions. Peroxyl radicals, produced by interactions with oxygen and PUFA, generate a cascade of free radicals that make the oxidative process unavoidable. In a recent paper, we also showed that hydroxyl

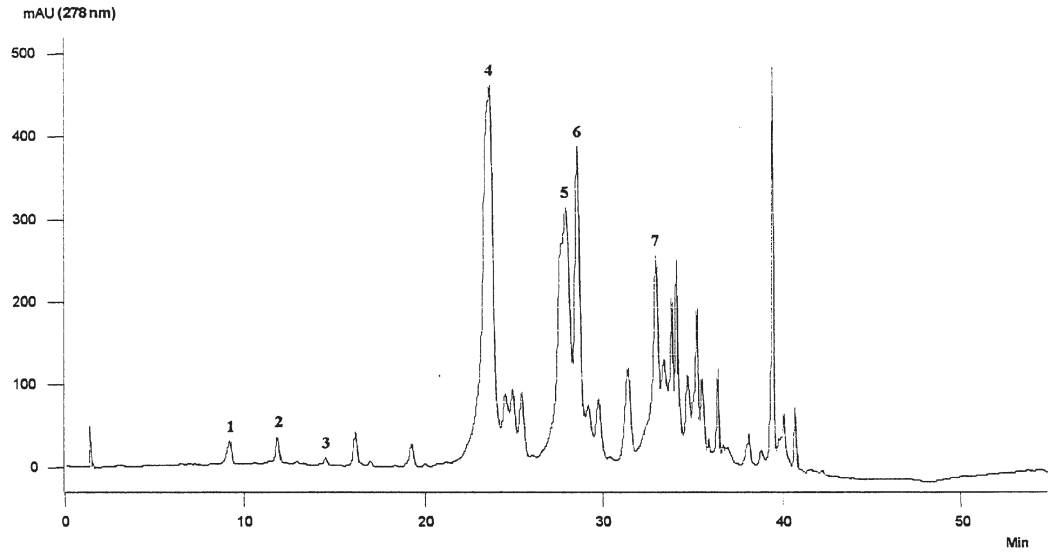

FIG. 1. HPLC chromatogram of the phenolic extract of virgin olive oil recorded at 278 nm. Peak numbers correspond to the following compounds: 1 = 3,5-dihydroxyphenylethanol (3,4-DHPEA); 2 = *p*-hydroxyphenylethanol (*p*-HPEA); 3 = vanillic acid; 4 = dialdehydic forms of elenolic acid linked to 3,4-DHPEA (3,4-DHPEA-EDA); 5 = dialdehydic forms of elenolic acid linked to *p*-HPEA (*p*-HPEA-EDA); 6 = *p*-HPEA ester; 7 = isomer of oleuropein aglycon (3,4-DHPEA-EA).

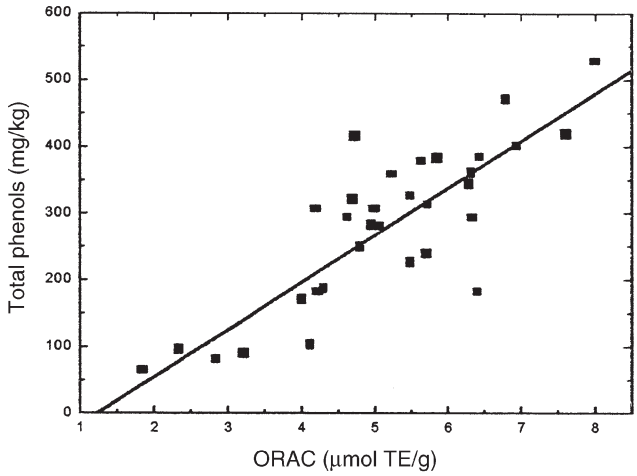

FIG. 2. Regression analysis between oxygen radical absorbance capacity (ORAC) values and total phenolics for 33 virgin olive oils. Values are the average of four determinations. TE, Trolox equivalents. The SD are less than 5%.

radicals, located in water drops of the oil matrix, escape from the water and oxidize the fat moiety (25).

Although unavoidable, the oxidation process can be delayed by endogenous antioxidants of the oil, which scavenge free radicals and metal ions. Many types of antioxidants are found in olive oils, including tocopherols, carotenoids, and phenolic compounds. The analysis of 33 virgin olive oils, reported here, showed that the oils have very similar tocopherol, β-carotene, and lutein values, and therefore these parameters cannot be used to determine an oil's stability against lipoperoxidation. The only significant difference among the groups of oils was found in the tocopherol and β-carotene values, which were highest in the Umbrian oils. These differences may be related to the cultivar origin of this virgin olive oil. β-Carotene markedly enhances the stability of oil exposed to sunlight or fluorescent light (9,26) since the molecule is able to scavenge singlet oxygen, but the effect of β-carotene may be considered negligible when the oil is aged under normal storage conditions, i.e., at 15°C in the dark. The incidence of higher values of β-carotene in Umbrian oils also was irrelevant in terms of its effect on the IP values, probably because at 120°C the molecule is rapidly denaturated and loses its antioxidant properties. Tocopherols too are unstable at high temperatures, so they contribute little to defining the IP value (12). Therefore, the main difference among the oils effectively is related to phenolics content, as shown by the differences between minimal and maximal values in each group (Table 2) and by the concentrations of *o*-diphenols, which demonstrate both the quantitative and qualitative differences

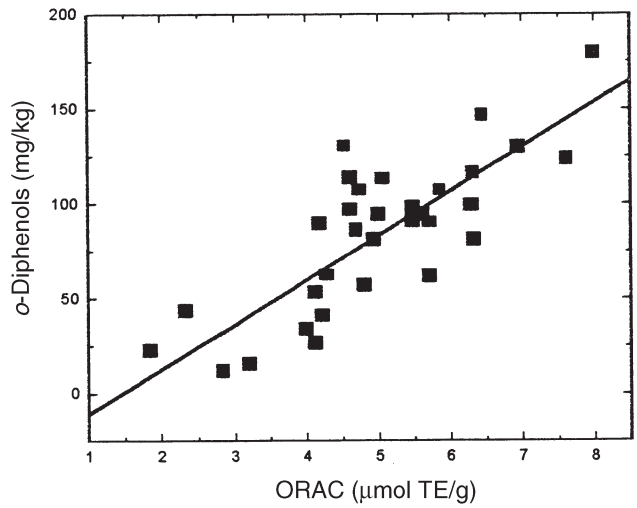

FIG. 3. Regression analysis between ORAC values and *o*-diphenolic compounds for 33 virgin olive oils. Values are the average of four determinations. The SD are less than 5%. For abbreviations see Figure 2.

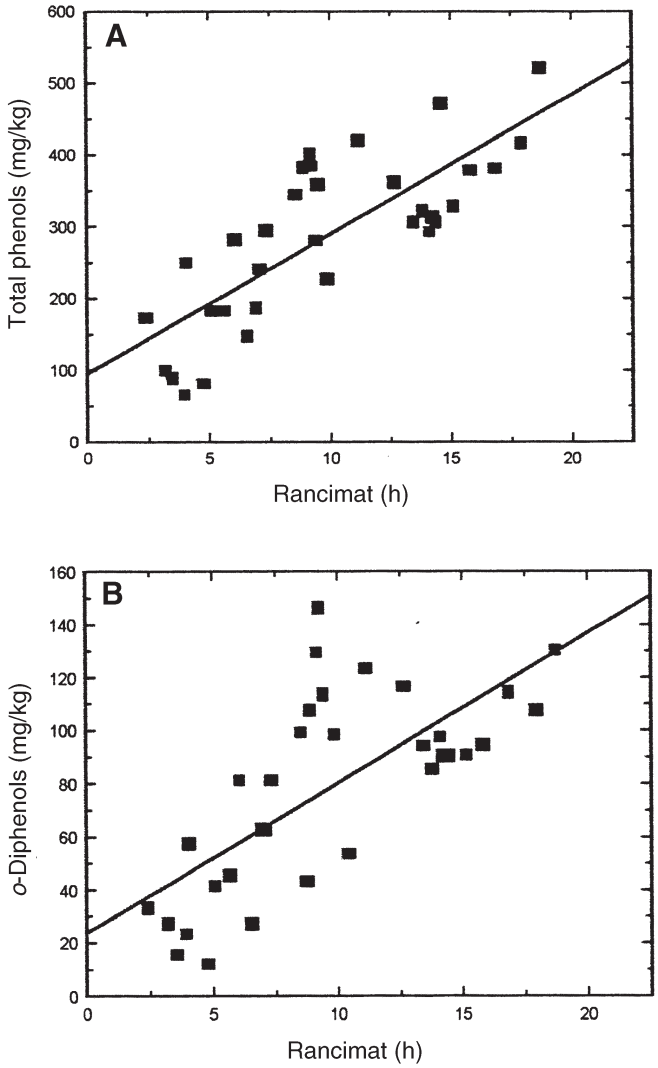

FIG. 4. Regression analysis between the Rancimat induction periods and the total phenols (A) and *o*-diphenols (B). Values are the average of four determinations. The SD are less than 5%.

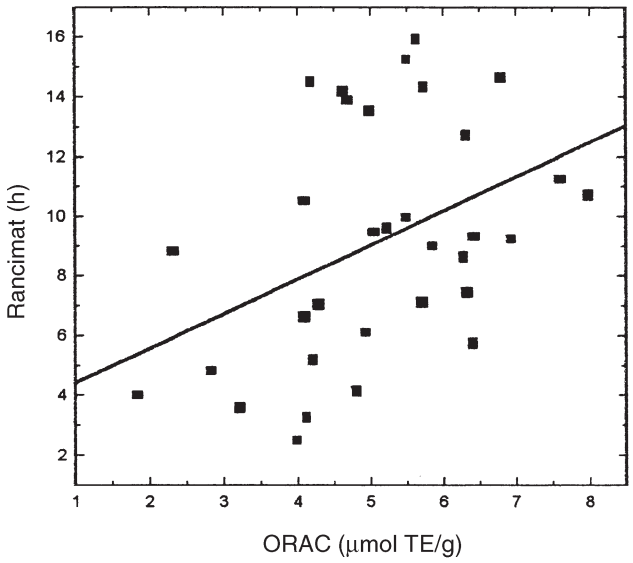

FIG. 5. Regression analysis between the ORAC and Rancimat induction period for 33 virgin olive oils. Values are the average of four determinations. The SD are less than 5%. For abbreviations see Figure 2.

of the phenolic pool. The linear correlations, shown in this paper, between total phenols or *o*-diphenols and the ORAC or IP values strongly suggest the importance of phenolics in protecting against the oxidation of olive oils. Both ORAC and IP values depend markedly on the phenolic pool, even though the Rancimat value takes into account the whole oil moiety, whereas the ORAC value considers any amphipathic antioxidant extracted with 80% methanol as shown by the HPLC profile of this extract of Figure 1. The methanolic extract did not contain lipophilic compounds such as tocopherol (4); consequently, the relatively low correlation between ORAC and the Rancimat method can be related to the antioxidant effect of tocopherol, which is evaluated by the Rancimat test but not by ORAC. The second aspect that can justify the low correlation between the two methods is related to the FA composition, which affects the oxidative stability of oil and is generally different according to their area of origin (27). When the two parameters (Rancimat and ORAC) were correlated with each other, their correlation coefficients were low but significant. Therefore, both may be considered as parameters of stability against oxidation.

The IP parameter has long been used in the assesment of oil quality and stability; the ORAC method is quite new but shows some advantages over IP. These advantages, which lead to the suggestion of using this test together with the IP, are the following: (i) The ORAC value is determined in standard and fixed conditions, i.e., at 37°C in the presence of peroxyl radicals, the radicals which naturally accumulate in the oil during lipoperoxidation. IP values are determined at different temperatures and air fluxes, depending on the stability of the oils. (ii) The assay may be performed within 2–3 h with a spectrofluorimeter, an instrument that is common and widely used in many laboratories. An automatic device has been constructed by Caldwell (28) that can perform 45 ORAC analyses per run. IP values are determined with the Rancimat equipment during 24 h. (iii) The results of the ORAC test are expressed in a way that is as simple as the expression of IP values. In fact, while IP values are generally shown on a scale from 1 to 30 h, the ORAC values range between 1 and 10 μmoL TE/g of oil. The oils in the interval 6–8 μmoL TE/g may be ranked as high-quality oils with a long shelf life.

Consumers are looking for parameters able to measure the healthfulness of a food product; the ORAC method meets this need by providing the potency of the oil to condition the *in vivo* antioxidant status. In samples of plasma or urine from humans, other mammals, and birds are found many compounds that exhibit antioxidant activity, and the ORAC value can be used to detect if particular foods increase the overall antioxidant capacity of biological fluids (29). In this respect, the ORAC value of foods can be correlated with the change of the ORAC value of the plasma (17,30–32), so the potential benefits of an oil can be determined by its effect on the ORAC plasma values. This comparison cannot be performed with the IP test, which sometimes gives values higher than 20 h, but the linkage between this value and the health benefits remains unknown. A high IP value may be due, in fact, to a number of physicochemical interactions among the saponifiable and nonsaponifiable components of the oil, meaning it is very hard to account for an IP value through the identification of single components. On the contrary, the ORAC value is attributable to the amphipathic compounds extracted with methanol, which are the true determinants of the oil shelf life. The ORAC assay distinguishes between oils with maximal or low phenolic antioxidant capacity, and therefore one may be able to compare the ORAC value of the plasma of individuals using the former oils with the ORAC plasma values of subjects using the latter.

The ORAC method provides a way to determine the quality and shelf life of virgin olive oil. This parameter allows us to correlate food intake with the antioxidant capacity of body fluids, thus providing an index of the health-protecting benefits of the phenolic compounds contained in virgin olive oil.

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