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Bioactive compounds and antioxidant potential in fresh and dried Jaffa® sweeties, a new kind of citrus fruit

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Abstract: Bioactive compounds in the edible parts of fresh and dried Jaffa® sweeties, a new kind of citrus fruit, were analysed and their antioxidant capacities were assessed. Antioxidant-rich fractions were extracted from fresh and dried sweeties with 1.2 M HCl in methanol/water (1:1 v/v), and the antioxidant activities of these extracts were evaluated. Using the β-carotene/linoleate model system, the extracts from equivalent quantities of fresh and dried sweeties showed 89 and 87% antioxidant activity respectively. Similarly, using the DPPH radical-scavenging method, the extracts showed 87 and 85% antioxidant activity respectively. The best correlations were between caffeic acid content and β-carotene and DPPH antioxidant activity values ($r = 0.9849$ and 0.9798 respectively, $p = 0.005$). Both fresh and dried sweeties are bioactive natural products; when fresh fruits are not available, properly dried sweeties could be used as a substitute.
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Keywords: fresh and dried sweeties; antioxidant compounds; antioxidant potential

INTRODUCTION

Fruits in general and citrus fruits in particular have many healthful properties.[1,2] The positive influence of these natural products is attributed to their antioxidant compounds.[3] Citrus fruits have high contents of phenolic and ascorbic acids.[4–6] The international scientific community is searching for new natural products that could enrich diets and potentially reduce the incidence of atherosclerosis and other diseases.[7–10] In recent years, Israel has produced and exported a new kind of citrus fruit called Jaffa sweetie. The size of Jaffa sweeties is similar to that of grapefruits with a thick peel, and the edible part is juicy and very tasty.

In a previous investigation[7] we found that fresh sweeties possess high amounts of bioactive compounds, which positively influenced plasma lipid levels of laboratory animals. However, fresh fruits are not available all year round, being harvested in Israel only in December–April. Therefore it is important to find a proper substitute that could be used when fresh sweeties are not available. It was decided to prepare dried sweeties, to determine the contents of some important bioactive compounds and their antioxidant potential therein and to compare them with the same parameters in fresh fruits.

MATERIALS AND METHODS

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), β-carotene, butylated hydroxyanisole (BHA), caffeic, ferulic and *p*-coumaric acids and Folin–Ciocalteu reagent were

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purchased from Sigma Chemical Co (St Louis, MO, USA). All reagents were of analytical grade.

Fruits and sample preparation

Ripe Jaffa® sweeties at the same degree of maturity were purchased from an Israeli farmer and randomly divided into two groups, one for use as fresh and the second as dried fruits. The peels and edible parts of the sweeties were separated manually and the edible parts were used in this investigation. After drying (Virtis 10-324 lyophiliser (VirTis Industries, Gardiner, NY, USA), 48 h, 1 mmHg, −3 °C), 100 g of fresh fruits yielded 19.7 g of dried fruits. The dried sweeties were ground to a 40-mesh powder.

Determination of fibres and trace elements

Total, soluble and insoluble dietary fibres were determined as described previously.[10] Determination of trace elements (Fe, Cu, Zn and Mn) was performed as follows. A 0.8 g sample of lyophilised fruit was mineralised in a microwave oven (4 h, 102 °C) with 1 ml of concentrated HNO_3. The concentration of each element was determined in a Perkin-Elmer 5100 ZL atomic absorption spectrometer (Perkin-Elmer Ltd, Beaconsfield, UK) using the flame ionisation method for Fe, Cu and Zn and the flameless method for Mn.[10]

Extraction of phenols

A 50 mg sample of fresh or dried fruit was accurately weighed in a screw-capped tube. Total phenols were extracted with 5 ml of 1.2 M HCl in methanol/water (1:1 v/v); the sample was vortexed for 1 min and heated at 90 °C for 3 h with vortexing every 30 min. After cooling, the sample was diluted to 10 ml with methanol and centrifuged for 5 min at $4000 \times g$ in a benchtop centrifuge to remove solids.[11] Total polyphenols were determined at 765 nm using Folin–Ciocalteu reagent with gallic acid as standard.[12]

Individual antioxidants

Phenolic acids were determined by HPLC[13,14] using a C_{18} column (250 mm × 4.6 mm, 10 µm, Supelco, Inc, Bellefonte, PA, USA) with a solvent of water/acetic acid/methanol (86:4:10 v/v/v) at a flow rate of 2 ml min^{-1}. The column eluate was monitored at 320 nm. For ascorbic acid determination the detector was set at 195 nm and the mobile phase consisted of a filtered and degassed solution of 0.2 M $NaHCO_3$.[15]

Total antioxidant determination

There are many methods for total antioxidant determination and each has its limitations,[16] with different assay methods often showing different antioxidant activity trends.[17] We have previously used various antioxidant tests,[18] including (1) TAA using the ferrylmyoglobin/ABTS method,[19] (2) scavenging activity against nitric oxide (NO test)[20,21] and (3) total radical-trapping antioxidant potential (TRAP) measurement.[18,22,23]

All these tests are relatively non-specific markers of free radical-scavenging activity. Therefore in the present investigation we used two other methods which have been successfully applied to both fresh and dried fruits.[24]

1. Antioxidant assay using the β-carotene/linoleate model system.
2. Radical-scavenging activity using the DPPH method.

Antioxidant assay using β-carotene/linoleate model system

β-Carotene (0.2 mg in 0.2 ml of chloroform), linoleic acid (20 mg) and Tween 40 (polyoxyethylene sorbitan monopalmitate) (200 mg) were mixed. The chloroform was removed under vacuum at 40 °C and the resulting mixture was diluted with 10 ml of water and mixed well. To this emulsion was added 40 ml of oxygen-saturated water. Aliquots (4 ml) of the emulsion were pipetted into test tubes each containing 0.2 ml of fresh or dried sweetie extract (50 or 100 µl) or the synthetic antioxidant BHA in ethanol (for comparative purposes). A control containing 0.2 ml of ethanol and 4 ml of the emulsion was also prepared. The tubes were placed in a water bath at 50 °C. The absorbance at 470 nm was measured at zero time ($t = 0$) and thereafter at 15 min intervals until the colour of β-carotene disappeared in the control tubes ($t = 180$ min). This period of time was checked with kinetic studies as a function of antioxidant activity. A mixture prepared as above without β-carotene served as blank. The antioxidant activities (AA) of the extracts were evaluated in terms of the bleaching of β-carotene using the formula

$$AA = 100[1 - (A_0 - A_t)/(A_0^o - A_t^o)]$$

where A_0 and A_0^o are the absorbance values measured at zero time for the test sample and control respectively and A_t and A_t^o are the corresponding values measured after incubation for time t (maximum 180 min).

Radical-scavenging activity using DPPH method

Different amounts of extracts (50 and 100 µl, equivalent to 50 and 100 µg of fresh or dried sweeties) and BHA (25 and 50 µl) were placed in separate test tubes. The volume was adjusted to 100 µl by adding MeOH. A 5 ml aliquot of a 0.1 mM methanolic solution of DPPH was added to each tube and shaken vigorously. The tubes were allowed to stand at 27 °C for 20 min. The control was prepared as above without any extract, and MeOH was used for baseline correction. Changes in the absorbance of the samples were measured at 517 nm. Radical-scavenging activity was expressed as percentage inhibition and was calculated using the formula

$$\%\text{radical-scavenging activity} = [(\text{control OD} - \text{sample OD})/\text{control OD}] \times 100$$

On the basis of the results of the two tests, the methanolic extract of the edible parts of sweeties which showed significant activity by both methods was selected for further studies.

Statistical analysis

The results of this study are quoted as mean ± standard deviation (SD). All determinations were carried out in triplicate. Analysis of variance and a least significant difference test were conducted to identify differences among means, while a Pearson correlation test was conducted to determine correlations among means, using a statistical software package (Instat, GraphPad Software, San Diego, CA, USA). Statistical significance was declared at $p < 0.05$.

RESULTS

Total, soluble and insoluble dietary fibres

The dietary fibre contents in the edible parts of fresh and dried sweeties are summarised in Table 1, the contents of insoluble dietary fibre in both fresh and dried fruits being significantly higher than those of soluble dietary fibre ($p < 0.05$).

Trace elements

The contents of trace elements in fresh fruits were 1079–1331, 661–822, 348–532 and 99–171 μg kg^{-1} for Fe, Zn, Cu and Mn respectively, the contents in equivalent quantities of dried sweeties being similar.

Total polyphenols

The mean total polyphenol content in fresh fruits was 1.051 ± 0.1 g kg^{-1}, the content in equivalent quantities of dried sweeties being similar.

Phenolic and ascorbic acids

The mean contents of phenolic and ascorbic acids in fresh fruits were 0.261 ± 0.02, 0.232 ± 0.02, 0.199 ± 0.02, 0.1 ± 0.01 and 1.01 ± 0.1 g kg^{-1} for ferulic, sinapic, *p*-coumaric, caffeic and ascorbic acids respectively. Among the phenolic acids the highest concentration was that of ferulic acid and the lowest that of caffeic acid. The contents of phenolic and ascorbic acids in equivalent quantities of fresh and dried sweeties were similar.

Table 1. Dietary fibre contents (g kg^{-1}) in edible parts of fresh and dry Jaffa sweeties

Fibre type	Fresh	Dry
Total	22.9 ± 2.1c	108.4 ± 9.2c
Soluble	8.8 ± 0.6a	44.2 ± 3.7a
Insoluble	14.1 ± 1.2b	64.2 ± 5.3b

Values are mean ± SD of three measurements. Means within a column without a common letter differ significantly ($p < 0.05$).

Free radical-scavenging activity

Using the β-carotene/linoleate model system, the methanolic extracts of fresh and dried sweeties showed 89 and 87% antioxidant activity respectively at the 50 μl level (Fig 1(a)). Similarly, using the DPPH radical-scavenging method, the extracts showed 87 and 85% antioxidant activity respectively at the 50 μl level (Fig 1(b)). Thus the free radical-scavenging activities of equivalent quantities of fresh and dried sweeties were similar.

Good correlation was observed between the β-carotene and DPPH values and the content of total polyphenols ($r = 0.9000$ and 0.8944 respectively, $p = 0.006$ and 0.007 respectively). Good correlation was also found between the β-carotene and DPPH values

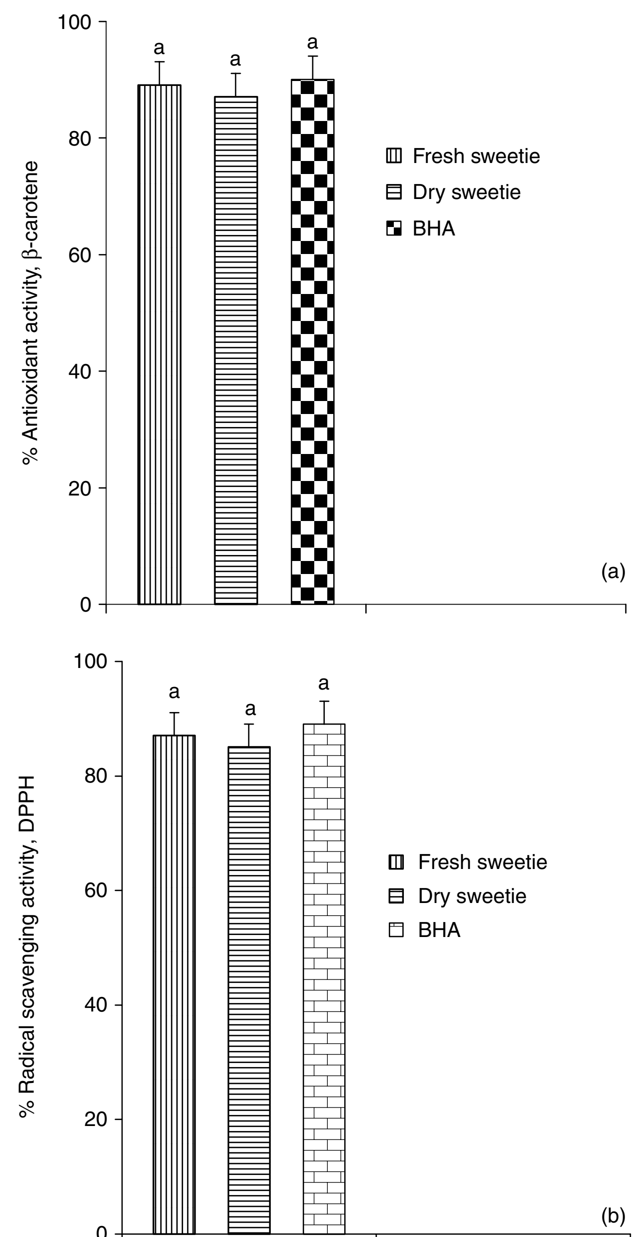

Figure 1. Antioxidant activity values (mean ± SD) of fresh and dried Jaffa sweeties using (a) β-carotene/linoleate model system and (b) DPPH radical-scavenging method. Different letters denote significant differences ($p < 0.05$).

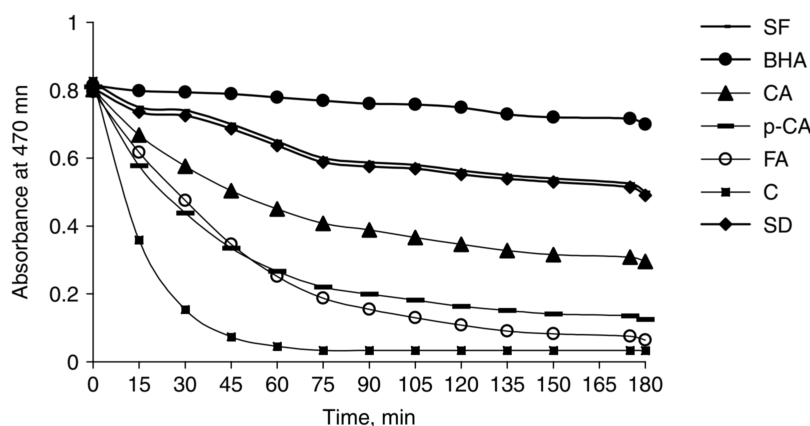

Figure 2. Reaction kinetics of fresh (SF) and dried (SD) Jaffa sweetie extracts, butylated hydroxyanisole (BHA), *p*-coumaric acid (p-CA), ferulic acid (FA) and caffeic acid (CA) in β-carotene bleaching. The β-carotene concentration was 0.004 mg ml^{-1} and the samples were at a level of 0.2 mg ml^{-1} in the reaction mixtures.

and the contents of individual phenolic acids: between caffeic acid content and β-carotene and DPPH values ($r = 0.9849$ and 0.9798 respectively, $p = 0.005$); between ferulic acid content and β-carotene and DPPH values ($r = 0.9592$ and 0.9539 respectively, $p = 0.006$); between *p*-coumaric acid content and β-carotene and DPPH values and between sinapic acid content and β-carotene and DPPH values ($r = 0.9274$ and 0.9220 respectively and $r = 0.9220$ and 0.9165 respectively, $p = 0.006-0.008$). It is important to note that the correlations between ascorbic acid content and β-carotene and DPPH values were relatively low ($r = 0.8$ and 0.7937 respectively, $p = 0.01$).

The antioxidant activities of sweetie extracts, standard antioxidants and some phenolic acids at a concentration of 0.2 mg ml^{-1}, as measured by the bleaching of β-carotene, are presented in Fig 2. Sweetie extracts prepared from equivalent quantities of fresh and dried fruits exhibited slight differences in antioxidant activity. BHA was found to give the maximum antioxidant activity. The antioxidant activity of sweeties was between those of BHA and caffeic acid. Among the three phenolic acids the lowest antioxidant activity was associated with ferulic acid and the highest with caffeic acid.

DISCUSSION

Coronary atherosclerosis is still a major cause of death in Western civilisation.[25] Diets rich in fruits and vegetables have proved to be effective in reducing the incidence of this disease,[8,9] and scientists continue to examine new kinds of these natural products.[7,26]

In the present study a new kind of citrus fruit, Jaffa sweetie, was investigated. In a previous investigation we found that fresh sweeties possess high amounts of bioactive compounds, which positively influenced plasma lipid levels of laboratory animals. However, fresh sweeties are not available all year round, and it was important to find an alternative form of this fruit that could be used when fresh sweeties are not available. Therefore, for the present investigation, a dried form was prepared. The contents of dietary fibre, total polyphenols and phenolic and ascorbic acids in fresh and dried sweeties were determined and their antioxidant capacities were evaluated. Equivalent quantities of fresh and dried Jaffa sweeties had comparable characteristics.

The antioxidant activity of sweeties was between those of BHA and caffeic acid. Among the three phenolic acids the lowest antioxidant activity was associated with ferulic acid and the highest with caffeic acid. Our results are in accordance with others which showed that the presence of different extracts can hinder β-carotene bleaching by neutralising the linoleate and other free radicals in the system.[27,28]

Therefore, according to our previous investigation *in vivo*, it could be supposed that the dried form of Jaffa sweeties would positively influence plasma lipid levels and plasma antioxidant capacity.

CONCLUSIONS

1. The contents of dietary fibre, total polyphenols and phenolic and ascorbic acids in equivalent quantities of fresh and dried Jaffa sweeties are similar.
2. The antioxidant values of equivalent quantities of fresh and dried Jaffa sweeties as determined by β-carotene and DPPH tests are similar and very high.
3. Therefore, when fresh fruits are not available, properly dried Jaffa® sweeties could be used as a substitute.

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