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Total Oxidant Scavenging Capacities of Common European Fruit and Vegetable Juices

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The total oxidant scavenging capacity (TOSC) assay in a modified and automated version was applied for a comparative and detailed survey of the antioxidant capacities of 14 common European fruit and vegetable juices (ACE, apple, beetroot, blueberry, carrot, elderberry, lemon, lingonberry, multivitamin, orange, pink grapefruit, sauerkraut, and tomato juices as well as sour cherry nectar). The juices were ranked according to their scavenging capacity against the three reactive oxygen species (ROS) peroxyl and hydroxyl radicals and peroxynitrite. These ROS are of physiological and technological relevance and cover a broad range of reactivity. Nonlinear correlations between concentrations of all studied samples and antioxidant capacity were taken into account for the assessment of the results. Due to the more complex assay design, results are only partially in accordance with those of the literature. Because of its outstanding TOSC values against two of the three ROS, lingonberry juice deserves special attention.

KEYWORDS: TOSC assay; oxidant scavenging capacities; antioxidant; peroxyl radicals; hydroxyl radicals; peroxynitrite; fruit juices; vegetable juices

INTRODUCTION

Epidemiological studies demonstrated that food can have beneficial effects on human health in addition to its mere nutritional value (*1*, *2*). In recent years, research in this area has focused on the detection of antioxidants in food, because there is evidence that they could play an important role in the prevention of several illnesses such as cardiovascular disease and cancer as well as in the retardation of the aging process. Fruits and vegetables have received particular attention, because they contain high amounts of known antioxidants such as polyphenols, vitamin C, vitamin E, β-carotene, and lycopene (*3*, *4*).

Several in vitro assays to assess antioxidant capacity against reactive oxygen species (ROS) are available and can be accomplished with relatively low time and labor consumption. The assay conditions should correspond as closely as possible to physiological conditions. ROS should be used that are relevant for biological systems (*5*, *6*), which cover a broad range of reactivity. However, that requirement is not fulfilled in common assays in many aspects. Mostly, they are based on only one and different ROS with different reaction kinetics and with different reaction conditions. Therefore, results obtained with different assays cannot be compared directly. Finally, no standardized assay has been proposed, to date, which could facilitate the comparison of the antioxidant capacities of different materials, for example, of fruit and vegetable juices. Wang and Jiao (*7*) as well as Vinton et al. (*8*) bypassed that drawback by using combinations of different in vitro assays; however, they centered their studies on special juice groups only—respectively, berries and citrus fruits.

A way out of the problems outlined above could be possible by means of the total oxidant scavenging capacity (TOSC) assay, which was introduced for environment-related studies on marine organisms not so long ago (*9*, *10*). It is based on the inhibition of the radical-dependent formation of ethylene from keto-methiolbutyric acid by antioxidants. The TOSC assay permits testing against three different ROS with physiological relevance and different reactivities (peroxyl and hydroxyl radicals as well as peroxynitrite). It can be accomplished at physiological temperature and pH; nonlinear concentration-dependent activity variations can be examined easily, and different types of antioxidant reaction (retardant or fast-acting) can be distinguished. To our knowledge, hitherto TOSC applications on fruits and vegetables are reported only for a few products (*11*, *12*) which, moreover, do not use all of the three above-mentioned ROS.

Therefore, the objective of this work was to examine if the TOSC assay in its recently published improved version (*13*) is suitable to characterize the antioxidant properties of commonly marketed European fruit and vegetable juices in more detail and in comparison to each other as well as to contribute to better understanding of the role of different antioxidant juice ingredients.

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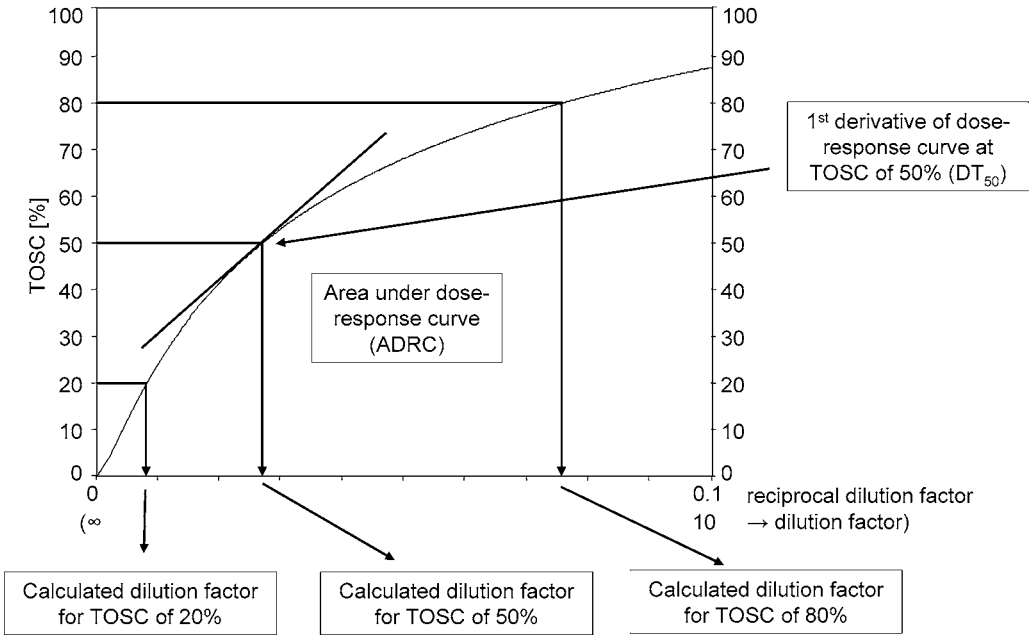

Figure 1. Overview of evaluation potentialities of TOSC assay data.

MATERIALS AND METHODS

Materials. *Chemicals.* Ultrahigh-quality (UHQ) water was prepared with a UHQ-II system (ELGA, Ubstedt-Weiher, Germany) and was used for all solutions. Diethylenetriaminepentaacetic acid (DTPA), 3-morpholinosydnonimine *N*-ethylcarbamide (SIN-1), and α-keto-γ-methiolbutyric acid (KMBA) were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). 2,2′-Azobis(2-methylpropionamidine) dichloride (ABAP), ferric chloride hexahydrate, ethylenediaminetetraacetic acid (EDTA), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Acros Organics (Geel, Belgium). Ascorbic acid was from Kraemer & Martin (Sankt Augustin, Germany).

Fruit and Vegetable Juices. ACE [vitamin A-, C-, and E-rich juice; mixture of orange, apple, grapefruit, passion fruit, acerola, lemon, and carrot juices with banana and rosehip pulp and a declared content of 30 mg of vitamin C, 5 mg of vitamin E, and 400 μg of vitamin A (added as provitamin A) per 100 mL], apple, beetroot, blueberry, carrot, elderberry, lingonberry, multivitamin [mixture of apple, orange, pear, pineapple, grape, passion fruit, and lemon juices with banana, nectarine, mango, guava, and papaya pulp and a declared averaged content of 45 mg of vitamin C, 6.5 mg of vitamin E, and 280 μg of vitamin A (added as provitamin A) per 100 mL], and sauerkraut and tomato juices as well as a sour cherry nectar (mixture of at least 50% cherry juice with water, sugar, and glucose syrup) were purchased as commercial products. For beetroot, carrot, sauerkraut, and tomato juices, samples from two different companies were bought. Beetroot juice I and carrot juice I were lactic-acid-fermented during the manufacturing process. Beetroot juice II and carrot juice II contained about 0.3 and 1−2% of lemon juice, respectively. Lemon, orange, and pink grapefruit juices were made by pressing fresh fruits from local supermarkets with a common juicer to avoid potentially added vitamin C in commercial juices. Carrot, tomato, lemon, orange, and pink grapefruit juices were filtered through a folded filter (Schleicher & Schuell, Dassel, Germany) before analysis to remove insoluble pulp parts. The juices were thinned with UHQ water to at least five different dilutions for each of the three ROS to cover the respective range from a low to a high antioxidant capacity as completely as possible. The diluting was done in duplicate in all cases, and each solution was measured at least twice.

Methods. *TOSC Assay Conditions.* The TOSC assay is based on the ethylene-yielding reaction of KMBA with peroxyl radicals, hydroxyl radicals, and peroxynitrite. The capacities of the different juices and Trolox to inhibit this ethylene production from the three different ROS were analyzed. Peroxyl radicals were generated by the thermal homolysis of ABAP. Hydroxyl radicals were formed during the iron plus ascorbate driven Fenton reaction. Peroxynitrite was produced by the decomposition of SIN-1. For details of assay conditions see refs *9*, *10*, and *13*.

Gas Chromatographic Analysis of Ethylene. The time course of ethylene formation at 37 °C during 1 h was monitored for six samples in parallel by repeated gas chromatographic analyses of 100 μL aliquots from the headspace of the 10 mL reaction vessels. Sampling was done by a CombiPAL autosampler (CTC Analytics AG, Zwingen, Switzerland) with a 1 mL headspace syringe, which was heated to 37 °C. The analyses of ethylene were carried out with a GC-17A gas chromatograph (Shimadzu, Duisburg, Germany) and a Chrompack PoraPLOT Q column (27.5 m × 0.53 mm × 20 μm; Varian, Darmstadt, Germany). Oven, injection port, and FID temperatures were 80, 100, and 220 °C, respectively. Nitrogen was used as carrier gas at a flow rate of 15 mL/min in split-off mode. The retention time of ethylene was 1.6 min. Peak integration was performed with EZChrom Elite v 2.8 (Scientific Software Inc., Pleasanton, CA). See ref *13* for more detailed information.

Data Evaluation. An overview of the various evaluation potentialities of the experimental TOSC data is provided in **Figure 1**.

Experimental TOSC Values. The kinetic curves that best fit the experimental GC data for ethylene production over a period of 60 min and the area beneath them were calculated with the data analysis software Root v 3.02/07 (developed at the CERN Particle Physics Centre, Genève, Switzerland). TOSC values were quantified by comparing the areas for the uninhibited control and the sample reactions. A TOSC value of 0%, for example, characterizes a sample without antioxidant properties; a solution that suppresses the ethylene formation completely achieves a TOSC value of 100% (*9*, *10*, *13*).

Calculated Data for TOSC of 20, 50, and 80%. The experimental TOSC values for Trolox were plotted versus the corresponding millimolar concentration of the added Trolox solutions. For the juices, the experimental TOSC values were plotted versus the reciprocal value of the dilution factor of the added juice. Dose−response curves were fitted that showed the best correlation for the respective data. On the basis of the resulting equations, the dilution factors of the juices and the concentrations of Trolox were calculated that match TOSC values of 20, 50, and 80%, respectively. Curve fits and TOSC calculations were accomplished with the software TableCurve 2D v 5.1 (SYSTAT Software Inc., Richmond, CA).

Area under Dose−Response Curves (ADRC) and First Derivative at TOSC of 50% (DT_{50}). The ADRC of the juices from the zero point

Table 1. Peroxyl Radicals: Calculated Dilution Factors and Concentrations for Different TOSC Values, ADRC, DT_{50}, and Fit Correlation for Juices and Trolox

	TOSC of					
	20%	50%	80%	DT_{50}	ADRC	r^2 of fit
diagonal defined as				1.0	1.0	
	calcd dilution factor					
lingonberry juice	1667	556	238	19.0	>2.0	1.0000
blueberry juice	833	357	167	14.3	>1.9	0.9995
elderberry juice	769	286	137	11.4	>1.9	1.0000
beetroot juice I (lactic acid fermented)	500	185	100	8.1	>1.9	1.0000
sour cherry nectar	526	185	79	6.7	>1.9	0.9991
beetroot juice II	337	114	57	4.5	>1.8	1.0000
ACE juice	213	69	30	2.4	1.6	0.9990
multivitamin juice	217	68	28	2.2	1.6	1.0000
orange juice	125	41	20	1.5	1.4	1.0000
carrot juice I (lactic acid fermented)	128	41	19	1.5	1.4	1.0000
pink grapefruit juice	133	40	17	1.4	1.3	1.0000
lemon juice	105	38	18	1.5	1.3	1.0000
apple juice	100	35	14	1.2	1.2	0.9996
sauerkraut juice I	99	26	12	0.8	1.1	1.0000
tomato juice I	81	25	11	0.8	1.0	0.9997
tomato juice II	71	23	10	0.8	1.0	0.9989
carrot juice II	69	21	9	0.8	1.0	1.0000
sauerkraut juice II	66	21	9	0.7	0.9	0.9999
	calcd concn (mM)					
Trolox	0.026	0.072	0.166			1.0000

of the coordinate system up to a reciprocal dilution of 0.1 (i.e., a dilution factor of 10) were as well calculated as the DT_{50} (the first derivative of the juice curves at a TOSC of 50%). DT_{50} values were normalized by defining the corresponding area and the first derivative of the diagonal of the coordinate system as 1. These calculations were performed using TableCurve 2D v 5.1 from SYSTAT Software Inc.

RESULTS AND DISCUSSION

The juices included in this study were chosen in such a manner that samples with high concentrations of different antioxidant groups are included; for example, different berry juices known to be rich in anthocyanins, beetroot juice containing betalains, and citrus juices containing flavanones as well as juices enriched in ascorbic acid, tocopherols, and β-carotene. Thus, the influences of these particular antioxidant groups on the overall antioxidant capacity can be considered.

For all of the studied juices TOSC assays against peroxyl and hydroxyl radicals as well as against peroxynitrite were performed. For each juice specific dilutions were prepared to cover TOSC values (degree of inhibiting ethylene formation) from 20 to 80%, at least. On the basis of these experimental data dilution factors have been calculated that correspond to TOSC values of 20, 50, and 80%. Furthermore, to give a more demonstrative overview of the concentration-dependent variation of the antioxidant capacities, the dose−response curves (ADRC) from the zero point of the coordinate system to a dilution factor of 10 and the first derivative of the curves at a TOSC of 50% (DT_{50}) are assessed (for explanation see **Figure 1**). The obtained results are summarized in **Tables 1**−**3**.

It is noticeable that, in all cases, nonlinear correlations between sample concentrations and antioxidant capacities were observed, which in many cases vary substantially from each other. Thus, simple descriptions of the antioxidant capacities by a single parameter, such as indication of Trolox equivalents for a distinct juice dilution, are not sufficient for the assessment and comparison of the antioxidant capacity of juices. Therefore, dose−response curves of selected juices that are representative for the complete range of the antioxidant capacity spectrum as well as for Trolox are displayed in **Figures 2**−**5**.

Table 2. Peroxynitrite: Calculated Dilution Factors and Concentrations for Different TOSC Values, ADRC, DT_{50}, and Fit Correlation for Juices and Trolox

	TOSC of					
	20%	50%	80%	DT_{50}	ADRC	r^2 of fit
diagonal defined as				1.0	1.0	
	calcd dilution factor					
beetroot juice I (lactic acid fermented)	500	135	34	3.7	1.6	0.9998
blueberry juice	909	141	29	2.8	1.6	1.0000
elderberry juice	606	128	26	2.7	1.5	0.9994
beetroot juice II	357	109	29	2.8	1.5	1.0000
lingonberry juice	588	106	22	1.7	1.5	1.0000
sour cherry nectar	435	82	16	1.7	1.4	1.0000
multivitamin juice	161	40	14	1.1	1.3	0.9999
ACE juice	143	37	13	1.0	1.2	1.0000
lemon juice	108	34	14	1.1	1.2	1.0000
orange juice	105	33	13	1.1	1.2	0.9991
pink grapefruit juice	115	33	12	0.9	1.2	0.9999
carrot juice I (lactic acid fermented)	123	31	5	0.6	1.0	1.0000
sauerkraut juice I	76	22	7	0.6	1.0	1.0000
apple juice	103	22	4	0.4	0.9	0.9994
tomato juice I	64	18	4	0.4	0.8	0.9999
carrot juice II	62	19	3	0.5	0.8	0.9998
sauerkraut juice II	56	17	6	0.5	0.8	0.9997
tomato juice II	55	17	4	0.4	0.8	0.9997
	calcd concn (mM)					
Trolox	0.036	0.103	0.216			0.9991

Table 3. Hydroxyl Radicals: Calculated Dilution Factors and Concentrations for Different TOSC Values, ADRC, DT_{50}, and Fit Correlation for Juices and Trolox

	TOSC of					
	20%	50%	80%	DT_{50}	ADRC	r^2 of fit
diagonal defined as				1.0	1.0	
	calcd dilution factor					
lingonberry juice	214	90	33	3.4	1.6	1.0000
sour cherry nectar	161	45	15	1.3	1.3	0.9999
blueberry juice	141	48	13	1.5	1.3	1.0000
ACE juice	123	34	13	1.1	1.2	1.0000
elderberry juice	99	32	13	1.1	1.2	1.0000
multivitamin juice	154	36	10	0.9	1.2	1.0000
beetroot juice I (lactic acid fermented)	105	32	10	0.9	1.1	1.0000
pink grapefruit juice	145	33	10	0.8	1.1	0.9999
apple juice	139	36	8	0.9	1.1	1.0000
carrot juice I (lactic acid fermented)	100	36	8	0.9	1.1	0.9998
sauerkraut juice I	106	33	9	0.7	1.1	1.0000
beetroot juice II	100	30	9	0.8	1.1	1.0000
carrot juice II	100	30	8	0.8	1.1	1.0000
sauerkraut juice II	112	28	8	0.7	1.1	1.0000
orange juice	106	27	8	0.7	1.0	0.9992
tomato juice I	64	19	5	0.5	0.9	0.9995
tomato juice II	52	16	5	0.5	0.8	0.9998
lemon juice I	36	11		0.3	0.6	1.0000
	calcd concn (mM)					
Trolox	0.622	2.599	11.51			0.9999

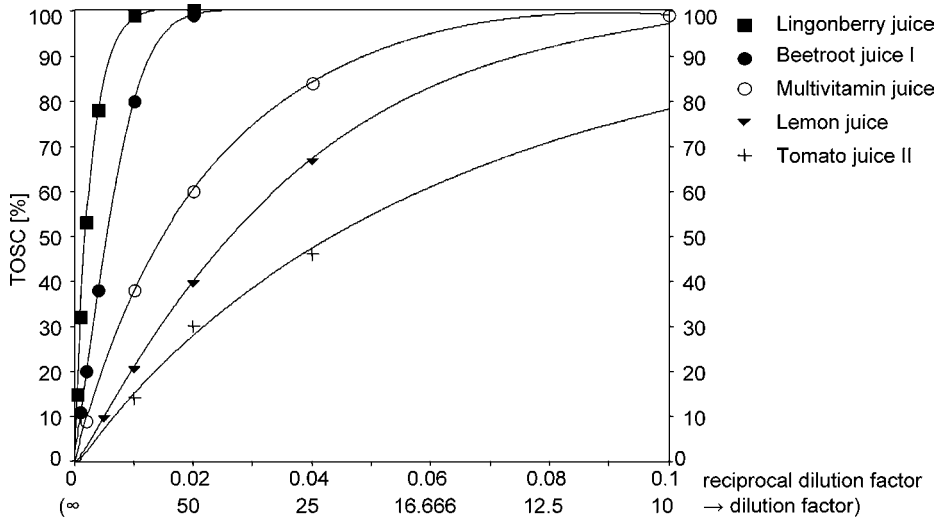

Figure 2. Characteristic dose–response curves of selected fruit and vegetable juices against peroxyl radicals.

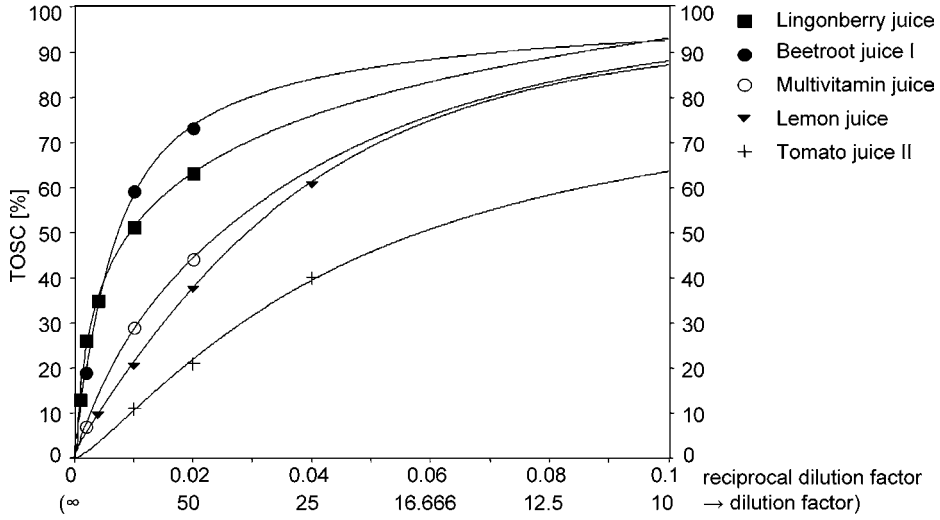

Figure 3. Characteristic dose–response curves of selected fruit and vegetable juices against peroxynitrite.

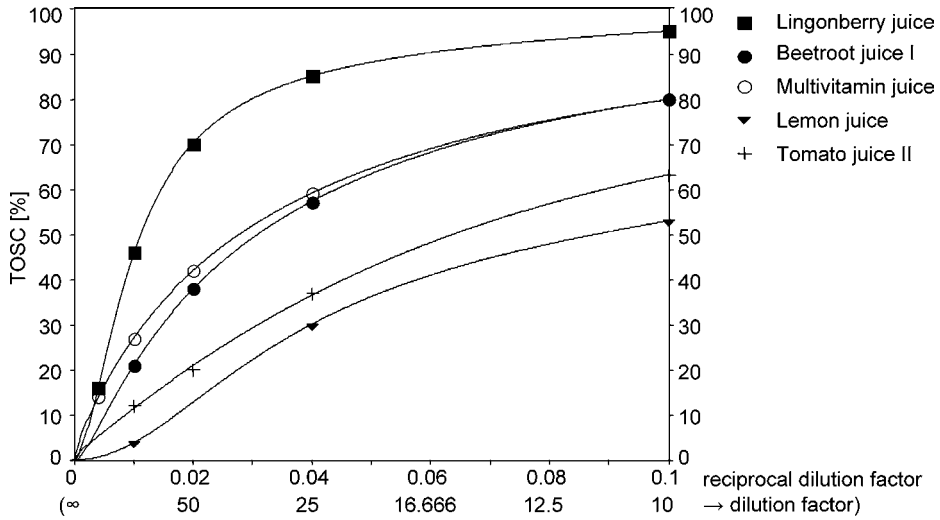

Figure 4. Characteristic dose–response curves of some fruit and vegetable juices against hydroxyl.

Before conclusions could be drawn from the results, it had to be proved that the test conditions are comparable and adequate for all of the juices. Mainly the pH dependence of antioxidant activities and possible adverse effects of sample filtration had to be examined. Furthermore, the possibilities and limits of comparison of antioxidant capacity of juices with standard compound solutions are discussed.

pH Dependence of Antioxidant Capacities. Protonating or deprotonating a molecule can affect its ability to donate an electron or a hydrogen radical and, therefore, its efficiency to

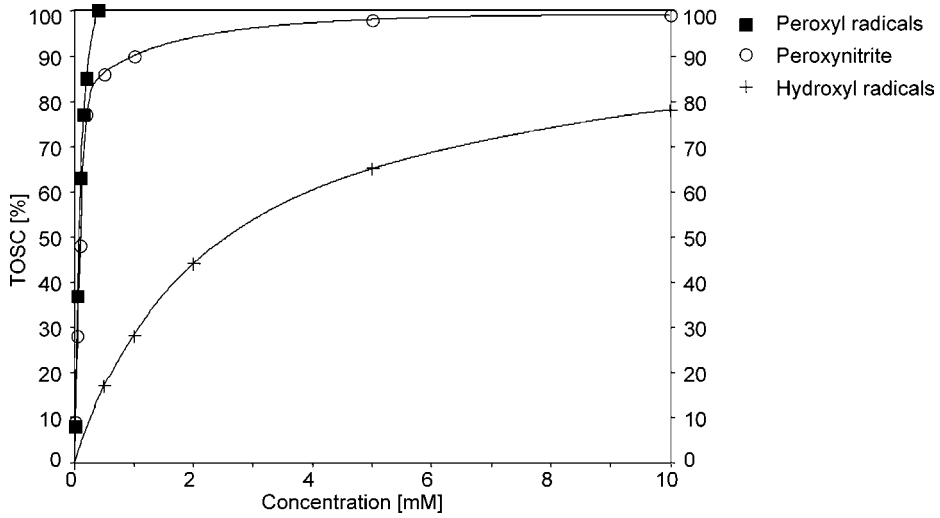

Figure 5. Dose–response curves of Trolox against peroxyl radicals, peroxynitrite, and hydroxyl radicals.

work as an antioxidant. For some flavonoids it was shown that their antioxidant activity could be increased significantly by increasing the pH (*14*); the capacity of some hydroxybenzoates was affected only at high pH, whereas Trolox was not influenced over the whole tested pH range (*15*). Therefore, results for the antioxidant activity of solutions should be compared only if they have the same pH or if it is proven that they consist of just pH-independent antioxidants.

Thus, some pure and only little-diluted juices of our study could not be taken into account, because their acidity exceeded the capacity of the buffer in the reaction vessels: Only the juices with a pH >4 (beetroot, carrot, and tomato juices) could be analyzed in pure form. The juices with a pH between 2.5 and 4 (ACE, apple, blueberry, elderberry, lingonberry, multivitamin, orange, pink grapefruit, and sauerkraut juices as well as the sour cherry nectar) had to be diluted to at least 1:5. For the lemon juice (pH <2.5) the minimum required dilution for performing the TOSC assay was 1:10. Because the experimental lemon juice TOSC values against hydroxyl radicals were found to be comparatively low (dilution factor 1:11 for a TOSC of 50%), it was not possible to calculate the corresponding dilution for a TOSC of 80% against this ROS.

Consequently, the assay conditions are to be considered as adequate for nearly all juices with the exception of very acid samples with very low TOSC values.

Influence of Filtering Juices on TOSC Values. The TOSC assay in the herein performed form covers only water-soluble antioxidants, although the antioxidant capacity of samples could be affected by filtration. Possible reasons could be, for example, a longer exposure of the samples to air at ambient temperature or an interaction with the filter material. This assumption was checked by analyzing carrot juices from two different companies as well as filtered or nonfiltered.

No significant difference could be found between the antioxidant capacities of filtered and nonfiltered samples. Some smaller differences at certain points of the dose−response curves were leveled when a broader range of data was taken into account (i.e., the value for the ADRC).

From these findings it was gathered that juices with high pulp content (tomato, lemon, orange, and pink grapefruit juice) can be filtered before the TOSC assay is performed, so the advantages of easier handling and better homogeneity of filtered samples can be capitalized.

Comparison of TOSC Values from Juices with Those from Standard Compounds such as Trolox (Possibilities and Limits). From the unequal nonlinear correlations between concentrations of different samples and antioxidant capacities it can be concluded that comparison of TOSC values of juices with those of Trolox is valid only for distinct dilution factors or rather selected points of the respective dose−response curve. Therefore, calculations of global Trolox equivalents (as performed in some other literature assays) are not possible and not allowable, in general. Answers to questions such as "Which concentration of Trolox or dilution of juice corresponds to a definite TOSC value?" or "Which Trolox concentration and which juice dilution is necessary to obtain a desired TOSC value?" can be taken mathematically from the respective dose−response curves as done for the dilutions of juices or concentrations of Trolox, respectively, corresponding to TOSC values of 20, 50, and 80% (see **Tables 1**−**3**).

ADRC and DT_{50} are useful parameters for comparison of the TOSC values of juices or standard compounds among each other; however, that is not the case for comparisons between the antioxidant capacities of juices and standard compounds because of the impossibility to choose the same concentration dimensions for the heterogeneous juices and pure standard compounds. Juices usually contain antioxidants with different molecular weights in different concentrations and possible synergistic or antagonistic effects. Therefore, their concentrations can be quoted only in units of weight per volume (such as grams of dry mass per liter, e.g.), not in molar units. Likewise, it is not suggested that standard compounds be measured in weight per volume units because comparison would be possible only if all of the antioxidant compounds within the juice would contribute equally to the antioxidant activity. Furthermore, illustrating the Trolox concentration in terms of dilution factors based on a specific starting concentration is not helpful in solving that problem, bearing in mind that, naturally, the resulting ADRC values depend on the concentration range which was chosen for such a starting point. Because the Trolox dose−response curves for the three studied ROS are very different (see **Figure 5**), it is not possible to choose the same starting point for calculation of ADRC and DT_{50} values. Therefore, the choice of dilution factors as dimension is out of the question, also.

In conclusion, mathematical calculations from the respective dose−response curves enable the comparison of the antioxidant capacities of juices with that of standard compounds such as Trolox for distinct juice dilutions and standard compound concentrations. It is not possible to define Trolox equivalents

which cover a range of concentrations of the samples. ADRC and DT_{50} are useful parameters only for the comparison of TOSC values of juices or standard compounds among themselves.

Comparison of Juices among Themselves. *TOSC against Peroxyl Radicals.* The group of the red juices rich in anthocyanins or betalains (i.e., the berry, cherry, and beetroot juices) showed the highest antioxidant capacities against peroxyl radicals. Among them, the lingonberry juice was the most effective one. In this group of juices the results for the concentrations corresponding to the different TOSC values as well as the DT_{50} were noticeably higher than for all other analyzed samples. Their capacities were so high that their TOSC values achieved 100% already at dilution factors of ~50 or even higher; the other juices do not achieve complete inhibition even at a dilution factor of only 10.

Consequently, in comparison to the other juices, ADRC values for the red juices are comparatively very high, also. However, the values presented for the red juices in **Table 1** are underestimated because the dose−response curves for the red juices are defined correctly only in the range from very high dilution (∞) to dilution factors near 50 when the TOSC values reach 100%; to get at least approximate values for their ADRCs, the same TOSC values (100%) were taken for the range of dilution factors between 50 and 10, although the antioxidant capacity surely increases with decreasing dilution factors. Therefore, in **Table 1** the ">" symbol was added to the calculated ADRC values of the red juices. The group that follows the red juices concerning the antioxidant capacity against peroxyl radicals is that of the two vitamin-added juices (ACE and multivitamin juice). All of their results (dilution factors for the specified TOSC values and DT_{50} and ADRC values) are very close to each other. It is noticeable that there is a large gap to the values for the red juices. Another distinct gap exists for the values of the group of citrus juices (orange, pink grapefruit, and lemon juices). Their antioxidant capacities are very similar, as well, yet approximately only half in comparison to the vitamin-added juices. Apple, tomato, carrot, and sauerkraut juices follow with even lower antioxidant capacities against peroxyl radicals.

Samples of tomato and sauerkraut juices from two different producers were studied (indicated in **Tables 1**−**3** as I and II), and very similar antioxidant capacities were observed. In the case of carrot and beetroot juices one normal commercial juice sample (indicated as sample I) and another one that was lactic acid fermented (indicated as sample II) were studied. The values for both lactic acid fermented samples showed considerably higher antioxidant capacities than the others.

TOSC against Peroxynitrite. For all of the measured juices the TOSC values against this ROS are remarkably lower than those against peroxyl radicals. Once more, the highest antioxidant capacities were found in the group of the red juices, yet the differences from the other juices are not so outstanding.

Obviously the reaction behavior against peroxynitrite is different for the anthocyanin-containing berry juices in comparison to the betalain-containing beetroot juice. A considerably higher concentration was required for the betalain juice to achieve a TOSC value of 20% in comparison to the anthocyanin juices. However, the dilution factors for TOSCs of 50 and 80% were quite similar for both groups (see **Table 2** and **Figure 3**). These results are in agreement with literature data, where for both types of antioxidants different reaction mechanisms against peroxynitrite, interfering at different stages of the multilevel formation of this ROS or regarding the reaction with the KMBA molecule (*16*, *17*), are proposed.

In the case of peroxynitrite the antioxidant activities for the vitamin-added and citrus juices are very similar, lower than that for the red juices and somewhat higher than for the others. With respect to the juices with lower TOSC values against peroxynitrite it is noticeable that the lactic acid fermented carrot juice and the apple juice dropped in the ranking of juices in comparison to that of the peroxyl values. In this group, the phenomenon of juices with different slopes for the dose−response curves but similar values for a TOSC of 50% also emerged (i.e., for sauerkraut juice I and apple juice). As for the anthocyanin and betalain juices, a different kind of reaction mechanism can be suggested herein. These findings emphasize the importance of analyzing a number of different dilutions for the same juice to get a well-founded characterization of its antioxidant capacities.

TOSC against Hydroxyl Radicals. The majority of the studied juices show similar, not very outstanding, antioxidant property capacities against hydroxyl radicals (see **Table 3** and **Figure 4**). Only some of the anthocyanin-containing juices, lingonberry juice, sour cherry nectar, and blueberry juice, demonstrated superior antioxidant properties. The tomato juices and also the lemon juice presented the lowest activity, in this case. The, at first sight, astoundingly low antioxidant capacity of the lemon juice against hydroxyl radicals is most likely due to its high vitamin C content. Depending on its concentration and the circumstances of reaction, vitamin C can act not only as an antioxidant but also as a prooxidant (*13*, *18*). Therefore, in this assay it is used as the radical starter for the formation of hydroxyl radicals, and so it was remarkable that the other juices rich in vitamin C (ACE, multivitamin, orange, and pink grapefruit) showed substantially higher inhibition against hydroxyl radicals than lemon juice. This suggests that in these juices the prooxidant features of vitamin C are overridden by effective antioxidant compounds to a greater extent than in lemon juice.

Comparison of TOSC Values for the Three Assayed ROS. Nearly all juices were most efficient against peroxyl radicals, much fewer against peroxynitrite, and fewest against hydroxyl radicals. Additionally, the biggest differences between the analyzed juices could be detected against peroxyl radicals. For peroxynitrite, the variations were much smaller. For hydroxyl radicals, the borders between different groups of juices had nearly vanished.

These findings can be explained by the highly different half-lives and reactivities of the three assayed ROS (*6*, *9*). Peroxyl radicals are the most stable ones with the least reactivity. Therefore, they can be simplest scavenged. If different antioxidants are present in a mixture, they have time enough to compete for the reaction with peroxyl radicals so that the most effective ones are able to dominate. Peroxynitrite is already much more reactive, but it is easily beaten by the hydroxyl radicals. They are so reactive that they combine with almost every molecule they can reach. Therefore, nearly every compound that is present in food can be seen as a hydroxyl scavenger, although they are not all very effective (*9*). In addition, different molecules such as vitamin C can act as prooxidants for the formation of hydroxyl radicals (*13*, *18*).

These facts explain why the results varied so much for peroxyl radicals and were so close together for hydroxyl radicals in most cases. The same conclusions may explain the course of the dose−response curves for Trolox (see **Figure 5**). It was not possible to obtain complete protection against peroxynitrite and

especially against hydroxyl radicals. The dose−response curves reach plateaus of inhibition (for peroxynitrite, ∼98%; for hydroxyl, ∼80%) that cannot be surmounted even when much higher concentrations of Trolox are present. Obviously, at least some of these highly reactive ROS are able to escape from the antioxidants and to react with some of the KMBA molecules.

In conclusion, all of the studied juices rich in anthocyanins and betalains deserve closer attention: they presented outstanding scavenging properties against peroxyl radicals and the comparatively best values against peroxynitrite. The ranking was more diverse for their ability to scavenge hydroxyl radicals. Lingonberry juice is worthy of special highlighting; among the studied juices it has the highest scavenging capacity against the highly reactive hydroxyl radical, by far.

For the two studied tomato and sauerkraut juices no significant differences, only minor differences, could be found between the antioxidant activities against all three assayed ROS. However, differences appeared between the lactic-acid-fermented and the nonfermented variants. The fermented samples showed significantly higher antioxidant capacities against peroxyl radicals and peroxynitrite than the nonfermented ones; for hydroxyl radicals the results were nearly equal. Therefore, an influence of the lactic acid fermentation can be suspected. This is in agreement with literature results where an improvement of the antioxidant capacity for sweet potato yogurt and milk (*19*, *20*) was reported. Fermentation enhanced also the antioxidant properties of a lot of other kinds of food such as pomegranate juice (*21*), soybean (*22*), or a special Asian antioxidant cocktail called EM-X (derived from fermentation of unpolished rice, papaya, and sea weeds) (*23*). Therefore, a positive influence of fermentation on the antioxidant capacities of the analyzed juices stands at least to reason.

Actually, a further important aspect for the evaluation of fruit and vegetable juices with respect to their antioxidant capacity, its alteration during storage, is being studied. Not yet published results indicate that the TOSC values of anthocyanin-containing juices against all three ROS do not follow the degradation velocity of the anthocyanins; surprisingly, the TOSC values remain almost on the initial level for a prolonged period.

Comparison of the Results with Literature Data. Many research results have been published about the antioxidant activity of berries, for example, for blueberries (*24*, *25*); most papers concerning berries from the genus *Vaccinium* are published about cranberries, but some surveys deal also with the smaller relative lingonberry (*26*, *27*). Analyses have also been done in the field of the antioxidant capacities of sour cherries (*28*, *29*) and elderberries (*30*). Consistently and in accordance with our findings in the literature, higher antioxidant capacities are reported for berry and cherry samples than for other kinds of fruits (*31*−*33*).

The good antioxidant properties of beetroot have also been stated in a couple of papers (*34*, *35*). However, to our knowledge, up to now no direct comparison is published for the antioxidant capacities of beetroot with those of berry or other fruit juices. Proteggente et al. (*31*) specified fruits rich in flavanones (e.g., oranges and grapefruits) to be second best after fruits rich in anthocyanins. For multivitamin and ACE juices Henn et al. (*36*) reported antioxidant capacities in the same range as for orange and grapefruit juices. These findings can be confirmed only partially by our results. Against peroxynitrite the antioxidant capacities of the vitamin-added juices are in the same level as the citrus juices; however, against hydroxyl and particularly against peroxyl radicals they are more effective.

The results for apple juices in the literature are more diverse: some authors came to the conclusion that their antioxidant capacities are rather poor (*31*, *32*, *36*), whereas others reported rather good antioxidant activities (*33*, *37*). Partially, this is explainable by the fact that a lot of apple varieties (*38*) with different amounts of antioxidants exist. On the other hand, comparison of the results is complicated when capacities based on an assay against only one ROS are evaluated. The ranking of the apple juice included in our TOSC assay based study was different depending on the regarded ROS. In comparison to the other juices its capacity against peroxyl and hydroxyl radicals was fair, against peroxynitrite, only poor.

For tomatoes, there are also contradictory results in the literature; those with weak results (*31*, *39*) dominate the few papers with more promising results (*32*). Carrots showed better antioxidant capacities than tomatoes and worse than white cabbage in the surveys of Gazzani et al. (*40*, *41*). This aligns partly with our findings for carrot, tomato, and sauerkraut juices. In relation to peroxyl and peroxynitrite the respective values were poor and close together (with the exception of the peroxyl value of one of the carrot juices); with respect to hydroxyl radicals carrot and sauerkraut values were close together and notably higher than those for tomato juice.

Altogether, our results are partially in good accordance with literature data, partially not. Obviously, this is because of basically different survey parameters. Most of the literature surveys are done with only one, rather stable ROS; variations due to dilution effects are not considered. Our data are based on the TOSC assay in which three different ROS with a broad spectrum of reactivities are used and the nonlinearity of the concentration dependence of the scavenging capacities is taken into account.

The data presented here confirm that the TOSC assay is a well-founded method and appropriate to survey the antioxidant capacities of juice samples in detail. Its main advantages are the fact that nonlinear correlation between juice concentrations and antioxidant capacities can be taken into account and that inhibition effects against three naturally occurring ROS with different reactivities can be studied.

ABBREVIATIONS USED

ABAP, 2,2′-azobis(2-methylpropionamidine) dichloride; ADRC, area under dose−response curve; DT_{50}, first derivative of dose−response curve at a TOSC of 50%; DTPA, diethylenetriaminepentaacetic acid; EDTA, ethylenediaminetetraacetic acid; KMBA, α-keto-γ-methiolbutyric acid; ROS, reactive oxygen species; SIN-1, 3-morpholinosydnonimine *N*-ethylcarbamide; TOSC, total oxidant scavenging capacity; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; UHQ, ultrahigh quality.

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