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Comparison of the contents of the main biochemical compounds and the antioxidant activity of some Spanish olive oils as determined by four different radical scavenging tests

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Abstract

The aim of this study was to compare the contents of the main biochemical compounds and the antioxidant capacity of five Spanish olive oils by four different antioxidant tests and to find out the most valuable oil for disease preventing diets. Fatty acids, sterols and individual antioxidant compounds in Arbequina, Hojiblanca, Extra Virgin, Picual and Lampante Spanish olive oils were determined. Antioxidant activities were done as well using different radical scavenging activities: total radical-trapping antioxidative potential by ABAP (TRAP-ABAP), radical scavenging activity by DPPH (RSA-DPPH), antioxidant assay by β-carotene-linoleate model system (AA-β-carotene) and total antioxidant status by ABTS (TAA-ABTS). The highest content of all studied antioxidant compounds (353; 329; 4.6 and 2.7 mg/kg for tocopherols, tocotrienols, polyphenols and o-diphenols, respectively) was found in Extra Virgin oil. Also the highest antioxidant capacity was observed in Extra Virgin oil (668nmol/ml; 29.4%; 40.4% and 2.64 mmolTE/kg for TRAP-ABAP, RSA-DPPH, AA- β-carotene and TAA-ABTS, respectively). The correlation between total phenols and antioxidant capacities measured by four methods was very high, but the highest for the β-carotene ($R^2 = 0.9958$). In conclusion, the best method for determination of the antioxidant capacity of olive oils is the β-carotene test. Extra Virgin olive oil has high organoleptic properties and the highest antioxidant activity. The above-mentioned makes this oil a preferable choice for diseases preventing diets.

Keywords: Olive oils; Fatty acids; Sterols; Antioxidant compounds; Antioxidant activity

1. Introduction

The olive oil supplemented diet is effective in prevention of some diseases, including coronary atherosclerosis-the principal cause of death in Western civilization [1-3]. It was observed in experiments *in vitro* and *in vivo* that vegetable oils: a) reduce the incidence and severity of arrhythmias [5,6] b) have antithrombotic properties [7] c) affect lipid peroxidation and antioxidant parameters, and lead to favorable changes in the plasma lipid status [8,9]. These data demonstrate that vegetable oils could be very important in prevention and even treatment of atherosclerosis and other diseases.

Therefore, we decided to compare contents of the main biochemical compounds and the antioxidant capacity of five Spanish olive oils using four different tests (total radical-

Abbreviations: AA β-carotene; antioxidant assay by β-carotene-linoleate model system; RSA-DPPH; radical scavenging activity by 2,2 −diphenyl-1-picrylhydrazyl; TAA-ABTS; total antioxidant status by 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonate); TE; trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalents; TRAP-ABAP; total radical-trapping antioxidative potential by 2,2-azo-bis-2-amidinopropane hydrochloride.

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trapping antioxidative potential by *ABAP (TRAP-ABAP*), radical scavenging activity by DPPH (RSA-DPPH), antioxidant assay by β-carotene-linoleate model system (AA β-carotene) and total antioxidant status by ABTS (TAA-ABTS) in order to find out the most valuable oil for disease preventing diets.

As far as we know, there are no investigations, which use four different antioxidant tests for determination of the antioxidant capacity of olive oils.

2. Methods and materials

2.1. Oil samples

Arbequina, Hojiblanca, Extra Virgin, Picual and Lampante Spanish olive oil samples were purchased in the same supermarket and were produced by various Spanish oil factories. The names of oils correspond to different Spanish olive varieties Picual and Hojiblanca are from South of Spain and Arbequina is growing in Catalonia.

2.2. Determination of fatty acids

Fatty acids were determined according to the analytical methods described in Regulations EEC/2568/91 and C/1429/92 of European Union Commission (EC 1991 Commission regulation EC No 2568/91 July 11, 1991. Official EC Journal L 8121. 10. 1991).

2.3. Determination of sterols

The content of sterols was determined according to procedures described by Gutiérrez et al., 2000 [10].

Fatty acids and individual sterols are given in %.

2.4. Determination of the phenolic compounds

Phenolic compounds were extracted with water-methanol 60:40. Folin-Ciocalteu reagent and sodium molybdate 5% in ethanol 50% reagent (both Merck), respectively, were added to suitable aliquots of the extracts. The absorption of the solution at 725 nm (phenolic compounds) and 370 nm (orthodiphenolic compounds) was measured on a spectrophotometer (Hewlett-Parkard 8450 A UV/vis). Results are given as mg/kg of caffeic acid [11,12].

2.5. Determination of tocopherols

Tocopherols were evaluated following the IUPAC Standard Method (IUPAC, 1992).

Determination of the total antioxidant capacity of oils was done by four antioxidant methods.

2.5.1. *Total radical-trapping antioxidative potential (TRAP by ABAP)*

Peroxyl radicals produced at a constant rate by thermal decomposition of 2,2-azo-bis-2-amidinopropane hydrochloride (ABAP-Polyscience, Warington, PA) are monitored by luminol-enhanced CL. The reaction was initiated by mixing 475 μl phosphate buffered saline, 50 μl 10 mM luminol in 100 mM borate buffer (pH 10.0), and 50 μl ABAP [13]. This mixture was incubated (37°C) in the temperature controlled sample carousel of the luminometer BioOrbit 1251 (BioOrbit, Finland) for 15 min. During this period of time a steady state of the chemiluminescence (CL) signal was reached. The extracts of oils were obtained in organic solvent. 0.5 ml of oil sample was mixed with 2 ml of acetone and was shaken for 1 hr. The acetonic extracts were then separated from oils in a freezer (−25°C, 1 hr) and 20 μl were added directly into the cuvette and the samples were measured for another period of time (τ) until a 50% recovery of the original steady state CL signal. 8.0 nM Trolox (Aldrich Chemical Co., Milwaukee, WI, 6-hydroxy-2,5,7,8-tetramethylchroman-2- carboxylic acid (a water soluble analogue of tocopherol), was used as a reference inhibitor instead of sample. The stoichiometric factor of Trolox (the number of peroxyl radicals trapped per added molecule of antioxidant) is 2.0. The TRAP value for sample measured can be obtained from the equation: TRAP = 2.0 . [trolox] . $\tau_{sample}/f \cdot \tau_{trolox}$, where f is the dilution of sample measured. The results obtained are expressed as nmol of peroxyl radicals trapped by 1 ml of sample. Solvents were verified to have negligible TRAP.

2.5.2. *Radical Scavenging Activity (RSA) Using DPPH Method (DPPH)*

Different concentrations (50 and 100 μl equivalent to 50 and 100 ppm) of oil extracts and BHA (25 and 50 ppm) were taken in different test tubes. The volume was adjusted to 100 μl by adding MeOH [14]. A 0.1 mM methanolic solution of 2,2 −diphenyl-1-picrylhydrazyl radical (DPPH) was added (5μl) to these tubes and shaken vigorously and stand at 27°C for 20 min. The control was prepared as above without any extract, and MeOH was used for the baseline correction. Changes in the absorbance of the samples were measured at 517 nm. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula: % radical scavenging activity = (control OD-sample OD/control OD) × 100.

2.5.3. *Antioxidant Assay (AA) Using β-Carotene Linoleate Model System (β-Carotene)*

β-Carotene (0.2 mg) in 0.2 ml of chloroform, linoleic acid (20 mg), and Tween-40 (polyoxyethylene sorbitan monopalmitate) (200 mg) were mixed [15]. Chloroform was removed at 40°C under vacuum, and the resulting mixture was diluted with 10 ml of water and mixed well. To this emulsion was added 40 ml of oxygenated water. Four milliliter aliquots of the emulsion were pipetted into different test tubes containing 0.2 ml of oil extracts (50 and 100 ppm) and BHA (25 and 50 ppm) in ethanol. BHA was used for comparative purposes. A control containing 0.2 ml of eth-

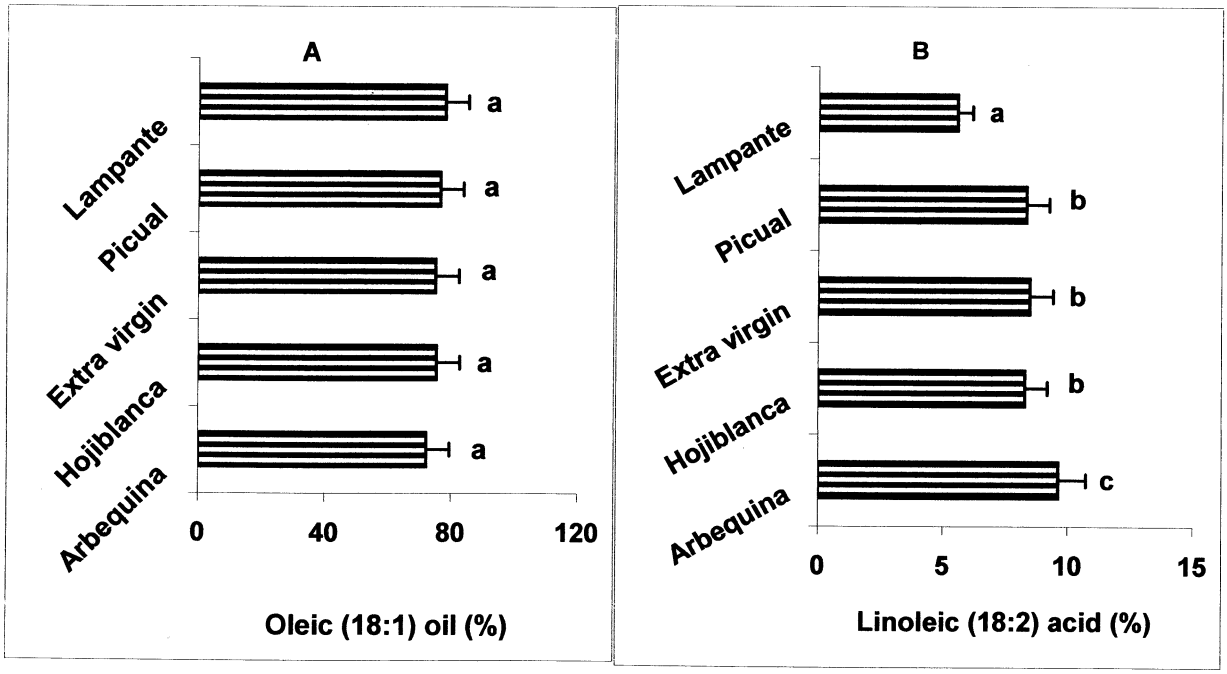

Fig. 1. The contents of oleic (**A**) and linoleic (**B**) fatty acids in 5 studied olive oils. Means ± SD (horizontal lines). Bars with different letters are significantly different ($p < 0.05$).

anol and 4 ml of the above emulsion was prepared. The tubes were placed at 50°C in a water bath, and the absorbance at 470 nm was taken at zero time ($t = 0$).

Measurement of absorbance was continued until the color of β-carotene disappeared in the control tubes (t = 180 min) at an interval of 15 min. A mixture prepared as above without β-carotene served as blank. The antioxidant activity (AA) of the extracts was evaluated in terms of bleaching of the β-carotene using the following formula, $AA = 100\,[1 - (A_0 - A_t)/(A°_0 - A°_t)]$, where A_0 and $A°_0$ are the absorbance values measured at zero time of the incubation for test sample and control, respectively, and At and $A°t$ are the absorbance measured in the test sample and control, respectively, after incubation for 180 min.

2.5.4. *Total antioxidant status (TAA Test with ABTS)*

The TAA was estimated using the ferrylmyoglobin/ABTS method [16]. This technique measures the relative ability of antioxidant substances to scavenge the 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonate) radical cation ($ABTS^{\bullet+}$), compared with Trolox. The radical cation $ABTS^{\bullet+}$, generated in the aqueous phase from ABTS through the peroxidation action of metmyoglobin, is a blue/green chromogen with characteristic absorption at 734 nm. Results are expressed as mmol Trolox equivalents (TE) per kg.

3. Statistics

Values are given as the means ± SD of five measurements. Where appropriate, data were tested by two-way ANOVA using GraphPad Prism, version 2.0 (GraphPad Software, San Diego, CA, following by Duncan's new multiple range test to assess differences between groups means. Differences of $P < 0.05$ were considered significant.

4. Results

The content of myristic acid (14:0) in all studied oils was comparable and his concentration was 0.05%. The highest content of palmitic acid (16:0) was in Arbequina oil (12.47%) and the lowest–in Picual (9.1%).

Also highest content of palmitoleic acid (16:1) was in Arbequina oil (1.15%) and the lowest–in Picual (0.49%).

The highest content of stearic oil (18:0) was in Picual, Hojiblanca and Extra Virgin oils (about 3.5%) and the lowest–in Arbequina oil (1.39%).

The content of linolenic (18:3) oil varied from 0.51 to 0.78% - the lowest in Lampante oil.

The contents of oleic (18:1) and linoleic (18:2) acids in all 5 studied oils are summarized in Fig. 1 A and B.

As can be seen the contents of oleic acid in all oils (Fig. 1 A) is very high (from 72.28% to 78.14% for Arbequina and Lampante oils, respectively). However, these differences (ANOVA) are statistically not significant ($P > 0.05$).

The content of linoleic acid (Fig. 1 B) in three (Hojiblanca, Extra virgin and Picual) of the studied oils are comparable (from 8.3% to 8.49%). However, the content of linoleic acid in Arbequina oil is significantly higher and the content of linoleic acid in Lampante oil is significantly lower ($P < 0.05$).

Table 1
Content of sterols in different Spanish olive oils

Spanish olive oils	Sterols					
	Cholesterol	Brassicasterol	Campesterol	Stigmasterol	β-sitosterol	Δ-7-stigmasterol
Arbequina	0.5 ± 0.05	0.1 ± 0.01	3.8 ± 0.41	0.90 ± 0.1	94.5 ± 10.1	0.37 ± 0.04
Hojiblanca	0.5 ± 0.05	0.1 ± 0.01	3.0 ± 0.31	0.55 ± 0.6	95.3 ± 10.2	0.28 ± 0.03
Extra virgin	0.5 ± 0.05	0.1 ± 0.01	3.7 ± 0.40	0.86 ± 0.9	94.6 ± 10.1	0.30 ± 0.03
Picual	0.5 ± 0.05	0.1 ± 0.04	3.2 ± 0.33	0.40 ± 0.5	95.2 ± 10.2	0.40 ± 0.04
Lampante	0.5 ± 0.05	0.1 ± 0.01	3.0 ± 0.31	0.84 ± 0.9	95.5 ± 10.3	0.28 ± 0.03

Values are means of 5 measurements ± SD. Data are expressed as percentage of total sterols.

According to some publications, cholesterol, which was considered to be an indicator of animal fat, also occurs in small amounts in plants [17].

β-sitosterols and campe-sterols, which are structurally related to cholesterol, dominate the sterol fraction (Table 1). The combined content of these two sterols is about 100% in all oil samples. The content of sterols in all five oils is comparable (ANOVA). The results of the contents of antioxidant compounds and antioxidant capacities of all studied Spanish olive oils by four different tests are summarized in Table 2 and Fig. 2. As can be seen, the Extra virgin oil possesses the highest contents of the antioxidant compounds and the highest antioxidant capacity, whereas the Lampante oil - the lowest.

The correlation between the contents of total polyphenols and results of the determination of the antioxidant capacity were used to compare the sensitivity of the relevant tests (Fig. 3 A, B, C and D).

As can be seen, the correlation between the content of total polyphenols and antioxidant capacity, as determined by antioxidant Assay (AA) Using β-Carotene Linoleate Model System (β-Carotene) is the highest ($R^2 = 0.9958$) and with TAA test is the lowest ($R^2 = 0.9197$).

5. Discussion

As was mentioned [18], during the last 15 years, our international team of cardiologists, biochemists and dietitians has been studying various kinds of nutritional products in order to improve the diet for patients suffering from coronary atherosclerosis [19–22].

The aim of this study was to compare the contents of the main biochemical compounds and the antioxidant capacity of five Spanish olive oils using four different methods in order to find out the most valuable oil for disease preventing diets and the more sensitive test.

To the best of our knowledge, there are no other such comprehensive comparative investigations using four different tests.

The quality of oils is affected by soil conditions, ripeness of olives and the length of storage. Therefore, it was expected that some results of our investigation could be different of other authors.

It was found that the combined contents oleic and linoleic acids in all studied oils exceed 80% and the difference is statistically not significant ($P > 0.05$). These results are in accordance with the results of others [17].

The campe-, stigma- and β-sitosterols, which are structurally related to cholesterol, dominate the sterol fraction. The combined content of these three sterols was 95.5% in all five oil samples. The content of β-sitosterols in all oils was high and exceeds 94.0%. It is known that β-sitosterols are poorly absorbed and lower absorption of cholesterol [23]. Therefore, the high content of this phytosterol in olive oils is an additional advantage.

There are many methods for total antioxidant determination. Every one has its limitations [24,25]. It was shown that some antioxidant assay methods give different antioxidant activity trends [26]. Therefore, the free radical scavenging properties of the studied samples were determined by four tests. According to all four used tests, the highest antioxidant potential was observed in Extra virgin oil.

Table 2
Total polyphenols and antioxidant capacity of all studied Spanish olive oils as determined by four different tests

Samples	Total polyphenols (mg/kg)	TRAP (nmol/ml)	DPPH (% RSA)	β-carotene (% AA)	TAA[d] (mmol TE/kg)
Arbequina	4.1 ± 0.4	541 ± 67	23.1 ± 2.3	33.2 ± 3.3	1.76 ± 0.3
Hojiblanca	4.4 ± 0.4	660 ± 81	26.8 ± 1.7	38.1 ± 3.5	2.14 ± 0.2
Extra Virgin	4.6 ± 0.4	668 ± 49	29.4 ± 1.4	40.4 ± 2.9	2.64 ± 0.4
Picual	4.5 ± 0.5	661 ± 102	27.2 ± 1.2	38.7 ± 2.0	2.31 ± 0.6
Lampante	2.1 ± 0.2	225 ± 42	9.8 ± 0.9	13.2 ± 1.1	0.78 ± 0.1

Values are means ± SD of 5 measurements.

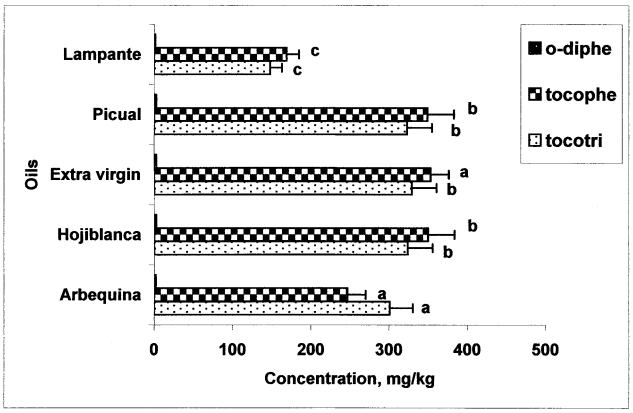

Fig. 2. The content of some antioxidants in 5 studied olive oils. Tocotrienols, tocotri; tocopherols, tocopher; o-diphenols, o-diphen. Means ± SD (horizontal lines). Bars with different letters are significantly different ($p < 0.05$).

It was important to examine the correlation between the content of the main antioxidant compounds (total polyphenols) and the total antioxidant capacity of the studied oils.

Some authors claim that there is no correlation between the total phenolic content and the radical scavenging capacity [25]. The obtained by us results did not support these claims. The correlation of the total phenolic content and the radical scavenging capacity as determined by all four used tests was very high (from $R^2 = 0.9197$ to $R^2 = 0.9958$ for TAA and β-carotene, respectively). Therefore, the best method for determination of the antioxidant capacity of olive oils is the β-carotene test.

These data are in accordance with others, who has shown that high total polyphenols content increases antioxidant activity and there is a linear correlation between phenolic content and antioxidant activity [27–30].

In conclusion, all olive oils have high organoleptic properties and high antioxidant capacity. However, the highest antioxidant capacity makes Extra Virgin oil preferable for diseases preventing diets. Of course, this has to be confirmed in investigations *in vivo*. Therefore, we are planning to conduct an experiment on laboratory animals, in which two olive oils with different contents of antioxidant compounds and different antioxidant capacity will be used.

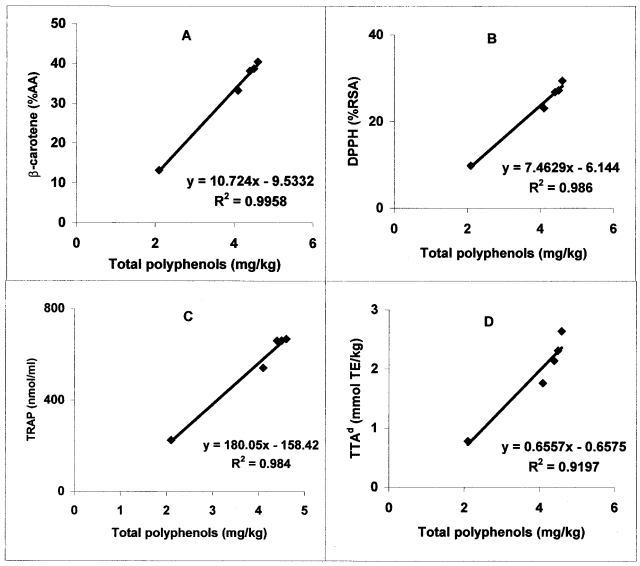

Fig. 3. Correlation between the contents of total phenols in olive oils and their antioxidant capacity as determined by Antioxidant Assay (AA) Using β-Carotene (**A**), DPPH Method (**B**), TRAP (**C**) and TAA Test with ABTS **(D).**

6. Conclusion

1. The most bioactive components of olive oils are their phenolic compounds.
2. According to results of this investigation, the best method for determination of the antioxidant capacity of olive oils is the β-carotene.
3. Extra Virgin olive oil has high organoleptic properties, the highest content of antioxidant compounds and the highest antioxidant capacity. The above-mentioned makes this oil a preferable choice for atherosclerosis preventing diets.

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