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Adaptation and Validation of Quick, Easy, New, Cheap, and Reproducible (QUENCHER) Antioxidant Capacity Assays in Model Products Obtained from Residual Wine Pomace

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Supporting Information

ABSTRACT: Evaluation of the total antioxidant capacity of solid matrices without extraction steps is a very interesting alternative for food researchers and also for food industries. These methodologies have been denominated by QUENCHER from quick, easy, new, cheap, and reproducible assays. To demonstrate and highlight the validity of QUENCHER (Q) methods, values of Q-method validation were showed for the first time, and they were tested with products of well-known different chemical properties. Furthermore, new QUENCHER assays to measure scavenging capacity against superoxide, hydroxyl, and lipid peroxyl radicals were developed. Calibration models showed good linearity ($R^2 > 0.995$), proportionality and precision (CV < 6.5%), and acceptable detection limits (<20.4 nmol Trolox equiv). The presence of ethanol in the reaction medium gave antioxidant capacity values significantly different from those obtained with water. The dilution of samples with powdered cellulose was discouraged because possible interferences with some of the matrices analyzed may take place.

KEYWORDS: *antioxidant activities, ABTS, Folin−Ciocalteu, free radical scavenging assays, cellulose*

INTRODUCTION

Natural antioxidants are increasingly demanded by the food industry to prevent oxidative degradation reactions while satisfying consumers' demands. Furthermore, there is growing interest in knowing the antioxidant value of foods, food supplements, and diets. Therefore, in recent decades, several in vitro and in vivo assays have been developed to determine the total antioxidant capacity (TAC) of foods and biological samples.[1−3] It is generally recognized that effects of food materials on the redox balance in vivo cannot be merely extrapolated from their activities in vitro, with the possible exception of their antioxidant action in the gastrointestinal tract.[2,4] However, the determination of the in vitro TAC can be achieved by simple and relatively fast chemical methodologies and may give more relevant information than that obtained from measuring each antioxidant compound separately, as possible synergistic interactions may not be considered.[4] Therefore, these methods have been largely used to estimate and compare the antioxidant capacity of food items.[5,6] Because most natural antioxidants are multifunctional, a reliable antioxidant protocol requires the measurement of more than one property relevant to either foods or biological systems.[7,8]

The most commonly applied in vitro TAC methodologies are based on diverse strategies to evaluate (1) the reducing ability of antioxidants, such as the Folin−Ciocalteu (FC) and ferric reducing/antioxidant power (FRAP) assays; (2) the scavenging of stable free radicals by antioxidants, including the 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays; and (3) the scavenging of short-lived radicals by competition mechanisms, such as peroxyl radicals in the oxygen radical absorbance capacity (ORAC) assay. Other less widely used methods assess the free radical scavenging capacity (FRSC) of antioxidants using radicals that are responsible for the oxidative stress damage observed in vivo, such as the superoxide, hydroxyl, and lipid peroxyl radicals. The respective FRSC methods have been named the superoxide radical scavenger capacity (SRSC), hydroxyl radical scavenger capacity (HRSC), and lipid peroxyl scavenger capacity (LPSC) assays.

Most of the TAC and FRSC determinations in foods and antioxidant products have been limited to their soluble compounds, and the extraction procedure has been considered a critical step.[9,10] This reason motivated the development of the quick, easy, new, cheap, and reproducible (QUENCHER) assays to measure the antioxidant activity of food materials.[11] These methods are a very interesting approach that avoids time-consuming solvent extraction steps of the classical protocols. The basis of QUENCHER (Q-) protocols is to place in direct contact the solid powdered food materials and the reagent solutions. Thus, the soluble antioxidants of the sample quench the radicals present in the reaction medium according to usual liquid−liquid reactions, whereas the antioxidants bound to the insoluble particle matter exert their antioxidant activity by taking advantage of surface reactions occurring at the solid−liquid interface.[11]

Several of the frequently used TAC methodologies (ABTS, DPPH, ORAC, FRAP, and FC) have recently been adapted to

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the QUENCHER approach.[12−15] Up to now, these assays have been successfully applied to some foods for which the antioxidant activity is largely dependent on the insoluble part, such as cereals and bakery products, seeds, nuts, pulses, and dietary fibers.[12−19] It must also be noted that a mixture of ethanol/water (50:50, v/v) has been suggested as the most appropriate reaction medium to carry out QUENCHER assays, and "neutral" powdered materials such as cellulose have been proposed as suitable diluting agents when products under study are very rich in antioxidants.[11,12]

Despite the advantages and previous applications of the QUENCHER methods, these assays are not yet extensively used, probably due to a lack of validation studies of these methodologies. In addition, to the best of our knowledge, the QUENCHER concept has still not been adapted to the methodologies that target biologically relevant radicals (SRSC, HRSC, and LPSC), and a statistical validation and correlations between the different Q-TAC and Q-FRSC assays have not yet been performed. These verifications are important to encourage the applicability of QUENCHER protocols as valuable tools for the in vitro antioxidant capacity assessment of different products such as foods, food ingredients, and others.

Therefore, to increase the use of the QUENCHER approach, the first aim of the present study was to develop new QUENCHER protocols to evaluate the scavenger capacity of some of the most biologically relevant radicals (superoxide, hydroxyl, and lipid peroxyl); the second aim was to optimize and, for the first time, validate different QUENCHER assays (Q-FC, Q-FRAP, Q-ABTS, Q-DPPH, Q-ORAC, Q-SRSC, Q-HRSC, and Q-LPSC) using three model products of different chemical properties. Secondarily, the best combination of QUENCHER assays for routine analysis was identified, and several factors that might influence the Q-TAC results, such as the presence of ethanol in the reaction medium and the use of cellulose as a diluting agent, were assessed. The study was carried out with three powdered model products, all of them rich in antioxidant compounds, but each containing antioxidants of different polarity. Powdered products were made in our pilot plant from wine pomace so that we could work with familiar products for which characteristics and composition were well-known.

MATERIALS AND METHODS

Chemicals. ABTS, cellulose, DPPH, deoxyribose (2-deoxy-D-ribose), 2,2′-diazobis(2-aminodinopropane) dihydrochloride (AAPH), gallic acid (GA), 6-hydroxyl-2,5,7,8-tetramethyl-2-carboxylic acid (Trolox), 4-nitroblue tetrazolium chloride (NBT), phenazin methosulfate (PMS), 2,4,6-tris(2-pyridyl)-*S*-triazine (TPTZ), and 2-thiobarbituric acid (TBA) were obtained from Sigma-Aldrich Co (St. Louis, MO, USA). Ethylenediaminetetraacetic acid (EDTA), iron(III) chloride acid ($FeCl_3$), iron(II) sulfate ($FeSO_4$), FC reagent, hydrogen peroxide (H_2O_2), L-ascorbic acid ($C_6H_8O_6$), potassium persulfate ($K_2O_8S_2$), sodium acetate ($NaC_2H_3O_2$), sodium carbonate (Na_2CO_3), sodium pyrophosphate anhydrous ($Na_4P_2O_7$), and trichloroacetic acid (TCA) were purchased from Panreac (Barcelona, Spain).

Samples. Red wine pomace was kindly supplied by different wineries located at Burgos (Spain). All red wine pomace was mixed and dehydrated until reaching a final water content of <10%. Dried materials were separated, ground, and sieved, thus obtaining three different powdered products,[20] which were used as model matrices. One of them was derived from grape seeds (Sd) separated from the wine pomace; this model product represented foods with a significant content of fat and rich mainly in hydrophobic antioxidants. Another product was obtained from the wine pomace free of seeds, which was mainly constituted by grape skins (Sk); this model product represented food matrices rich in hydrophilic antioxidant and without or with very low levels of fat. The third powdered product was obtained from whole (W) wine pomace; this model product represented food materials with some fat and rich in both hydrophilic and hydrophobic antioxidants. The particle size of these model products was <0.250 mm in the cases of Sk and W and <0.355 mm for Sd.[21]

QUENCHER Antioxidant Capacity Methods. The adapted Q-TAC and Q-FRSC methodologies were fixed end-point procedures with the exception of the Q-ORAC method, which was a kinetic assay. Results are given as mean values ± standard deviation of three independent samples.

Q-FC Assay. This method was adapted from the FC assay developed by Singleton and Rossi.[22] Briefly, 1 ± 0.005 mg of the tested products was weighed in a microbalance (MicroPro11, Sartorius) and mixed with 0.2 mL of Milli-Q (MQ) water and 0.2 mL of FC reagent. After 5 min of reaction, 4 mL of a 0.7 M Na_2CO_3 solution was added, and the final volume was made up to 10 mL with MQ water. After 1 h of incubation in an orbital shaker, the absorbance at 750 nm was measured using an UV−vis spectrophotometer (U-2000 Hitachi). The FC index was expressed as micromoles of gallic acid equivalents per gram of product (GAE/g) by means of a dose−response curve for different quantities of the standard.

Q-FRAP Assay. The Q-FRAP procedure was adapted from the method described by Benzie et al.[23] The reactive mixture was freshly prepared by mixing 10 mM TPTZ and 20 mM $FeCl_3$ in 300 mM $NaC_2H_3O_2$ buffer (pH 3.6) at a ratio of 1:1:10 (v/v/v) and diluted 10:1 (v/v) in MQ water. Ten milliliters of the FRAP solution was added to 1 ± 0.005 mg of the samples and incubated at 37 °C for 30 min with continuous stirring. The absorbance at 593 nm was measured. The results were expressed as micromoles of iron(II) equivalents per gram of product (Fe(II)E/g) using linear calibration obtained with different amounts of $FeSO_4$.

Q-ABTS Assay. The method was adapted from the assay developed by Re et al.[24] and modified by Rivero-Pérez et al.[25] The stock solution of $ABTS^{\bullet+}$ was diluted with MQ water to an absorbance of 0.70 ± 0.02 at 734 nm; 1 ± 0.005 mg of the assessed products was weighed, and the reaction was started by adding 10 mL of $ABTS^{\bullet+}$ working solution. After 30 min of incubation in darkness with continuous stirring, the absorbance of the samples at 734 nm was measured and subtracted from the absorbance of the $ABTS^{\bullet+}$ working solution. The results are expressed as micromoles of Trolox equivalents per gram of product (TE/g), using the dose−response curve described by different amounts of this standard.

Q-DPPH Assay. This method is based on the scavenging of the radical $DPPH^{\bullet}$.[26] A 0.1 mM $DPPH^{\bullet}$ working solution in pure methanol was prepared and diluted until an absorbance at 517 nm of 0.70 ± 0.02.[25] Then, 1 ± 0.005 mg of the powdered products was transferred into a test tube and mixed with 10 mL of $DPPH^{\bullet}$ working solution. The absorbance at 517 nm was measured after 30 min of continuous stirring at room temperature in the samples and subtracted from the absorbance of the $DPPH^{\bullet}$ working solution. Trolox was used as standard to perform a calibration curve, and the results were expressed as micromoles of TE per gram of product.

Q-ORAC Assay. This assay was adapted from the method described by Ou et al.[27] The analysis was conducted in a four-cell fluorometer (Cary-Eclipse, Varian) with continuous stirring in the cuvette and at 37 °C. Briefly, 0.2 ± 0.005 mg of the assessed products was weighed and resuspended in 0.8 mL of 75 mM phosphate buffer (pH 7.0, warmed to 37 °C) just before use. A Trolox quality control (T10, equivalent to 10 μg of Trolox) and a 300 mM AAPH solution in phosphate buffer were also freshly prepared. At least one T10 and one blank were analyzed with every set of samples (same AAPH solution). In a glass cuvette, 0.4 mL of the samples (the resuspended products, the T10 control or just phosphate buffer for the blank) was mixed with 2 mL of a freshly prepared 87.5 nM fluorescein solution in phosphate buffer. The fluorescence reading (λ_{exc} = 493 nm and λ_{em} = 511 nm) was started, and 0.1 mL of the AAPH solution was added after 2.5 min. The fluorescence decay curve was monitored for 100 min. The net area under the curve (net AUC) was calculated for each sample by

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subtracting the blank AUC from the sample AUC. Regression equations between net AUC and different quantities of Trolox were obtained and used for the expression of the Q-ORAC values as micromoles of TE per gram of product.

Q-SRSC Assay. The Q-SRSC procedure was adapted from the method described by Liu et al.[28] and Rivero-Pérez et al.[25] Briefly, 1.5 ± 0.005 mg of the products was added to the test tube and mixed with a 1.5 mL final volume of 78 μM NADH, 50 μM NBT, and 10 μM PMS in 16 mM buffer Tris-HCl (pH 8.0). After the mixture had been shaken for 10 s, the absorbance (A) of the samples (S) at 560 nm was measured. "Oxidized controls" (OC; without products) and "sample controls" (SC; products and buffer) were also prepared. The results are expressed as oxidation inhibition percentages by the equation

$$\text{oxidation inhibition } \% = (A_{OC} - (A_S - A_{SC}))/A_{OC} \times 100 \quad (1)$$

Q-HRSC Assay. The Q-HRSC assay was adapted from the method developed by Halliwell et al.[29] and modified by Rivero-Pérez et al.[25] Briefly, 1 ± 0.005 mg of the powdered products was weighed and transferred into a screw-top test tube. A 1 mL final reaction volume contained a mixture of 1 mM deoxyribose, 0.1 mM $C_6H_8O_6$, 1 mM H_2O_2, 0.1 mM $FeCl_3$, and 0.1 mM EDTA in 5 mM phosphate buffer (pH 7.4). OC and SC were also prepared. The tubes were incubated for 60 min at 37 °C with continuous stirring. Then, 1.5 mL of TCA (28% w/v) and 1 mL of TBA (1% w/v) were added, and the capped tubes were shaken and heated at 100 °C for 15 min. The absorbance was recorded at 532 nm, and the results were expressed as oxidation inhibition percent following eq 1.

Q-LPSC Assay. This assay was adapted from the method described by Rivero-Pérez et al.[25] The experiments were carried out in rat liver microsomal preparations,[30] and lipid peroxidation was induced using AAPH as oxidant. The total microsomal protein content was determined using the Bradford method.[31] A microsomal solution containing 10 mg/mL protein in 0.1 M $Na_4P_2O_7$ buffer (pH 7.4) was prepared and stored at −80 °C until the day of analysis; 0.2 ± 0.005 mg of the tested products was placed into a screw-top tube, and 400 μL of 50 mM Tris-HCl buffer (pH 7.0), 50 μL of the microsomal solution, and 50 μL of 100 mM AAPH solution in the Tris-HCl buffer were added. OC and SC (replacing the AAPH solution by Tris-HCl buffer) were also prepared. The tubes were incubated at 37 °C for 90 min under continuous stirring. Then, 750 μL of TCA (2.8% w/v) and 500 μL of TBA (1% w/v) were added, and the capped tubes were shaken and heated at 100 °C for 15 min. Seven hundred and fifty microliters of the reaction mixture was transferred to another tube, and the TBA reactive substances were extracted with the same volume of butanol. The absorbance at 532 nm of the butanol extract was measured, and the results were expressed as oxidation inhibition percent with respect to the OC sample following eq 1.

Statistical Analysis. The statistical validation of the methods was performed using Statgraphics Centurion XVI version 16.2.04 software (Statpoint Technologies, Inc., Warranton, VA, USA). The statistical analysis of the Q-TAC data was carried out using one-way analysis of variance (ANOVA). Fisher's least significant difference (LSD) test was applied to determine the statistical significance of differences among various groups. A minimum significance level of $p < 0.05$ was considered. Pearson's correlation coefficients were determined to study linear correlations between pairs of the Q-TAC methodologies.

RESULTS AND DISCUSSION

Adaptation and Optimization of the Q-TAC and Q-FRSC Methods. This study shows some modifications with respect to Q-TAC protocols (Q-ABTS,[12] Q-DPPH,[12] Q-FRAP,[14] Q-ORAC,[13] and Q-FC[15]) previously published. Moreover, three new QUENCHER methodologies were developed (Q-SRSC, Q-HRSC, and Q-LPSC), which were adapted from validated classical FRSC methods.[25]

The main modification from other Q-TAC assays was the selection of aqueous reaction mediums whenever the methodologies allowed for the use of both organic and aqueous solvents. Aqueous medium might better represent the real environment surrounding any food matrix or biological system. Therefore, MQ water or aqueous buffer was initially used in all protocols except in the Q-DPPH assay, which required an organic solvent to dissolve the radical.

The particle size of the powdered products is a factor that might influence the results of the Q-TAC and Q-FRSC measurements, and this aspect should be taken into account for comparisons among powders of different particle size. Nevertheless, Serpen et al.[17] did not find a remarkable effect of this factor, and Gökmen et al.[11] suggested the use of powdered samples of particle size ranging from 0.3 to 0.1 mm in QUENCHER assays.

All protocols in this study were optimized for the direct measurement of small quantities of powdered products, considering the final reaction volume and amount of sample weighed.

The Q-FC, Q-FRAP, Q-ABTS, and Q-DPPH methods are rather inexpensive and simple methodologies, which allow for the adjustment of the assay volumes (maintaining the proportionality among the reagents) without a relevant increase in the cost of the analysis. Preliminary studies were conducted to establish 1 mg as an advisible amount of product, although satisfactory results can also be obtained with smaller or larger quantities (0.2−5 mg), depending on the TAC of the products and the sensitivity of the assay.

In the case of the Q-ORAC method, the reaction volume is restricted by the maximum volume of the cuvette because the fluorescence decay needs to be continuously monitored (kinetic assay). Amigo-Benavent et al.[13] proposed that the Q-ORAC assay be carried out in alternating stages of incubation at 37 °C in a test tube with centrifugation, transfer to a cuvette, and fluorescence reading. The Q-ORAC method proposed in the present study avoids several centrifugation/transfer steps, is more similar to classical protocols, and reproduces more effectively the advantages of the ORAC assay with respect to other TAC methods.[2,4,8] However, a fluorometer with a temperature control chamber and constant stirring in the cuvette is required, and the assay might not be suitable for products of very high Q-TAC.

With regard to the Q-SRSC, QHRSC, and Q-LPSC methods, the final reaction volume and amount of sample weighed must be adjusted to give results in the range of 0−100% oxidation inhibition. The Q-SRSC assay is a rather simple and quick methodology that can be directly performed in the cuvette or in a test tube. However, the Q-HRSC and the Q-LPSC assays involve more tedious protocols. It is suggested that the amount of sample used be adjusted rather than increasing the final volume, although it must be remarked that the precision of the QUENCHER methods might be compromised when <0.2 mg of the samples is weighed.

QUENCHER fixed end-point procedures allow for transferring an aliquot of the reaction medium to a microplate for the absorbance reading step. This alternative increases the repeatability and reduces the time between different measurements, so it is especially appropriate for the simultaneous analyses of multiple samples. On the other hand, QUENCHER kinetic assays cannot be carried out directly in a microplate due to the interference of solid products on the absorbance measurement. Furthermore, the extremely small quantity of solid sample required, proportional to the small volume of wells, could put at risk the validity of this procedure.

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Table 1. Calibration Models of the QUENCHER Total Antioxidant Capacity (Q-TAC) Methods

method	standard[a]	range (μmol)	calibration model[b]	R^2[c]
Q-FC	GA	0.059−0.588	$A_{750\ nm}$ = (1.79 ± 0.009) μmol of GA + (0.005 ± 0.004)	0.999
Q-FRAP	Fe(II)	0.036−0.285	$A_{593\ nm}$ = (2.76 ± 0.046) μmol of Fe(II) − (0.009 ± 0.008)	0.996
Q-ABTS	T	0.083−0.499	$(A_{WS} - A_S)_{734\ nm}$ = (1.20 ± 0.015) μmol of T + (0.007 ± 0.005)	0.998
Q-DPPH	T	0.040−0.200	$(A_{WS} - A_S)_{517\ nm}$ = (3.20 ± 0.033) μmol of T + (0.007 ± 0.004)	0.999
Q-ORAC	T	0.010−0.050	net AUC = (3361 ± 70.3) μmol of T + (4.78 ± 2.25)	0.996

[a]GA, gallic acid; Fe(II), iron(II); T, Trolox. [b]$3A_{WS}$, absorbance of the radical working solution; A_S, absorbance of the sample; net AUC, net area under the curve. [c]R^2, coefficient of determination.

Table 2. Linearity Test, Proportionality, and Detection Limits of the QUENCHER Total Antioxidant Capacity (Q-TAC) Methods

	linearity test[a]				proportionality test[b]				detection limits[c]	
			ANOVA							
method	CV(Fr) (%)	CV(b) (%)	F_{exp}	F_{tab}	$a \pm t_{tab}S(a)$	$t_{cal}(a)$	$t_{cal}(b)$	t_{tab}	y_d[d]	x_d[e]
Q-FC	2.99	0.48	42730	4.38	0.005 ± 0.006	1.45	207	1.73	0.016	12.0 nmol of GAE
Q-FRAP	4.79	1.68	3537	4.67	−0.009 ± 0.015	1.12	59.5	1.77	0.012	15.3 nmol of Fe(II)E
Q-ABTS	3.27	1.25	6328	4.49	0.007 ± 0.009	1.49	80.2	1.75	0.020	20.4 nmol of TE
Q-DPPH	2.57	1.03	9384	4.67	0.007 ± 0.008	1.53	96.1	1.77	0.017	6.68 nmol of TE
Q-ORAC	4.86	2.10	2271	4.97	4.78 ± 4.09*	2.12*	47.7	1.81	10.1	3.08 nmol of TE

[a]CV(Fr), coefficient of variation of the response factor; CV(b), coefficient of variation of the slope; ANOVA by means of the F test (F_{exp}, experimental F; F_{tab}, tabulated F (1, n − 2, 0.05)). Linearity test acceptance criteria: CV(Fr) < 5%; CV(b) < 2%; ANOVA $F_{exp} > F_{tab}$. [b]$a \pm t_{tab}S(a)$, confidence interval of the ordinate at origin; t_{cal}, calculated Student's t; t_{tab}, tabulated Student's t (n − 2, 0.05). Proportionality acceptance criteria: interval should include 0; $t_{cal}(a) < t_{tab}$; $t_{cal}(b) > t_{tab}$. *, results do not meet the acceptance criteria. [c]y_d, detection signal; x_d, capability of detection. Detection limits calculated for α and β = 0.05. [d]y_d units, absorbance units (Q-FC, Q-FRAP, Q-ABTS, Q-DPPH); net area under the curve (net AUC) (Q-ORAC). [e]GAE, gallic acid equivalents; Fe(II)E, iron(II) equivalents; TE, Trolox equivalents.

Table 3. Precision of the QUENCHER Total Antioxidant Capacity (Q-TAC) and QUENCHER Free Radical Scavenging Capacity (Q-FRSC) Methods

		repeatability			intermediate precision	
method	units of S_r and S_R[a]	CV[b] (%)	$S_{r\ (Rp)}$[c]	$S_{r\ (IP)}$[d]	CV[e] (%)	S_R[f]
Q-FC	μmol GAE/g product	2.06	1.23	1.17	2.37	1.49
Q-FRAP	μmol Fe(II)E/g product	4.98	10.8	10.7	6.48	16.0
Q-ABTS	μmol TE/g product	2.13	2.24	2.73	3.05	3.26
Q-DPPH	μmol TE/g product	3.12	1.24	1.26	3.85	1.59
Q-ORAC	μmol TE/g product	5.45*	2.94	2.79	5.48	3.01
Q-SRSC	oxidation inhibition %	3.27	1.05	1.46	6.35	1.62
Q-HRSC	oxidation inhibition %	5.31*	2.79	3.09	6.32	3.28
Q-LPSC	oxidation inhibition %	5.26*	4.01	4.44	6.35	4.87

[a]GAE, gallic acid equivalents; Fe(II)E, iron(II) equivalents; TE, Trolox equivalents. [b]Repeatability acceptance criteria: CV < 5%. *, results do not meet the acceptance criteria. [c]Estimate of repeatability (S_r) determined by one-variable analysis of seven replicates 1 day (Rp, repeatability experiment). [d]Estimate of repeatability (S_r) determined by one-way ANOVA of three replicates on three different days (IP, intermediate precision experiment). [e]Intermediate precision acceptance criteria: CV < 10%. [f]Estimate of intermediate precision (S_R) determined by one-way ANOVA of three replicates on three different days (IP, intermediate precision experiment).

Validation of the Q-TAC and Q-FRSC Methods. The validation study was done by the examination of the following parameters: linearity, proportionality, detection limits, and precision (repeatability and intermediate precision).[32] In the case of Q-FRSC assays (Q-SRSC, Q-HRSC, and Q-LPSC), only the precision was evaluated because in these methods a calibration curve is not needed. In the Q-TAC methodologies (Q-FC, Q-FRAP, Q-ABTS, Q-DPPH, and Q-ORAC), a dose−response curve was obtained by testing different amounts of the standard used in each method. The calibration was performed using linear models and the least-squares (LS) method. All of the models presented coefficient of determination (R^2) values >0.995 (Table 1).

Once the parameters were estimated, the models were validated by verifying the assumptions related to the residuals and the functions.[33] The studentized residuals were normally distributed (Kolmogorov−Smirnov, chi-square, and Shapiro−Wilk tests), independent (Durbin−Watson test), and homoscedastic (Cochran and Bartlett tests) in all cases. The functional part of each linear model was first validated in a linearity test. According to the results (Table 2), all of the models were suitable for describing the linear relationship between the standard concentration and the response of each assay. The Q-FC method obtained the best values for all of the parameters tested, whereas the Q-ORAC assay linearity response was the least satisfactory, although within the acceptance criteria. In addition, a proportionality test to determine whether the mathematical model could be used for predictive purposes was performed (Table 2). The conditions of proportionality were corroborated for all methods except Q-

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Table 4. QUENCHER Total Antioxidant Capacity (Q-TAC) and QUENCHER Free Radical Scavenging Capacity (Q-FRSC) of the Model Products Obtained from Skins (Sk), Seeds (Sd), and Whole (W) Wine Pomace[a]

		Sk		W		Sd	
method	units[b]	value	CV (%)	value	CV (%)	value	CV (%)
Q-FC	μmol GAE/g product	74.4 ± 1.07 b	1.43	78.3 ± 2.37 b	3.02	52.6 ± 2.53 a	4.80
Q-FRAP	μmol Fe(II)E/g product	275 ± 4.18 c	1.52	224 ± 13.6 b	6.05	140 ± 4.92 a	3.52
Q-ABTS	μmol TE/g product	120 ± 5.21 a	4.33	150 ± 2.66 b	1.78	120 ± 0.83 a	0.69
Q-DPPH	μmol TE/g product	51.9 ± 1.10 a	2.11	110 ± 3.60 b	3.27	174 ± 4.16 c	2.39
Q-ORAC	μmol TE/g product	236 ± 9.92 b	4.19	243 ± 4.17 b	1.72	181 ± 7.96 a	4.39
Q-SRSC	oxidation inhibition %	31.7 ± 1.19 a	3.76	50.5 ± 1.88 b	3.72	30.1 ± 1.20 a	3.98
Q-HRSC	oxidation inhibition %	50.1 ± 2.59 a	5.17	59.0 ± 0.64 c	1.08	53.8 ± 1.08 b	2.01
Q-LPSC	oxidation inhibition %	52.2 ± 2.57 b	4.92	62.5 ± 3.38 c	5.41	33.7 ± 2.02 a	7.27

[a]Q-TAC and Q-FRSC values are the mean ± standard deviation (n = 3). Roman letters indicate significant differences among the model products (Sk, W, Sd) within each QUENCHER methodology. [b]GAE, gallic acid equivalents; Fe(II)E, iron(II) equivalents; TE, Trolox equivalents.

ORAC, where the ordinate at origin did not meet any of the criteria. In this assay the Q-TAC is assessed by the net AUC determination and a lag time is observed during oxidation of the fluorescein in the presence of an antioxidant.[1,27] The unsatisfactory result of the ordinate at origin is explained by the lack of proportionality of this lag time at very small amounts of Trolox. However, a linear response was observed at higher quantities of the standard, as previously observed by Ou et al.[27] in the classical approach of this assay.

Having studied the acceptability of the linear models, the detection limits y_d (detection signal) and x_d (capability of detection) of the assessed methodologies were determined as described by Ortiz et al.[33] In general, low limits of detection were observed (Table 2), which confirmed the acceptability of all methodologies to determine the TAC of very small quantities of antioxidants. Among the assays using Trolox as standard, the lowest x_d value corresponded to the Q-ORAC assay, which was corroborated as a highly sensitive methodology, and the highest limits of detection were obtained by the Q-ABTS method. A similar observation was previously reported for a comparison of classical TAC methodologies.[25]

Finally, two different terms of the precision were evaluated, the repeatability (Rp) and the intermediate precision (IP), determining their estimates (S_r and S_R, respectively) and coefficients of variance (CV).[32] The IP refers to the precision under reproducibility conditions when only some of the factors are varied. In this study a single factor (different days) was investigated. The results of the Rp and IP experiments for each methodology are shown in Table 3. It can be seen that similar S_r values were obtained in both Rp and IP experiences for all of the methods. The CV of the assays was the criteria selected to evaluate their acceptability. The Rp of the longest protocols (Q-ORAC, Q-HRSC, and Q-LPSC) exceeded the CV < 5% cutoff value, whereas those assays with just one or two steps (Q-FC, Q-ABTS, and Q-DPPH) showed a high Rp between measurements. By comparison with the Rp obtained in classical protocols validated using different wines as samples,[25] slightly higher CV were observed in the Q-TAC, and especially in the Q-FRSC methods, as all classical assays obtained CV < 5%. The only exception was the Q-ABTS assay, which showed better Rp than the classical approach. In terms of IP (Table 3), all of the methodologies presented satisfactory results, with the lowest CV (%) obtained again by two of the simplest methods (Q-FC and Q-ABTS). Therefore, the differences in the Rp and IP among the methodologies were mainly attributed to the complexity of the protocols. The sensitivity of the assays could also affect the precision observed, as might be the case for the Q-ORAC method mentioned above.

Q-TAC and Q-FRSC of the Model Products. To provide comprehensive information on the actual TAC of food, it is recommended that at least two TAC assays be used.[6,8] Similarly, in this study, a broad overview of the antioxidant capacity of the three model products (Sk, W, and Sd) was achieved with the different QUENCHER methodologies under study.

As shown in Table 4, model product W (rich in both hydrophobic and hydrophilic antioxidant compounds) showed the highest Q-TAC and Q-FRSC values in most of the methods, with the exception of the Q-FRAP assay, where Sk (model product especially rich in hydrophilic antioxidant) presented the most elevated antioxidant capacity and the Q-DPPH assay, where Sd (model product especially rich in hydrophobic antioxidant) exhibited the highest Q-TAC values. The difference between the antioxidant capacity of Sk and W was not significant in the Q-FC and Q-ORAC protocols. No significant differences were found between Sk and Sd in the Q-ABTS and Q-SRSC assays. From a comparison of the responses obtained by the three methodologies where Trolox was used as standard, Q-ORAC obtained the highest TAC values, but it must be noted that the sensitivity of this method to Trolox was also more elevated than in the Q-DPPH and Q-ABTS assays.

The obtained results were contrasted with those obtained by classical determinations, and considerable differences were found, except with the Q-DPPH method. Several studies comparing extracts from wine pomace reported a higher antioxidant capacity exhibited by seeds than skins, obtaining the same tendency using different assays (FC,[34−37] FRAP,[34,36,37] ABTS,[36] DPPH,[34−37] ORAC,[36] SRSC,[35] and HRSC[34]). This fact may be explained because classical protocols involve extraction with solvents, which are capable of extracting both hydrophobic and hydrophilic antioxidants. Therefore, the extracts obtained might not be representative of the antioxidant capacity that solid foods could actually exert, whereas QUENCHER assays may provide more reliable information.[11]

Correlation Study between the QUENCHER Methodologies. Pearson's correlation coefficients (r) between pairs of the assays were determined using the Q-TAC and Q-FRSC values obtained for Sk, W, and Sd by the different QUENCHER methodologies.

In the literature, due to the wide spectrum of analytical techniques and reaction conditions available, it is difficult to find an agreement on the correlations among different TAC methodologies.[2,8,38] To the extent of our knowledge, the

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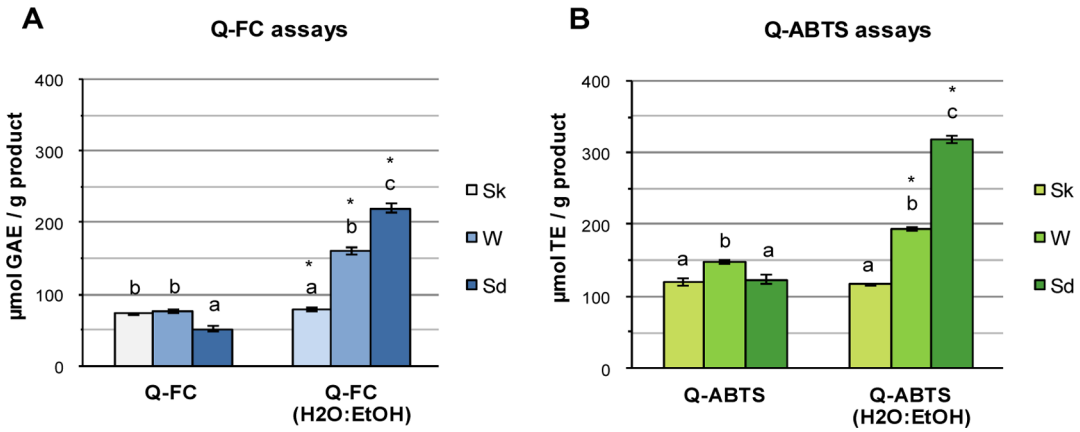

Figure 1. QUENCHER total antioxidant capacity (Q-TAC) of the model products (Sk, skins; W, whole; Sd, seeds): (A) Q-FC assays; (B) Q-ABTS assays. GAE, gallic acid equivalents; TE, Trolox equivalents. Data are expressed as mean values ± standard deviation (n = 3). Roman letters indicate significant differences among the model products (Sk, W, Sd) within each assay. The asterisk (*) refers to significant differences between the Q-TAC and Q-TAC (H_2O:EtOH) approaches for each wine pomace product.

correlation between different QUENCHER methodologies has not been described yet. In this study, an interesting correspondence among the assays was observed, especially for the Q-FC and Q-ABTS assays with the rest of methods. A highly significant ($p < 0.01$) positive correlation was detected between Q-FC and Q-FRAP (r = 0.847), between Q-FC and Q-ORAC (r = 0.974), and between Q-FC and Q-LPSC (r = 0.926). The correspondence between Q-FC and Q-FRAP is due to the fact that both methods measure the reducing capacity of the sample,[38] also detected between the classical approaches.[36,37] Furthermore, the significant correlation between Q-ORAC and Q-LPSC (r = 0.914) may be explained because peroxyl radicals are involved in both assays.[39] The positive correlation of the Q-FC with the Q-ORAC and Q-LPSC assays is very convenient as they are more biologically relevant methods, but these assays are also more complicated, and their validation study showed some unsatisfactory results. However, the obtained results contrast with the weaker correlation (R^2 = 0.409) found between the classical approaches of the FC and the ORAC assays by Ky et al.[36] using also wine pomace as samples. In the present study, interesting high positive correlations ($p < 0.01$) between Q-ABTS and Q-SRSC (r = 0.935) and between Q-ABTS and Q-HRSC (r = 0.903) were also observed. According to Rivero-Pérez et al.,[25] such strong correlations were not detected in the classical approaches of these assays using wines as samples. On the other hand, negative correlations ($p < 0.05$) between Q-DPPH and Q-FC (r = −0.781), between Q-DPPH and Q-FRAP (r = −0.986), and between Q-DPPH and Q-ORAC (r = −0.808) were found in the present study, and the Q-DPPH method was not significantly correlated with any of the biologically relevant Q-FRSC assays. In classical methodologies, the DPPH and HRSC assays were not found to be correlated either,[34] but high positive correlations were usually detected between the DPPH and other TAC methods (FC,[35−37] FRAP,[34,37] and ABTS[25]). It must be noted that the Q-DPPH is the only QUENCHER methodology that was performed in an organic reaction medium, and its lack of positive correlation with the rest of the protocols indicates the elevated influence of the solvent used in these assays.

The different contents of hydrophilic and lipophilic compounds[40,41] of the three model products used in this study allow for assessing the response of each method toward matrices with compounds of high or low water solubility. Anyway, it would be advisible to confirm the correlations found in the current study by evaluating a wider range of products.

With the results of both the validation and correlation studies taken into account, the combination of both Q-FC and Q-ABTS assays is suggested as a good selection for a general Q-TAC analysis of powdered foods. The Q-SRSC, Q-HRSC, and Q-LPSC methods, first adapted to the QUENCHER approach in this study, might give a more biologically relevant overview of the antioxidant capacity of the samples. However, the Q-HRSC and Q-LPSC assays are complicated protocols for routine analysis, and it has been shown that the combination of the Q-FC and Q-ABTS methods covers the correspondence with the results obtained by the three biologically relevant Q-FRSC assays.

Effects of the Reaction Medium in the Q-FC and Q-ABTS Assays. In the QUENCHER methods there is no extraction step, but the reaction medium is a key factor that determines the solubility of the compounds present in the solid powdered product, as well as the interactions between the antioxidants and the radicals used as probes in the assays.

Serpen et al.[42] proposed a water/ethanol (H_2O:EtOH) 50:50 (v/v) ratio as a suitable working solution, providing accurate Q-TAC results for most of the food matrices studied. Therefore, the effect of using water or a mixture H_2O:EtOH 1:1 (v/v) as reaction medium was assessed in the current study using the two previously selected Q-TAC methods (Q-FC and Q-ABTS).

The Q-FC (H_2O:EtOH) and Q-ABTS (H_2O:EtOH) assays were validated as previously described for the rest of the methodologies and presented satisfactory results for all parameters evaluated (Supporting Information 1). Slight formation of white solids was observed in the Q-FC (H_2O:EtOH) method, probably due to the precipitation of Na_2CO_3 in the presence of ethanol. Nevertheless, these solids did not interfere in the measurement. The responses of the Q-FC and Q-FC (H_2O:EtOH) assays to different standard concentrations were not significantly different (similar slopes in the calibration models), whereas a 2.54 times lower response to Trolox in the Q-ABTS than in the Q-ABTS (H_2O:EtOH) method was detected. In contrast, Serpen et al.[42] found similar slopes of the dose−response lines obtained in the Q-ABTS assays using different solvent ratios H_2O:EtOH (0:100, 25:75, 50:50, 75:25, 100:0). A possible explanation might be that the

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Table 5. Influence of the Amount of Sample Weighed and the Use of Cellulose (C) as a Diluting Agent in the Q-ABTS Assays Evaluated in Model Products Obtained from Skins (Sk) or Seeds (Sd) Separated from Red Wine Pomace[a]

method	Q-TAC values (μmol TE/g product)				
Q-ABTS	Sk 3 mg	Sk 2 mg	Sk 1 mg	Sk:C (2:1) 3 mg	Sk:C (1:2) 3 mg
	116 ± 3.97	119 ± 3.41	122 ± 3.77	121 ± 1.88	118 ± 8.32
	Sd 3 mg	Sd 2 mg	Sd 1 mg	Sd:C (2:1) 3 mg	Sd:C (1:2) 3 mg
	126 ± 5.47 a	139 ± 5.83 b	148 ± 3.30 c	153 ± 3.44 c	167 ± 5.11 d
Q-ABTS (H_2O:EtOH)	Sk 1.5 mg	Sk 1 mg	Sk 0.5 mg	Sk:C (2:1) 1.5 mg	Sk:C (1:2) 1.5 mg
	124 ± 4.10 cb	131 ± 4.56 c	116 ± 8.89 b	117 ± 4.13 b	93.4 ± 4.75 a
	Sd 0.75 mg	Sd 0.5 mg	Sd 0.25 mg	Sd:C (2:1) 0.75 mg	Sd:C (1:2) 0.75 mg
	314 ± 5.45	320 ± 9.30	318 ± 18.1	323 ± 10.1	320 ± 17.4

[a]Q-TAC values are the mean ± standard deviation (n = 3). TE, Trolox equivalents. Roman letters indicate significant differences among the different amounts of sample and the dilutions with cellulose tested within each method (Q-ABTS or Q-ABTS (H_2O:EtOH)) for each product (Sk or Sd).

calibration curves of Q-TAC methodologies carried out in this study were performed by weighing directly the standards, without their previous dilution in any solvent. Thus, the lower solubility of Trolox in water than in organic solvents played a critical role in the response observed in each method, as previously described by other authors.[9] This fact may lead to lower Q-TAC values in the Q-ABTS than in the Q-ABTS (H_2O:EtOH) assay due intrinsically to the methods used rather than the samples analyzed. Therefore, as the numeric Q-TAC values obtained by the Q-ABTS assays may not be directly comparable, the tendency observed among the samples (higher or lower Q-TAC values) was the main interest of these analyses.

The Q-TAC of Sk, W, and Sd obtained using the Q-FC and Q-ABTS assays can be seen in Figure 1. As previously described for classical methods,[1,39] the reaction medium greatly influenced the Q-TAC results, with both soluble compounds and insoluble antioxidants attached to the powdered products being affected by the solvent used in the Q-TAC assays. A similar tendency was observed in both Q-FC and Q-ABTS assays when water was replaced by a mixture of H_2O:EtOH (1:1, v/v) as reaction medium. A significant increase in the Q-TAC values of Sd was observed, reaching higher antioxidant capacity than W, for which Q-TAC was also enhanced, but to a lesser extent. The Sk product showed the lowest Q-TAC in both Q-FC (H_2O:EtOH) and Q-ABTS (H_2O:EtOH) assays. Similar results were obtained in previous studies[21] carried out using conventional methodologies including extraction steps with organic solvents. Indeed, a good correlation between general results obtained using Q-TAC (H_2O:EtOH) and classical methods was observed. In contrast, this fact was only found between Q-TAC (carried out in water) and classical methods when the matrices under study were rich in hydrophilic antioxidants (Supporting Information 2).

Differences among results obtained for the three model products using the different Q-TAC methods can be partly explained by their chemical composition, the solubility of their antioxidant compounds in the reaction medium, and their redox reactions with the radical probes or reagents used in each assay. With regard to the chemical composition of the model products tested, it is important to have in mind that skins of grapes are a rich source of anthocyanins and hydroxycinnamic acids, although they also contain some flavanols and flavonol glycosides, whereas gallic acid, flavanols, and proanthocyanidins are mainly present in the seeds of grapes.[35,37,43] According to these facts, the Sk model product was rich mainly in anthocyanins and hydroxycinnamic acids, whereas Sd was rich in flavanols from monomers to polymers (proanthocyanins and tannins). A description of the solubility of food antioxidants, including main phenolic classes, can be found in Gökmen et al.[11] In general, the water solubility of polyphenols increases with the number of glycosylated hydroxyl groups and with the amount of sugars constituting the carbohydrate moiety. On the other hand, in the case of polymerized polyphenols (such as proanthocyanidins or tannins), the water solubility considerably drops with an increased number of units and with the formation of intramolecular bonds.[44] Thus, the higher polarity of the polyphenols found in the grape skins allows that they are well solubilized when water is used as reaction medium,[44] whereas the presence of ethanol seems to favor the extraction of hydrophobic antioxidants present in the grape seeds. It must be pointed out that lower H_2O:EtOH ratios are not advised because it may cause the shrinking of the major constituents of the food matrices, such as cellulose and proteins.[17,42] This could hamper the release of the compounds or the diffusion of the radicals into the solid matrices where they can react with inner bound insoluble antioxidants, leading to lower Q-TAC values.[11]

The reaction medium also affects the interactions between the antioxidants and reagents involved in the methods by enhancing or decreasing the equilibrium constant of their redox reactions. In the QUENCHER approaches, when the redox equilibrium constant of an antioxidant compound in a determinate medium is higher than its solubility constant, the extraction of this antioxidant from the insoluble matrix and its solubilization in the reaction medium can be thermodynamically enhanced.[42]

The study of the correlations between the Q-TAC assays carried out in the same reaction medium showed a strong correspondence ($p < 0.01$) between Q-FC (H_2O:EtOH) and Q-ABTS (H_2O:EtOH) (r = 0.971), whereas there was no significant correlation between Q-FC and Q-ABTS (r = 0.496). This finding shows that, in the Q-TAC (H_2O:EtOH) methods, the factors related to the assay reaction medium (solubilization of the antioxidant compounds) play a more critical role than those related to the method used (reaction with the probes or reagents). Therefore, the mixture H_2O:EtOH 50:50 (v/v) proposed by Serpen et al.[42] might be adequate to evaluate the global TAC of food materials, but the Q-TAC of hydrophobic

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antioxidants could be overestimated under certain conditions and, then, their real antioxidant role in food matrices.

Moreover, the correlations between the biologically relevant Q-FRSC assays and both Q-TAC (H_2O:EtOH) methods were not significant in the case for the Q-SRSC and Q-HRSC methods, and not even a significant ($p < 0.01$) negative correlation between Q-LPSC and Q-ABTS (H_2O:EtOH) ($r = -0.716$) was found. These results further support the selection of the Q-FC and Q-ABTS assays in water as preferred protocols to assess a potentially more biologically relevant Q-TAC of food products and dietary supplements.

Effects of Using Cellulose as a Diluting Agent in the Q-ABTS Assays. Cellulose has been suggested as a diluting agent in previous Q-TAC protocols.[11,12] However, the affinity of polyphenols to cellulose is well-known,[45] so it is possible that interactions between the cellulose and these antioxidant compounds interfere in the quantification.

The possible influence of cellulose on the Q-TAC measurements was evaluated with the Q-ABTS assays (the most widely used Q-TAC assay up to now), and it was compared with the effects of weighing different amounts of sample. The two model products that presented more differences between them (Sk and Sd) were chosen to carry out this study. Two dilutions or product/cellulose ratios (2:1 and 1:2) were used. The quantities of product weighed were adapted to those required by each method to give results within their detection range. As can be seen in Table 5, no influence of the amount of sample or the presence of cellulose on the Q-TAC values was observed for Sk in the Q-ABTS assay and for Sd in the Q-ABTS (H_2O:EtOH) method. However, Q-ABTS results of Sd showed significantly higher Q-TAC values when lower amounts of sample were used, and this effect was more marked when the product was diluted with cellulose. In contrast, the opposite tendency was found for Sk in the Q-ABTS (H_2O:EtOH) assay. In this case, inferior Q-TAC values were obtained when a lower amount of Sk was used, which were only significant in Sk/cellulose (1:2) with respect to the nondiluted Sk sample.

The observed effect of weighing different amounts of Sd in the Q-ABTS assay could be due to a better water extraction of its slightly soluble hydrophobic polyphenols[11,36] when they are at a lesser concentration. Moreover, possible hydrophobic interactions between the antioxidant compounds (favored in the aqueous medium)[46] could be more relevant when a higher amount of sample is present, thus decreasing their ability to scavenge the soluble $ABTS^{\bullet+}$ radicals present in the reaction medium.

With regard to the influence of cellulose in the measurements, absorbance values similar to those of the $ABTS^{\bullet+}$ working solution were obtained in both methods (Q-ABTS and Q-ABTS (H_2O:EtOH)) when only cellulose was used as sample (data not shown), corroborating that cellulose alone is inert toward the $ABTS^{\bullet+}$ reagent, as has been previously described.[12] Therefore, the results obtained in this study could be due to the effects of cellulose in the solubility of the hydrophilic or lipophilic compounds present in the products and their capacity to interact with the $ABTS^{\bullet+}$ radicals, both factors influenced by the reaction medium. Hydrogen-bonding and hydrophobic interactions between cellulose and polyphenols have been described,[45] which are also dependent on the solvent systems used. Hydrophobic interactions are favored in hydrophilic solvents, whereas hydrogen bonding is favored in the more hydrophobic ones. Thus, the affinity of polyphenols (such as gallotannins and ellagitannins) to cellulose is expected to correlate with their hydrophobicity, their number of galloyl groups, and their molecular size.[44,45] This evidence explains that cellulose effects are of particular importance in the hydrophilic solvents when hydrophobic compounds are tested, as happened when Sd was assessed by the Q-ABTS assay. The monomeric and oligomeric proanthocyanidins of the grape seeds might remain insoluble in aqueous reaction mediums.[40,41] However, their interaction with cellulose through their hydrophobic moieties might raise their water solubility and leave the hydroxyl groups available to interact with the $ABTS^{\bullet+}$ radicals, leading to the increased Q-TAC values observed when the product/cellulose ratio was decreased. On the other hand, in the case of Sk in the Q-ABTS (H_2O:EtOH) assay, the contrary effect may take place. Hydrogen bonding between cellulose and the hydrophilic phenolics present in wine pomace skins could be favored in the presence of organic solvents. Thus, the number of hydroxyl groups available in the molecules to scavenge the radical probes of the assay is reduced.

On the basis of the cited results, the use of cellulose as a diluting agent is discouraged in the Q-TAC methodologies, especially if the antioxidant products tested are a source of polyphenols. The main motive is the possible interaction of cellulose with the phenolic compounds involved in the assays, leading to an under- or overestimation of the Q-TAC of the products.

In summary, a wide range of QUENCHER analytical techniques to provide a comprehensive initial assessment in vitro of the TAC and FRSC of food materials have been adapted and validated (Q-FC, Q-ABTS, Q-DPPH, Q-FRAP, Q-ORAC, Q-SRSC, Q-HRSC, and Q-LPSC). The Q-TAC and Q-FRSC assays skip the extraction step of the classical antioxidant capacity protocols and are simple, quick, inexpensive, and precise methodological approaches. The QUENCHER methodologies validated in this paper working with powdered model products are suitable for application to other foods and food ingredients, both rich in fat (as seeds) and not, as skins, which are rich in fiber and minerals among others. The combination of the Q-FC and Q-ABTS assays using water as reaction medium is proposed as the best choice to analyze a large number of samples. They provide more biologically relevant information than the classical TAC methods using extracts or other Q-TAC approaches using nonaqueous solvents. It is suggested that the same amount of sample be weighed, especially when products rich in hydrophobic antioxidants are assessed. The use of cellulose as a diluting agent is not recommended in the Q-TAC methods. Finally, it must be pointed out that, despite the advantages of the proposed QUENCHER assays over other in vitro methodologies, the Q-TAC or Q-FRSC of food materials and dietary supplements cannot be directly translated into healthful effects provided in vivo.

■ ASSOCIATED CONTENT

Supporting Information

Validation of Q-FC (H_2O:EtOH) and Q-ABTS (H_2O:EtOH) methods; correlations between classical TAC, Q-TAC (H_2O:EtOH) and Q-TAC methods. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.5b01644.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); AUC, area under the curve; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FC, Folin−Ciocalteu; FRAP, ferric reducing/antioxidant power; FRSC, free radical scavenging capacity; GA, gallic acid; HRSC, hydroxyl radical scavenging capacity; LPSC, lipid peroxyl scavenging capacity; Q-, QUENCHER; OC, oxidized controls; ORAC, oxygen radical absorbance capacity; SC, sample controls; Sd, product from wine pomace seeds; Sk, product from wine pomace skins; SRSC, superoxide radical scavenging capacity; T, Trolox; TAC, total antioxidant capacity; W, product from whole wine pomace

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