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ORIGINAL PAPER

Direct Determination of Antioxidants in Whole Olive Oil Using the SIFT-MS-TOSC Assay

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Abstract A modified Selected Ion Flow Tube Mass Spectrometry Total Oxyradical Scavenging (SIFT-MS-TOSC) assay has been used to assess the antioxidants present in olive oil samples. The assay compares antioxidants in emulsified olive oil competing with added 2-keto-4-methylthiobutanoic acid (KMBA) for peroxyl radicals formed from thermal decomposition of 2,2′-azobis (2-amidinopropane) hydrochloride. No solvent extraction from the oil is required. The assay reaction is monitored by analysis of ethene, a break down product of KMBA, from attack by peroxyl radicals. A new simpler modification to the TOSC assay was found to produce reliable results requiring measurements of just a single concentration of emulsion. The results of the SIFT-MS-TOSC assay show correlation with results from the Folin Ciocalteu assay of total phenol content and HPLC analysis of several standard antioxidants. The use of hydroxyl radicals generated by the Fenton reaction of Fe^{3+} with ascorbic acid was also investigated, but with less repeatable results. A wide range of antioxidant levels differing by more than a factor of 10 were found in 20 different olive oils examined.

Keywords Antioxidants · Fats and oils · Emulsions · Colloids

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Introduction

Antioxidants are considered very important compounds. As many studies have suggested, they can have a beneficial effect on human health when consumed [1], even to lowering the risk of Alzheimer's disease [2]. This has sparked interest in foodstuffs which contain high concentrations of antioxidant compounds, such as olive oil [3]. The antioxidant species present in olive oil and their concentrations may vary greatly for oils of different origin, or even for oils of the same origin from one year to the next. There are many reasons for this variation including the oil extraction technology used, different olive cultivars and different environmental conditions [4–6]. Potential exists for an advantage for any olive oil producer who can demonstrate that their oil consistently contains higher than average concentrations of antioxidant compounds. The only way to show this is via a reliable test to evaluate these compounds.

A number of antioxidant assays exist to quantify the antioxidant capacity of food samples, but comparisons of antioxidant behaviour using different assay methods do not necessarily yield the same results. Schaich [7] has noted that "no single assay accurately reflects all of the radical sources or all the antioxidants in a complex system". Most of these assays are performed in aqueous solutions, as they are designed to detect aqueous radical scavengers [8]. Assays also exist which are conducted in lipid media or emulsions, and results from these assays are not easily compared with those of aqueous assays [9]. Aqueous assays may be used to measure moderately lipophilic antioxidants; however, the antioxidants must first be extracted from their lipid medium using a water-miscible solvent [7]. Solvent extraction does not remove all lipophilic compounds from the original matrix, meaning these compounds will be under-represented in the final assay

solution. A common solvent used to extract antioxidant species from lipid samples in aqueous radical scavenging assays is a mixture of methanol and water [10–12], whereby approximately 90% of the phenolic substances present in a lipid sample are extracted by the methanol/water mixture [13]. More hydrophobic antioxidant species (such as tocopherols and carotenoids) are largely left behind and therefore have little influence on these assay results.

Quite a number of different tests have been used to assess the antioxidant capacity of olive oils. It has been shown that tests of the antiradical properties of olive oils are significantly correlated with total phenolic and *o*-diphenolic content [14]. Sanchez et al. applied four different tests to gauge the antioxidant properties of 39 oils and compared the result to the total phenolic content as appraised by the Folin–Ciocalteu (FC) method [15]. Ninfali et al. examined methanolic extracts of 33 different samples of olive oil and found that the ORAC radical assay and the induction period from the Rancimat test correlated well with the total concentrations of phenolics and diphenolics [16]. Minioti and Georgiou also used radical scavengers on methanolic extracts from olive oil to assess the total antioxidant capacity of 50 Greek oils and found correlation with the total phenolic content as monitored by the FC method [17]. Cerretani and Bendini critically compared a number of the different methods that have been used to assess the antioxidant capacity of olive oil [18]. Frankel also has presented a comparative summary of different methods that have been used to evaluate antioxidants in olive oil [19].

The Selected Ion Flow Tube Mass Spectrometry Total Oxyradical Scavenging Capacity (SIFT-MS-TOSC) assay can be used for the evaluation of radical scavenging capacity of both aqueous and emulsified samples. SIFT-MS is a real-time technique for the analysis of trace gases in whole air. It has been shown to be ideal for analysis of TOSC assay results [20] and to provide a simple test for olive oil freshness [21]. An assay utilising evolved gas is required here as an emulsion does not allow optical absorbance or fluorescence measurements to be carried out on the assay mixture. Further, the ability to use whole oil, without being limited to an extract, means that no antioxidant species are left out of the assay mixture, enabling all antioxidants present to contribute to the results obtained.

The present article outlines preliminary characterisation of the SIFT-MS-TOSC assay for the purpose of defining the assay method and then develops a procedure for simplifying and shortening it. The simplified method was used to measure the antioxidant capacity of 20 olive oil samples. The results were then compared with analyses of the same oils performed by the FC total phenols assay and the concentrations of selected important phenolic compounds using HPLC with UV detection.

Method

SIFT-MS-TOSC Assay

Potassium dihydrogen phosphate, disodium monohydrogen phosphate, and ferric chloride, all AnalaR grade, were purchased from BDH Laboratory Supplies, Poole, England, UK. Diethylenetriaminepentaacetic acid (DTPA), ≥99%, ascorbic acid and α-keto-γ-methylthiobutanoic acid (KMBA) sodium salt were from the Sigma-Aldrich Co., St. Louis, MO, USA. Pluronic P 104 surfactant (a difunctional block copolymer) was from the BASF Corporation, Mount Olive, NJ, USA. 2,2′-Azobis (2-amidinopropane) hydrochloride (AAPH) 97% was from the Aldrich Chemical Company Inc., Milwaukee, WI, USA. Ethylene diamine tetraacetic acid was from Merck KGaA, Darmstadt, Germany.

The following solutions were prepared: Solution 1 was 0.20 mmol l^{-1} DTPA in potassium/sodium phosphate buffer at pH 7.4; solution 2 was 2.3×10^{-2} mol l^{-1} KMBA in distilled water; solution 3 was phosphate buffer at pH 7.4; solution 4 was AAPH at 0.20 mol l^{-1} in phosphate buffer; solution 5 was 0.45 mmol l^{-1} ascorbic acid in phosphate buffer; solution 6 was 3.6×10^{-5} mol l^{-1} $FeCl_3$ and 9.1×10^{-5} mol l^{-1} EDTA in distilled water.

In addition, an oil-in-water emulsion was prepared for each oil by adding 2.00 ml of the oil to 18.00 ml of surfactant solution. The surfactant solution was prepared by adding 20.00 g l^{-1} of surfactant to the phosphate buffer solution. The mixture of oil and surfactant solution was turned into an emulsion by mixing in a D-500 homogeniser (Wiggenhauser, Berlin, Germany) at 10,000 rpm for 10 min.

The total volume of the assay solution for both the peroxyl and hydroxyl radical assays was 10 ml. For the peroxyl radical assay, this was made up of 5.0 ml of solution 1, 100 µl of solution 2, the desired volume of oil-in-water emulsion and the rest made up with solution 3 to make 9.0 ml. 1 ml of solution 4 was added last, to coincide with the beginning of the assay.

For the hydroxyl radical assay, 100 µl of solution 2 was added to 4.0 ml of solution 5 along with the desired volume of oil-in-water emulsion and enough of solution 3 to give a total volume of 9.5 ml. The hydroxyl assay began with the addition of 0.5 ml of solution 6.

Analysis Technique

SIFT-MS-TOSC Assay

The olive oils examined in this study were obtained mainly from growers in the New Zealand region. Fourteen were provided by Olives New Zealand member growers and six were imported oils purchased at local supermarkets. For the initial study, seven bottles were used for each oil per

experiment: one control containing the assay mixture minus emulsion and six additional bottles containing different concentrations of oil emulsion. All bottles were kept at 37 ± 0.2 °C in a water bath throughout the experiment. In the TOSC assay, peroxy radicals generated by thermal homolysis of AAPH react with the added KMBA producing ethene, which is a gas [20, 22]. Any antioxidants present also compete with KMBA for the radical. Therefore, a higher antioxidant presence results in lower ethene production for the oil compared with the ethene produced in the control experiment. In the initial assay, each bottle was analysed for ethene concentration in the head space every 12 min for 1 h. Each analysis took approximately 20 s during which time approximately 10 ml of bottle headspace (1% of total) was sampled. The sample was drawn through a heated, stainless steel needle and inlet system. The pressure was equalized after each analysis by piercing the septum with a needle allowing room air to enter. The concentration of ethene in each bottle during every 12 min segment was calculated as an area (elapsed time multiplied by the average of concentrations at the beginning and end of the interval) [20]. The total area produced for each bottle was divided by the equivalent measurement in the control to give a percent inhibition. The percent inhibition displayed in each bottle was plotted against the volume of oil added to the corresponding bottle and the resultant function interpolated to obtain the volume expected to inhibit 50% of ethene production. This volume is called the VI_{50} value and is shown for one oil in Fig. 1.

Folin–Ciocalteu (FC) Total Phenols Assay

The Folin–Ciocalteu (FC) total phenols assay was performed on methanol/water (80/20 v/v) extracts of olive oil

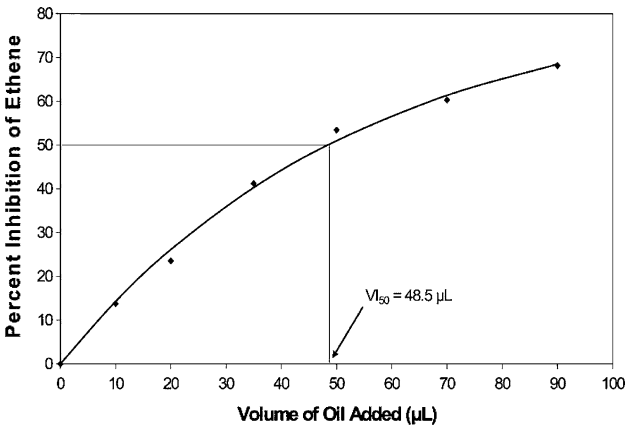

Fig. 1 SIFT-MS-TOSC assay results for one olive oil sample. The raw results consist of ethene head space concentrations over the course of 1 h for six bottles containing different concentrations of the same oil and one bottle containing no oil. From these results the VI_{50} value for that oil is then obtained as a percentage inhibition of ethene production by calculating the function (1-(oil sample area/area under control curve))

phenolic compounds and standard phenolic compounds dissolved in the same solvent mixture. 25 ml of olive oil was shaken vigorously with 10 ml of the methanol/water mixture and centrifuged for 10 min at 1,900 rpm. This was repeated using the same oil with another 10-ml aliquot of the methanol/water mixture and the two resulting 10-ml extracts were combined to give 20 ml. The assay procedure followed was that of Scalbert et al. [23]. Briefly, 100 µl of the extract was added to a 14-ml vial along with 400 µl of methanol/water (80/20 v/v). Then, 2.5 ml of the Folin-Ciocalteu reagent (Sigma-Aldrich Co., St. Louis, MO, USA) diluted 1:9 with water was added to the vial. The vial then stood for 8 min with occasional shaking, after which 2 ml of 75 g l^{-1} sodium carbonate solution was added. The reaction mixture was transferred to a water bath at 50 °C for 5 min then cooled to room temperature. The absorbance was measured at 760 nm.

HPLC Analysis

Selected phenolic compounds were quantified in olive oil extracts following the method of Tovar et al. [24]. A 20-ml aliquot of methanol/water 80:20 v/v was mixed with 45 g of olive oil and separated at 1,900 rpm in a centrifuge for 10 min. The methanol/water was decanted off and the oil was extracted again with another 20 ml of methanol/water. The two extracts were combined to give 40 ml. This extract was concentrated in a rotary evaporator at 40 °C until a syrup-like consistency was reached. This concentrate was dissolved in 5 ml of acetonitrile and washed with 20 ml of hexane three times. The total 60 ml of hexane were extracted with a further 5 ml of acetonitrile. The resulting 10 ml of acetonitrile was evaporated in a rotary evaporator and this syrup-like mixture was dissolved in 5 ml of acetonitrile. Of this solution, a further 2 ml was evaporated and the residue dissolved in 1 ml of methanol.

The HPLC system consisted of a Dionex ASI-100 autosampler, P680 HPLC pump, TCC-100 thermostatted column compartment and a UVD340U detector. The column was a Waters Spherisorb ODS-2 (5 µm, 25 cm × 4.6 mm i.d., Alltech Associates, Inc., Deerfield, IL, USA) maintained at 35 °C. The mobile phases were 0.2% acetic acid in water and methanol, flowing at 1 ml min^{-1}. The initial composition was 90% water and 10% methanol. This was changed to 30% methanol in 10 min and kept at 30% for 15 min. Methanol was raised to 40% in 10 min and maintained for 5 min. It was then raised to 50, 60, 70 and 100% in 5 min intervals. Initial conditions were reached in 15 min to give a total run time of 75 min. Chromatograms were obtained at 280 nm. Compounds were identified by comparison of retention times and UV/Vis spectra with commercially available olive oil antioxidant standards.

These included tyrosol, hydroxytyrosol, trolox, *p*-coumaric acid and vanillic acid.

Data Analysis

Data display and plotting were performed using Microsoft Excel. Function fitting and multivariate analyses were performed using Matlab 6.5 student version, release 13. The freely available Matlab functions leasqr.m and its companion dfdp.m [25] were used for least-squares fitting of univariate models. Principal Component Analysis (PCA) was performed using the algorithm presented by Reyment and Jöreskog [26].

Results and Discussion

All the oils analysed using the SIFT-MS-TOSC assay gave very similar inhibition functions and all were well described by the same inverted exponential decay function of the form: $y = a(1 - e^{-bx})$. The raw data consists of ethene concentrations relative to the control over the course of 1 h for six bottles that contain different volumes of the oil. From these data the VI_{50} value was found as shown in Fig. 1 for an individual oil. The results for each oil were then scaled to allow a direct comparison between oils by dividing all oil concentrations by the VI_{50} value measured for that oil. These scaled results for ethene generation by the peroxyl radical fit well to an inverted exponential function as shown in Fig. 2 ($R^2 = 0.97$). When the hydroxyl radical was used to generate ethene, the fit of the scaled results, although not matching those for peroxyl radicals, is still quite reasonable as shown in Fig. 3 ($R^2 = 0.94$). These results support the development of a routine SIFT-MS-TOSC assay for analysis of olive oil antioxidant capacity.

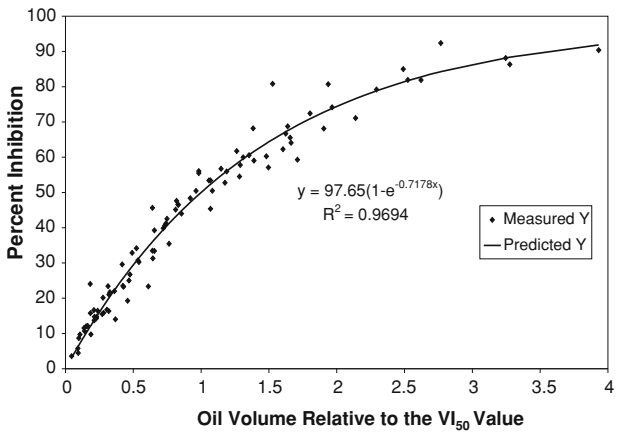

Fig. 2 The results obtained by recording the inhibition scores of 16 *peroxyl radical* assays of different oil emulsions are shown. Each assay was linearly adjusted to exhibit 50% inhibition at the arbitrary value of 1 for direct comparison with the other assays. The inverse exponential function that best fits the data is shown

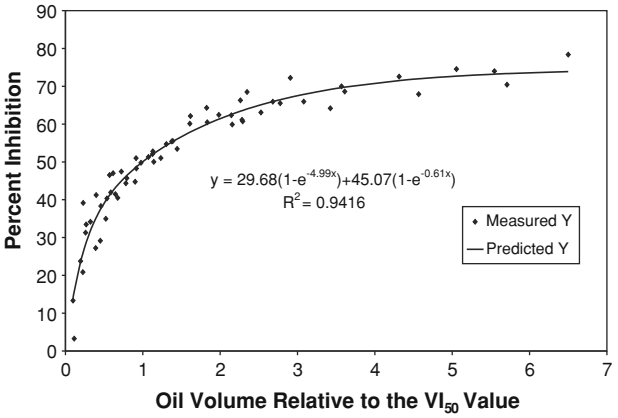

Fig. 3 Results corresponding to those of Fig. 2, are plotted here for 10 assays for the *hydroxyl radical*. These results were also adjusted so that each assay exhibits 50% inhibition at the arbitrary value of 1. The inverse function that best fits the data is shown

It may seem surprising that all oils produce the same inhibition function when scaled, as there may be large variations not only in the concentrations of antioxidant species in olive oils, but also in the types of antioxidants present. The results observed here suggest that the function describing the inhibition of ethene production with increasing antioxidant concentration is a characteristic of the SIFT-MS-TOSC assay and not the antioxidant compounds involved. The most noticeable difference between the actions of different antioxidant compounds is the speed at which they scavenge radicals. As the assay is conducted over the course of an hour, the final result does not discriminate between fast and slow-acting antioxidants. This is explained by Lichtenthäler et al. [27], where a similar assay was used to analyse several pure compounds. The function or functions used to fit the results of Lichtenthäler et al. were not stated.

The results reported thus far permit the routine analysis of olive oil samples by the SIFT-MS-TOSC assay using a simpler experimental analysis that requires measurement of only a single concentration of oil. Using the function developed in Fig. 2, a VI_{50} value can be derived from a single sample concentration. As the model was calibrated using a cross-section of olive oils commercially available in New Zealand (including locally produced and imported oils), it is equally applicable to all such oils. It is strongly suspected that the shortened assay will apply to all oils, no matter what their country of origin.

Twenty additional olive oils were obtained and analysed via the new SIFT-MS-TOSC assay using the peroxyl radical. The same oils were also analysed using the FC total phenols assay and the HPLC phenolic analysis method outlined earlier. In addition, six phenolic standard

compounds were analysed in parallel with the oils: trolox, gallic acid, *p*-coumaric acid, vanillic acid, tyrosol and hydroxytyrosol. These were added in varying amounts to a commercially obtained refined olive oil for SIFT-MS-TOSC analysis, and were dissolved in an 80/20 mixture of methanol/water for the FC and HPLC analyses. Gallic acid was included as it is commonly used for calibration in the FC assay. Trolox is analysed in many different investigations of antioxidant capacity and was included here to facilitate comparisons with other studies.

Dividing the concentration of each phenolic standard compound in each SIFT-MS-TOSC analysis by the concentration of that phenolic compound required to inhibit 50% of ethene production allows all results to be plotted on the same chart and compared directly. All compounds obey a function which is not only the same for all of these phenolic compounds, but is also the same as that described by the oils shown in Fig. 2. These data support the statement earlier that the inhibition/concentration function observed is a characteristic of the assay and not the antioxidants being analysed. The data showing the percent inhibition of the oils integrated with the percent inhibition of the standard phenolic compounds at different concentrations are shown in Fig. 4.

Assay Comparisons

The SIFT-MS-TOSC assay and FC assay results were compared, as both assays provide a measure of the sample's antioxidant capacity. Figure 5 shows the correlation observed. It is difficult to know what type of function to expect, however it is logical that a zero result for either assay would not obtain a sensible result for the other. This behaviour is exhibited by the chosen inverse function, where a zero value on either axis corresponds to an infinite value on the other. The R^2 value of 0.70 indicates that a significant correlation exists between the results from the two assays. The results of the SIFT-MS-TOSC assay for all 20 oils are summarized in Table 1.

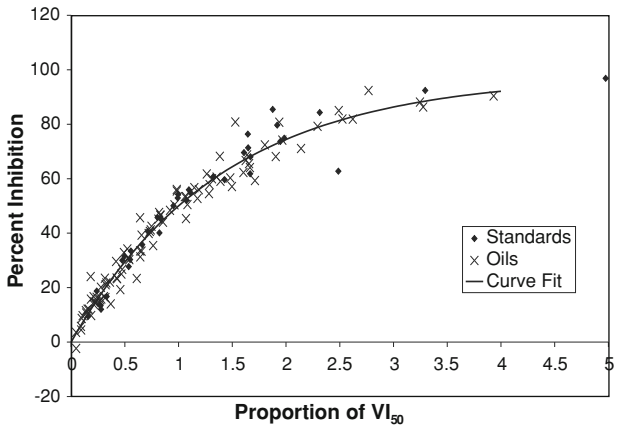

Fig. 4 The function that best fits the data ($y = 97.65(1 - e^{-0.7178x})$) using the new simplified single oil concentration data for all 20 oils combined with the standard phenolic compounds. The R^2 value for the oils is 0.97 while the R^2 value for the standard compounds is 0.96

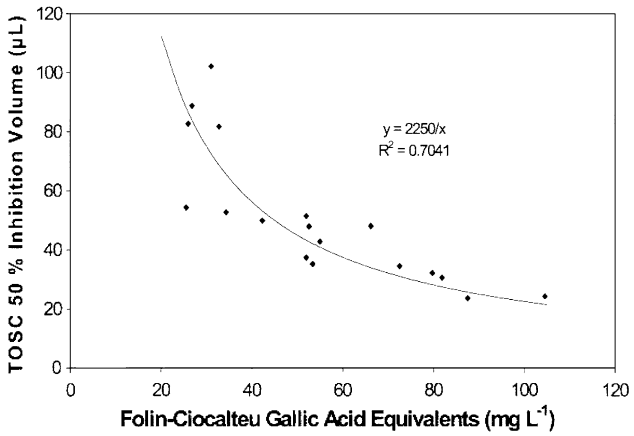

Fig. 5 Comparison of the response of 18 oils to the peroxyl radical SIFT-MS-TOSC assay and the F–C total phenol assay. A relationship is apparent with $R^2 = 0.70$

Table 1 SIFT-MS-TOSC assay results for peroxyl radicals for the 20 oils examined in this study

	VI_{50}	SD[a]	CV (%)[b]
Oil 1	30.6	2.4	7.7
Oil 2	47.9	10	21
Oil 3	23.7	0.38	1.6
Oil 4	48.1	2.4	5
Oil 5	102	5.5	5.4
Oil 6	81.8	9.2	11
Oil 7	82.7	6.1	7.4
Oil 8	88.8	7.2	8.1
Oil 9	54.3	0.8	1.5
Oil 10	52.7	4.4	8.4
Oil 11	49.9	2.5	5
Oil 12	34.6	0.94	2.7
Oil 13	24.2	0.85	3.5
Oil 14	32.2	0.29	0.92
Oil 15	51.5	4.9	9.6
Oil 16	126	20	16
Oil 17	42.9	2	4.6
Oil 18	37.4	2.2	5.8
Oil 19	35.2	2.3	6.6
Oil 20	233	0.91	0.39
CV mean			6.6

The VI_{50} amounts are the oil volumes (in µl) required to reduce the ethene concentration to half that found in the control

[a] Standard Deviation of 3 single point measurements

[b] Coefficient of Variation

Table 2 Curve fitting parameters for the pure phenolic compounds used in the SIFT-MS-TOSC assay

	a	*b*	R^2	IC_{50}
Gallic acid	187	−0.0333	0.987	9.33
p-Coumaric acid	100	−0.0768	0.986	8.99
Tyrosol	74.2	−0.0178	0.995	63
Hydroxytyrosol	95.2	−0.232	0.872	3.22
Vanillic acid	111	−0.0791	0.991	7.56
Trolox	161	−0.0408	0.992	9.12

The equation used to fit the curve was the same as that used for the emulsified oil reference function $y = a\ (1 - e^{-bx})$. The R^2 value for the fit is given along with the calculated concentration (μmol l^{-1}) required for 50% inhibition

The phenolic standards analysed by the SIFT-MS-TOSC assay were also analysed by the FC assay. All but one of the compounds behaved as expected, with their FC assay responses governed by the number of phenolic hydroxy groups available for reaction [28]. The response of vanillic acid was surprisingly less than 20% of that expected, as compared with the other compounds possessing one available phenolic hydroxy group.

The comparison of responses of the phenolic standard compounds between the SIFT-MS-TOSC and FC assays presents some interesting information. The response of these compounds as represented by their IC_{50} values (these correspond to the concentrations required for 50% inhibition) in the SIFT-MS-TOSC assay are shown in Table 2 and do not appear to be governed by the number of phenolic hydroxy groups they have available for reaction.

The highest antioxidant response as monitored by the radical scavenging ability, from the limited number of standard compounds included in the present study, originates from hydroxytyrosol in the SIFT-MS-TOSC assay. Hydroxytyrosol has been shown to be the most active olive oil polyphenol that scavenges reactive oxygen species produced by neutrophils that are involved in the induction of many diseases [29, 30].

The same standard compounds whose responses were determined in the two assays were quantified via HPLC in the 20 olive oil samples used for comparison between the assays and these are summarised in Table 3. The method used for extraction of phenolic compounds was similar to that used for the FC assay, however washing steps were added to purify the extract further before chromatographic analysis.

Tyrosol and hydroxytyrosol were found in most of the samples, while vanillic and *p*-coumaric acids were found at lower concentrations, each in only four oils. Neither gallic acid nor trolox were observed in any of the oils. None of the standard antioxidant compounds were detected at measureable quantities in the refined oil (oil 20 in Table 1) that was sold in supermarkets as "light" oil.

Table 3 Concentrations of the standard phenolic compounds hydroxytyrosol, tyrosol, vanillic acid and *p*-coumaric acid from HPLC measurements found in the 20 oils tested in this study

μg per ml extract				
	Hydroxytyrosol	Tyrosol	Vanillic acid	*p*-Coumaric acid
Oil 1	190	180	0	0
Oil 2	500	1400	0	0
Oil 3	920	650	0	0
Oil 4	110	450	0	0
Oil 5	90	900	20	5
Oil 6	0	140	0	0
Oil 7	0	130	0	0
Oil 8	0	810	0	0
Oil 9	80	400	30	10
Oil 10	125	500	0	10
Oil 11	0	190	0	0
Oil 12	220	740	50	0
Oil 13	190	224	0	4
Oil 14	130	257	0	0
Oil 15	50	90	0	0
Oil 16	0	189	0	0
Oil 17	150	520	21	0
Oil 18	240	450	0	0
Oil 19	150	320	0	0
Oil 20	0	0	0	0

Principal component analysis (PCA) was performed on the combined results from the SIFT-MS-TOSC and FC assays and the HPLC analyses of the 20 olive oils. The reciprocals of the SIFT-MS-TOSC assay results were used (1 was divided by each result) so as to be linear with respect to the FC assay results and expected to be linear with respect to the HPLC results. Figure 6 shows the loadings matrix from the PCA analysis.

Values close to one (either positive or negative) indicate a strong correlation with the corresponding principal components. Values close to zero indicate no correlation. It can be seen that the results from both assays and the total HPLC peak area show significant correlations with the first principal component. All three measures (SIFT-MS-TOSC, FC and HPLC) are supposed to approximate some measure of the antioxidant capacity of the oils, therefore it is reasonable to propose that the first principal component represents the antioxidant capacity of the olive oils under analysis. Of course, this does not necessarily assume that any one measure produces results closer to the true antioxidant capacity than any other, so the best approximation we can make based on the present data is to weight each

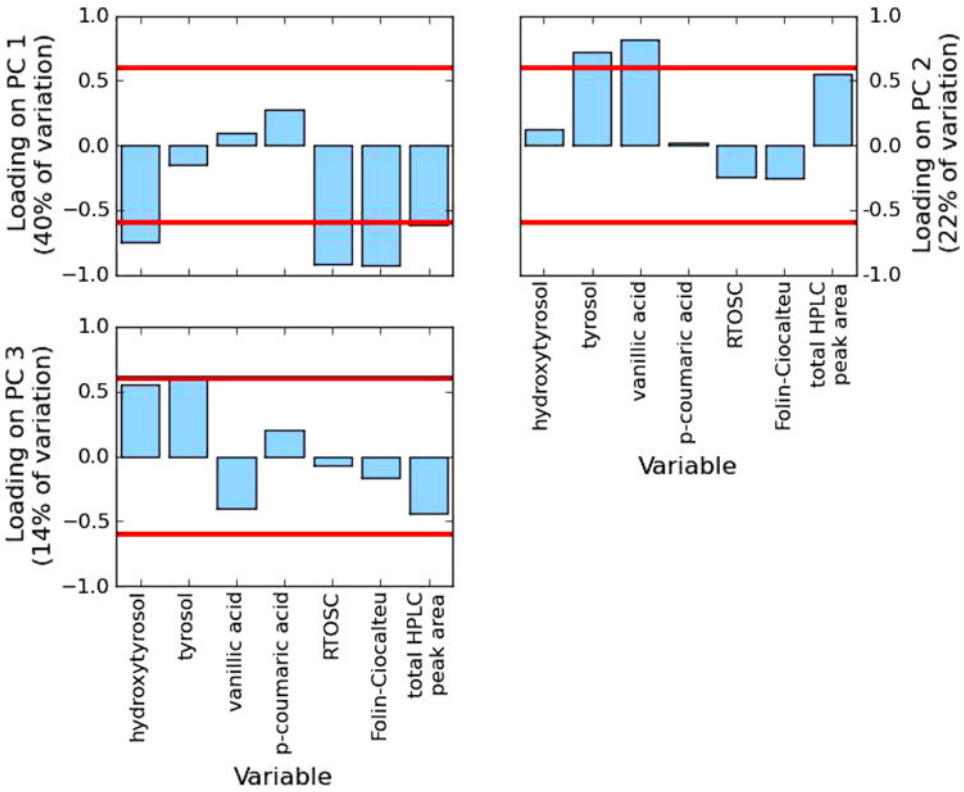

Fig. 6 Principal component loadings from principal component analysis performed on the concentrations of phenolic compounds determined in olive oil extracts using peroxyl radical SIFT-MS-TOSC VI_{50} values, FC gallic acid equivalents and total peak area from HPLC. RTOSC stands for "Reciprocal TOSC", and is 1/the SIFT-MS-TOSC VI_{50} value. Correlations above ±0.6 are considered significant

measure equally. It seems that analysing the entire oil gives a better appraisal of the antioxidant capacity than analysing an extract, so the SIFT-MS-TOSC assay is expected to give the best representation of the antioxidant capacity of the three techniques. The FC and HPLC methods both measure a polar extract of the oil and are therefore blind to any non-polar constituents, removing any contribution to the total antioxidant capacity of a sample by non-polar constituents.

Conclusions

The SIFT-MS-TOSC assay has been shown to provide a relevant measure of antioxidant capacity for lipid-based samples. In the process of this work a much simpler SIFT-MS-TOSC assay has been developed. For olive oil, where the majority of antioxidants are relatively polar, the SIFT-MS-TOSC assay results displayed a significant correlation with the widely used FC total phenols assay. The response of the FC assay is governed largely by the number of phenolic hydroxy groups in a sample and it appears from the correlation between the FC and SIFT-MS-TOSC assays that the antioxidant capacity in olive oil is controlled largely by the polar constituents. Although the presence of phenolic hydroxy groups should have some bearing on the SIFT-MS-TOSC assay response, the investigation of the limited set of antioxidant compounds analysed in this study suggests that features other than phenolic hydroxyl groups are also important. In the series of 20 oils examined by the shorter SIFT-MS-TOSC assay, oil 3 (Table 1), grown in the Marlborough region of New Zealand, was found to have the highest antioxidant capacity and oil 20, a refined commercial "light" oil, the lowest.

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