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Article *in* Journal of Agricultural and Food Chemistry · March 2006

DOI: 10.1021/jf052697j · Source: PubMed

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JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY

J. Agric. Food Chem. **2006,** *54,* 1271−1276 **1271**

Polyphenol Composition and Antioxidant Activity of Kei-Apple (*Dovyalis caffra*) Juice

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The polyphenolic and ascorbate (ASC) components as well as the antioxidant capacity of Kei-apple (*Dovyalis caffra*) juice were analyzed and compared to three other fruit juices. The Kei-apple juice had significantly the highest total polyphenolic concentrations (1013 mg gallic acid equivalent/L), and solid phase (C_{18}) fractionation identified the majority of these polyphenols to be phenolic acids. The Kei-apple juice also had significantly the highest ASC concentrations (658 mg/L), which showed exceptional heat stability with very little conversion to dehydroascorbate (DHA). Antioxidant capacities of both the unfractionated fruit juices and their solid phase-extracted fractions, as determined by oxygen radical absorbance capacity and ferric reducing antioxidant power analyses, correlated well to the polyphenol concentrations. Gas chromatography−mass spectrometry analyses showed caffeic acid as the most abundant polyphenol present (128.7 mg/L) in the Kei-apple juice; it contributed to 63% of the total antioxidant capacity (of all of the individual compounds identified). Other notable polyphenols identified in higher concentrations included *p*-coumaric acid, *p*-hydroxyphenylacetic acid, and protocatechuic acid. Our results therefore support the putative high antioxidant value linked to this fruit and better define this potential in terms of the major antioxidants that exist in the Kei-apple.

KEYWORDS: Kei-apple; *Dovyalis caffra*; antioxidant; polyphenols; ascorbate; GC-MS; ORAC; FRAP

INTRODUCTION

Polyphenols in foods have recently gained much attention, because of, among others, their antioxidant functions and their possible impact on human health. This is demonstrated by reports of their biological activity in cancer, cardiovascular diseases, and neurodegeneration (*1*). Berries and fruits contain a wide range of flavonoids and phenolic acids that show antioxidant activity. Flavonoid subgroups in fruits are anthocyanidins, flavonols, flavones, catechins, and flavanones (*2*). Phenolic acids occur as hydroxylated derivatives of benzoic acid and cinnamic acid (*3*). Many of these polyphenols have redox properties allowing them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers (*4*) and thus contribute to the antioxidant capacity of teas, wines, and a range of fruits and vegetables (*5*, *6*). Because of the beneficial effects attributed to polyphenols (*7*, *8*), there is new interest in finding vegetal species with high antioxidant content and relevant biological activity (*9*).

The Kei-apple (*Dovyalis caffra*) is native to the Kei River region of southwestern Africa. It also grows abundantly and wild in the eastern regions of South Africa. The nearly-round bright yellow fruit has a tough skin and an apricot-textured, juicy, highly acidic flesh with 5−15 seeds arranged in double rings in the center. Very little is known about the nutritional value of this fruit. It has, however, been reported to be rich in ascorbic acid (AA) (83 mg/100 g) and consists of 3.7% pectin (*10*). Additionally, the fruit has a frank taste and is subsequently thought to be rich in polyphenolic compounds, but no quantification of these has yet been reported.

In this study, we investigated and report the major compounds associated with antioxidant function in Kei-apple juice. This was done by evaluating the polyphenol and ascorbate (ASC) content, as well as the total antioxidant capacity of unfractionated and C_{18}-fractionated Kei-apple juice, and comparing these data with that obtained from three commonly used fruit juices, known to have a relatively high polyphenol and/or ASC content. We additionally report a more detailed characterization of the individual polyphenol components of the Kei-apple juice and discuss the possible health benefits of these.

MATERIALS AND METHODS

Preparation of Fruit Juices. Fruit juices were prepared from 100 Kei-apples randomly selected from a larger batch of 500 kg picked in the month of November from the Bloemhof area in South Africa. The other fruits used in the comparison were purchased from various South African fresh produce market places and included 100 strawberries, 200 red globe grapes, and 50 Valencia oranges. All fruits were immediately frozen at −85 °C until juice preparation. Small scale juice

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10.1021/jf052697j CCC: $33.50

Table 1. Total Polyphenols, Total ASC, ASC, and DHA Concentrations Determined in Kei-Apple, Grape, Strawberry, and Orange Juice

juice	total polyphenols (mg GAE/L)[a]	total ASC (mg/L)[a]	ASC (mg/L)[a]	DHA (mg/L)[a]
Kei-apple	1013 ± 3.0 abc	669 ± 15.7 ab	658 ± 0.7 abc	12 ± 0.2 ab
grape	269 ± 1.4 ad	14 ± 1.4 acd	12 ± 1.2 adf	1 ± 0.1 de
strawberry	567 ± 10.1 bde	693 ± 17.7 ce	211 ± 5.3 bde	482 ± 11.6 adf
orange	264 ± 0.9 ce	536 ± 12.83 bde	411 ± 10.2 cee	115 ± 2.9 bef

[a] Data presented as means ± standard deviation ($n = 3$). Means with a letter in common differ significantly from each other ($p < 0.001$).

preparation was done via a steam extraction process using a double-jacketed steam kettle. This technique of juice preparation is one of the many methods used commercially. Various smaller scale models have been sold for home juice production, which we used to model the large scale industrial juicing process.

In short, fruits were cut into segments and placed into the kettle. Oranges were the only fruit to be peeled before the juicing process. Although this juice extraction method effectively separates the juice from the other solid components of the fruit, various compounds in the peels of the oranges affect juice flavor; hence, the peels were removed before the steaming process (as done by commercial juicers). The kettle was divided into three levels. The first, uppermost level was a sieve/strainer onto which the cut fruits were placed. The second compartment was the juice collection reservoir, which was situated beneath the sieve, and the third compartment was the water reservoir used for steam production when heated. The fruit was totally pulped after 7 min of steaming on a hot plate. The juice was siphoned off from the juice collection reservoir by a tap for analysis, and the pulp (peals, seeds, etc.) remained on the sieve in compartment one.

Solid Phase Extraction. Solid phase extractions of the fruit juices were done on C_{18} cartridges (Waters) by adapting a method described by Oszmianski and co-workers (*11*). This method was based on multiple elutions at different pH values with three different mobile phases and results in four fractions containing (i) phenolic acids; (ii) procyanidins, catechins, and anthocyanin monomers; (iii) flavonols; and (iv) anthocyanin polymers. Briefly, 1 mL of sample was introduced into preconditioned C_{18} cartridges and the various fractions eluted at the specified pH values using 5 mL of the described elution solvents (*11*).

Determination of Total Polyphenols. The total polyphenol content in the fruit juices and fruit juices fractions was determined according to Folin−Ciocalteu's method (*12*). Unfractionated fruit juices were diluted 62 times prior to analysis and filtered through a Whatman no. 1 filter (Merck). Fractionated samples were used as collected from solid phase extraction. Samples (200 μL) were introduced into test tubes followed by 1 mL of Folin−Ciocalteu's reagent (Sigma). This was allowed to stand for 8 min at room temperature. Next, 0.8 mL of sodium carbonate (7.5%) was added, mixed, and allowed to stand for 30 min. Absorption was measured at 765 nm (Shimadzu UV−vis 1601 spectrophotometer). The total phenolic content was expressed as gallic acid (Aldrich) equivalents (GAE) in milligrams per liter (mg/L). Because total AA and sugars contribute to the response of the Folin−Ciocalteu assay, corrections for these were done as described by Asami et al. (*13*) and Slinkard et al. (*14*), respectively. Mean values of polyphenol content were expressed as GAE/L ± standard deviation ($n = 3$).

ASC Determination. ASC, dehydroascorbate (DHA), and total ASC (ASC + DHA) concentrations were determined in the fruit juices spectrophotometrically at 578 nm using a method described by Beutler (*15*). These measurements were necessary not only for the nutritional evaluation of the juice but also for correction of the polyphenol concentrations determined by the Folin−Ciocalteu assay. Mean values of ASC content were expressed as milligrams per liter (mg/L) ± standard deviation ($n = 3$).

Total Sugar Determination. Total sugars were determined in fruit juices according to the 16th edition of the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC) International (*16*). The sugar content for strawberry, orange, grape, and Kei-apple juices was 6, 12, 17, and 5%, respectively, and the values were used for the corrections for polyphenol concentrations determined via the Folin−Ciocalteu assay.

Oxygen Radical Absorbance Capacity (ORAC). The fruit juices were prepared as described earlier and further extractions as well as ORAC antioxidant capacity analyses of hydrophilic and lipophilic compounds were performed essentially as described by Prior et al. (*17*). The analysis of lipophilic compounds was aided by addition of randomly methylated β-cyclodextrin, kindly provided by Dr. Ronald Prior, as a solubility enhancer as described by Huang et al. (*18*). Briefly, the assay in a volume of 200 μL contained fluorescein (56 nM) as a target for free radical attack by 2,2′-azobis(2-amidino-propane) dihydrochloride (240 mM). A BioTEK fluorescence plate reader was used, and the decay of fluorescence of fluorescein (excitation, 485 nm; emission, 520 nm) was measured every 5 min for 2 h at 37 °C. Costar black opaque (96 well) plates were used in the assays. Trolox was used as a standard at a range between 0 and 20 μM with a polynomial (2nd order) curve fit analysis. Mean values of antioxidant capacities were expressed as mmol Trolox equivalents (TE) per liter of the extracts ± standard deviation ($n = 3$).

Ferric Reducing Antioxidant Power (FRAP). FRAP values were determined essentially as described before (*19*). Briefly, the reduction of an Fe^{3+}−2,3,5-triphenyltetrazolium complex in the assay by the antioxidants in the samples was monitored at 593 nm. L-AA was used as standard and the FRAP of the samples was expressed as mean μmol/L AA equivalents (μM AA) ± standard deviation ($n = 3$).

Gas Chromatography−Mass Spectrometry (GC-MS) Analysis. Kei-apple juice extraction and derivatization were carried out as previously described (*20*) and analyzed in triplicate. An Agilent 6890 GC ported to a 5973 mass selective detector (CA) was used for identification and quantification of individual polyphenols. For the acquisition of an electron ionization mass spectrum, an ion source temperature of 200 °C and an electron energy of 70 eV were used. The gas chromatograph was equipped with a SE-30 capillary column, a split/splitless injection piece (250 °C), and a direct GC-MS coupling (260 °C). The 10:1 split injection (0.6 μL) was used during the MS analysis. Helium (1 mL/min) was used as the carrier gas. An oven temperature of 100 °C, isometric for 1 min, was used as an initial temperature after which a rise of 10 °C/min was continued until a temperature of 200 °C was reached. This was followed by a temperature increase of 15 °C/min until a final temperature of 300 °C was reached. This temperature was then maintained for a further 5 min.

Statistical Analyses. Descriptive and other statistics were done using Statistica (StatSoft Inc., United States). To determine whether differences existed between the various fruits, analysis of variation was performed using the Tukey honest significant difference test for posthoc comparison. ORAC, FRAP, and polyphenol Pearson correlation analyses were performed using Statistica (Statsoft Inc., Tulsa, OK) with significance set at $p \leq 0.05$.

RESULTS

Total Polyphenols and ASC. The total polyphenols (mg GAE/L) and ASC values (mg/L) of the various fruit juices are given in **Table 1**. The Kei-apple juice has a significantly higher amount of total polyphenols (1013 mg GAE/L) as compared to the other juices prepared under identical conditions. The total ASC concentration of the Kei-apple juice is comparable to that of strawberry and more than 100 mg/L greater than that of orange juice. Interestingly, the ASC in Kei-apple juice is significantly higher than any of the other juices, with a comparable low DHA content.

Table 2. Concentrations of Various Solid Phase C_{18} Polyphenol Fractions of Kei-Apple, Grape, Strawberry, and Orange Juices Determined by the Folin–Ciocalteu Method

juice	phenolic acids (mg GAE/L)[a]	procyanidins, catechins, and anthocyanin monomers (mg GAE/L)[a]	flavonols (mg GAE/L)[a]	anthocyanin polymers (mg GAE/L)[a]
Kei-apple	495.5 ± 16.3 abc	214.9 ± 5.7 abd	13.0 ± 2.0 abd	23.7 ± 0.3 bde
grape	73.0 ± 5.2 a	101.9 ± 2.4 acf	29.6 ± 0.4 acf	27.8 ± 2.6 cef
strawberry	86.0 ± 5.7 b	282.0 ± 7.2 bcg	34.1 ± 1.6 bce	51.3 ± 0.5 bcg
orange	68.3 ± 2.3 c	75.7 ± 0.2 dfg	50.5 ± 1.5 dfe	41.7 ± 1.2 dfg

[a] Data presented as means ± standard deviation ($n = 3$). Means with a letter in common differ significantly from each other ($p < 0.001$); e ($p = 0.03$).

Table 3. ORAC of Fruit Juices

juice	total (mM TE)[a]	phenolic acids (mM TE)[a]	procyanidins, catechins, and anthocyanin monomers (mM TE)[a]	flavonols (mM TE)[a]	anthocyanin polymers (mM TE)[a]
Kei-apple	43.9 ± 1.3 abc	17.4± 1.0 abc	12.3 ± 1.9 ab	5.4 ± 0.1	2.2 ± 0.2 ab
grape	15.7 ± 0.7 ade	5.1 ± 1.6 af	5.8 ± 1.1 adg	0.9 ± 0.3	1.4 ± 0.01 dh
strawberry	33.0 ± 2.6 bdi	10.3 ± 0.4 bef	13.2 ± 0.5 dh	2.6 ± 0.6	4.2 ± 0.7 acd
orange	21.6 ± 1.4 cei	4.1 ± 0.1 ce	2.0 ± 0.01 bgh	1.1 ± 0.1	7.7 ± 0.3 bch

[a] Data presented as means ± standard deviation ($n = 3$); mM TE, mmol/L TEs. Means with a letter in common differ significantly from each other ($p < 0.002$); g ($p = 0.004$); e ($p = 0.01$); and f ($p = 0.04$).

Table 4. FRAP of Fruit Juices

juice	total (μM AA)[a]	phenolic acids (μM AA)[a]	procyanidins, catechins, and anthocyanin monomers (μM AA)[a]	flavonols (μM AA)[a]	anthocyanin polymers (μM AA)[a]
Kei-apple	6100 ± 191 abc	2270.7 ± 26.5 abc	566.4 ± 26.7 a	109.9 ± 6.6 bc	82.6 ± 7.6
grape	1514 ± 61 ade	90.8 ± 17.4 ad	461.4 ± 13.4 b	97.7 ± 1.9 de	0.6 ± 0.2
strawberry	3517 ± 179 bdf	416.2 ± 40.2 bdf	1545.1 ± 88.3 abd	21.0 ± 10.6 bdf	92.1± 18.1
orange	2253 ± 111 cef	97.8 ± 19.5 cf	563.2 ± 51.6 d	65.6 ± 9.4 cef	22.1 ± 1.2

[a] Data presented as means ± standard deviation ($n = 3$); μM AA, μmol/L AA equivalents. Means with a letter in common differ significantly from each other ($p < 0.001$); e ($p = 0.004$).

Polyphenol Fractionation. Solid phase (C_{18}) fractionation of the juices (**Table 2**) shows the Kei-apple juice phenolic acids to contribute to 66.3% of the total of the combined fractions, followed by the procyanidin, catechin, and anthocyanin monomers (28,8%) and then by anthocyanin polymers (3.2%) and flavonols (1.7%). Comparative to the other juices, the Kei-apple juice had significantly higher phenolic acids (approximately seven times more than other juices) and a relatively high procyanidin, catechin, and anthocyanin monomer fraction, which is comparable to that detected in strawberry juice. Its flavonols and anthocyanidin polymer content are, however, significantly the lowest of all of the juices used in the comparison, with orange juice having the highest flavonols and strawberry juice the highest anthocyanidin polymers.

Antioxidant Capacity. Antioxidant capacity of the various fruit juices and their fractions were determined by ORAC and FRAP analyses and summarized in **Tables 3** and **4**, respectively. The Kei-apple juice showed both significantly higher total ORAC and FRAP values as compared to the other fruits juices. In both assays, the decreasing order of total antioxidant capacity values was as follows: kei-apple, strawberry, orange, and grape juice. ORAC and FRAP analyses of the fractionated samples indicate that the Kei-apple juice phenolic acid fraction is the highest contributor of its total antioxidant capacity, followed by the procyanidin, catechin, anthocyanin fraction > flavonol > anthocyanin polymers. This is notably different from the three other juices where the procyanidin, catechin, and anthocyanin fractions were the highest contributors to antioxidant capacity, with the exception of the anthocyanin polymer fraction in orange juice.

GC-MS Polyphenol Characterization of Kei-Apple Juice. GC-MS is particularly suited for determining nonflavoniod components, which were identified by C_{18} fractionation to be the major components of the Kei-apple juice. Flavonoid components of molecular weights below 800 are also easily detected using this technique. A summary of the polyphenol compounds in the Kei-apple juice sample as identified by GC-MS is given in **Table 5**. Using the published Trolox equivalent antioxidant capacity (TEAC) of the various compounds, the contribution of individual polyphenols to the total antioxidant activity (TAA) of the sample was calculated (*4*, *21*).

Caffeic acid was by far the most prominent polyphenol present in the Kei-apple juice (128.7 mg/L). The order of other predominant nonflavonoid components was *p*-coumaric acid > *p*-hydroxyphenylacetic acid > protocatechuic acid > 3-methoxy-4-hydroxyphenylacetic acid. When considering the antioxidant potential of these compounds using their TEAC values, again caffeic acid predominates due to its high concentrations (alone contributing to 63% of the total TAA of the GC-MS identified polyphenols). This was followed by *p*-coumaric acid > protocatechuic acid > 3-methoxy-4-hydroxyphenylacetic acid. Although gallic acid was detected at much lower concentrations, it is also a major contributor to the TAA of the mixture due to its high antioxidant capacity.

Table 5. GC-MS Polyphenol Content, TEAC, and TAA of Kei-Apple Juice[a]

class	compound	concn (mg/L)	TEAC (mM)[b]	TAA (μM)[c]
		nonflavonoids		
hydoxybenzioic acids	salicylic acid	0.35 ± 0.02	0.04	0.10 ± 0.005
	m-hydroxybenzoic acid	4.27 ± 0.12	0.84	25.76 ± 0.07
	vanillic acid	3.64 ± 0.35	1.43	31.01 ± 3.0
	gallic acid	2.36 ± 0.11	3.01	41.74 ± 1.98
	α-resorcylic acid	0.57 ± 0.02	2.15	5.44 ± 0.22
	protocatechuic acid	9.51± 0.13	1.19	73.48 ± 1.01
	syringic	0.66 ± 0.03	1.36	4.93 ± 0.25
hydroxycinnamic acids	*m*-coumaric acid	1.67 ± 0.02	1.21	12.29 ± 0.12
	p-coumaric acid	15.70 ± 0.30	2.22	212.5 ± 4.0
	ferulic acid	0.81 ± 0.02	1.91	7.98 ± 0.19
	caffeic acid	128.7 ± 1.03	1.26	900.8 ± 7.19
hydroxyhydrocinnamic acids	hydro-*p*-coumaric acid	0.35 ± 0.04	*d*	*d*
hydroxyphenylacetic acids	*p*-hydroxyphenylacetic acid	10.62 ± 0.62	0.34	23.76 ± 1.38
	3-methoxy-4-hydroxyphenylacetic acid	6.24 ± 0.11	1.72	58.94 ± 1.0
		flavonoids		
catechins	catechin	2.71 ± 0.02	2.4	22.49 ± 0.18

[a] Concn, concentration expressed as mean ± standard deviation ($n = 3$). [b] Ref *4.* [c] TAA determine by calculation (*4*). [d] Data unavailable.

Correlation Analyses. Comparing the unfractionated fruit juice samples, ORAC ($r = 0.95$) and FRAP ($r = 0.98$) both correlated significantly with the polyphenol content. In the fractionated samples, slightly lower ORAC and FRAP correlations with polyphenols were observed ($r = 0.87$ and 0.96, respectively); however, they were still highly significant ($p \leq 0.05$). To compare the two methods used for determining antioxidant capacity, all ORAC and FRAP values were compared and showed significant correlations ($r = 0.97$).

DISCUSSION

Over the past 10 years, there has been a growing interest in the value of polyphenols among researchers and food manufacturers. This is mainly because of their antioxidant properties, their abundance in our diet, and their probable role in the prevention of various diseases associated with oxidative stress, such as cancer, cardiovascular disease, and neurodegeneration (*22*). The Kei-apple is a fruit that has been associated with high antioxidant properties, although little scientific data exists to support anecdotal reports. In this study, we investigated the major compounds associated with the antioxidant potential in the fruit juice. Total polyphenol and ASC content of the Kei-apple juice was determined and antioxidant capacities evaluated using two separate techniques. To better interpret the data obtained from the Kei-apple juice, we compared these values with that of three commonly used fruit juices.

The total polyphenol content of the Kei-apple juice was almost twice that of strawberry juice and almost four times that of grape and orange juice. The low polyphenol content of the grape juice can be attributed to the fact that ∼70% of its total polyphenol content occurs in the seeds and the majority of the remaining ∼30% occurs in the peel (*23*). Both of these fruit components are excluded during the juicing process. The total ASC content of the Kei-apple juice compared well to that of strawberries and was 100 mg/L more than that of orange juice. Of particular interest, however, was that the Kei-apple juice ASC showed exceptional stability with very little oxidation to DHA. This is of particular importance to both health and industry. Although DHA is easily taken up by erythrocytes and other cells in vivo and reduced to ASC, which is the active form of vitamin C (*24*), it is not readily absorbed across the intestinal mucosa (*25*) and has little antiscorbutic activity (*26*).

The crude polyphenol composition of the various fruit juices was compared by C_{18} fractionation. The majority of the polyphenols in the Kei-apple juice was identified as phenolic acids, followed by procyanidins, catechins, and anthocyanin monomers > anthocyanin polymers > flavonols. This is in line with a report by Miller et al. associating acidic fruits with a high phenolic content (*27*). The low flavonol content of the grape juice can be attributed to the fact that these polyphenols are concentrated in the seeds of these fruits (*23*), which are excluded in the juicing method. High concentrations of polyphenols were measured in the strawberry juice fractions containing anthocyanins, which are associated with the rich red color seen in these fractions (*28*).

Both the ORAC and the FRAP analyses of the unfractionated fruit juices showed that, in comparison to the other juices included in this study, Kei-apple juice has a significantly higher antioxidant capacity. It has been reported that total polyphenol content correlates well with antioxidant capacity (*29*). This high correlation of ORAC and FRAP analyses with the polyphenol concentration of the unfractionated samples was indeed also observed in our study ($r = 0.95$ and $r = 0.98$, respectively). The amount of polyphenols is, however, not the only factor influencing antioxidant capacity. The structural arrangements (number and position of hydroxyl groups, double bonds, and aromatic rings) of the various individual polyphenols also play a role (*4*). This would explain some of the slightly lower correlations observed with ORAC or FRAP and polyphenol content correlation analyses ($r = 0.87$ and $r = 0.96$, respectively) for the fractionated fruit juice samples. An example of these variations is the higher ORAC value in the Kei-apple flavonol fraction (**Table 3**) despite it having the lowest concentration (**Table 2**) as compared to the same fraction of the other fruit juices. A similar anomaly can be seen in the anthocyanin polymer fraction of orange juice. Another clear correlation was obtained when comparing all ORAC and FRAP values for the combined samples ($r = 0.97$), indicating both ORAC and FRAP to be good predictors for measuring antioxidant capacity. Similar correlations are reported by Moyer et al. (*29*). Ou et al. (*30*), however, showed discrepancies in these comparisons with certain fruit and vegetables showing exceptionally good correlations and others showing the opposite. In our study, trend outliers showing a higher FRAP and a lower

ORAC value (Kei-apple phenolic acid and orange juice flanonol fractions) or a higher ORAC and a lower FRAP value (orange juice anthocyanin polymer fraction) may be attributed to the varying composition of the individual components of these fractions. As the FRAP value is an indication of the ferric ion reducing power of the mixture and the ORAC value indicates ability to scavenge free radicals, the various individual polyphenol components of the mixture may have stronger free radical scavenging abilities than a ferric ion reducing power or visa versa.

Further characterization of the individual polyphenols components by GC-MS showed caffeic acid to be the most prominent compound contributing to 63% of the TAA of all of the individual compounds identified using this technique. Caffeic acid, in addition to other polyphenols, has been associated in the possible preventions of cancer, cardiovascular disease, and neurodegeneration. It has been reported to inhibit the growth of human-derived breast and colon cancer cells (*31*). Cancer is a hyperproliferative disorder, in which invasion and angiogenesis, leading to tumor metastasis by the activation of nuclear transcription factor κB (NF-κB) occurs (*32*). There are various mechanisms by which caffeic acid may exert its proposed health benefits. It is seen to have a direct interaction with the aryl hydrocarbon receptors, nitric oxide inhibition, and proapoptotic effects in cancer cells (*31*), and it has been shown to inhibit tumor cell invasion and metastasis by the inhibition of methalloproteinase-2 and -9 (*33*). Caffeic acid has also been reported to protect DNA against damage by nitrite and proxynitrite (*34*). Furthermore, it is thought to promote health due to its antioxidant effects. It is seen to strongly inhibit lipid hydroperoxide formation, lipid hydroperoxide formation, and aqueous peroxyl radical-induced oxidation of low-density lipoproteins while sparing α-tocopherol (*35*). Apart from being able to act as a free radical scavenger, caffeic acid is shown to inhibit monoamine oxidase (*36*) and 5-lipoxygenase (*37*) implicated in various neurodegenerative diseases.

Although the results indicate caffeic acid to be by far the most predominant polyphenol component of Kei-apple juice, the other polyphenol components occurring also contribute to possible health-promoting effects of this juice. It is well-known that the protective health benefits of polyphenols are mainly through a combination of additive and/or synergistic effects (*38*). Additionally, other polyphenol compounds, which were detected in the Kei-apple juice, have also been associated with various beneficial health effects (*1*) and, although these occur in lower concentrations, may also prove to be beneficial.

From this study, we conclude that Kei-apple juice is a rich source of plant-derived antioxidant compounds (polyphenols and vitamin C) with strong antioxidant capacity, which is generally associated with health-promoting properties. Although the plant is not widely known or cultivated, it may be an interesting alternative natural source of these compounds.

ABBREVIATIONS USED

ASC, ascorbate; DHA, dehydroascorbate; ORAC, oxygen radical absorbance capacity; FRAP, ferric reducing antioxidant power; GC-MS, gas chromatography−mass spectrometry; GAE, gallic acid equivalents; AOAC, Association of Official Analytical Chemists, TE, trolox equivalent; AA, ascorbic acid; TEAC, trolox equivalent antioxidant capacity; TAA, total antioxidant activity.

ACKNOWLEDGMENT

We thank the following institutions and persons for their kind contributions: Dr. Ronald Prior for kindly donating the randomly methylated β-cyclodextrin for the ORAC analyses and Chricilia Visser at J. Muller Laboratories (PTY) Ltd. (Cape Town, South Africa) for sugar analyses.

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Received for review October 31, 2005. Revised manuscript received December 5, 2005. Accepted December 6, 2005.

JF052697J