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Influence of Postharvest Hot Water Treatment on Nutritional and Functional Properties of Kumquat (*Fortunella japonica* Lour. Swingle Cv. Ovale) Fruit

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The present study investigated the influence of a hot water dip (HWD) for 2 min at 50 °C, a standard and effective treatment for postharvest decay control of citrus fruit, on the nutritional and health-related properties of kumquats. The results show that most of the parameters examined, including titratable acidity, soluble solids content, maturity index, glucose, fructose, sucrose, ascorbic acid, dehydroascorbic acid, α- and γ-tocopherols, β-carotene, zeaxantin, rhoifolin, and antioxidant activity, were not significantly affected by treatment. The levels of β-cryptoxanthin, narirutin, and total flavonoids increased after HWD, whereas lutein and total phenols decreased. The concentration of the essential oil and the relative percentage of the individual components of the essential oil were not affected by HWD except for the minor compound *p*-menta-1,5-dien-1-ol, which increased after HWD. After storage, lower levels of glucose, total sugars, β-carotene, β-cryptoxanthin and lutein were recorded in HWD fruit. A decrease in antioxidant activity and increases in α-tocopherol and total vitamin E were found both in control and HWD fruit. The influence of HWD at 50 °C for 2 min on individual nutraceuticals and health-related properties was thus generally low and may depend on storage conditions.

KEYWORDS: *Citrus*; essential oils; ascorbic acid; dehydroascorbic acid; tocopherols; carotenoids; flavonoids

INTRODUCTION

The many benefits to human health of citrus fruit, including those against common cold diseases, have long been known. Moreover, recent discoveries indicate additional therapeutic properties against allergies and other inflammatory diseases, anticancer activity, beneficial effects on capillary fragility and arteriosclerosis, and antiviral activity (*1, 2*).

Kumquats (*Fortunella* spp.), the smallest of the true citrus fruits (*3*), are usually eaten raw as a whole fruit, excluding the seeds. The exocarp is fleshy, sweet, and edible with a typical aroma due to the presence of flavonoids and terpenoids (*4*). As other citrus fruit, kumquats can be candied, marinated, prepared as marmalade, added to fruit salad, preserved as a whole in sugar syrup, or used as a decoration. Kumquats are also an excellent source of nutrients and phytochemicals, including ascorbic acid, carotenoids, flavonoids, and essential oils. From a dietary viewpoint, essential oils represent an added value for kumquat fruit because, in addition to their contribution to the flavor, they play an important role in human health, like other non-nutritive phytochemicals such as polyphenols and flavonoids.

The postharvest life of kumquat fruit is greatly offset by its high perishability under shelf-life conditions as a result of high transpiration rate and spoilage losses caused mainly by *Penicillium* spp. (*5*). Cold storage can extend kumquat's postharvest life for some weeks (*5*), and postharvest fungicide treatments consistently suppress pathogen development (*6*). However, the use of fungicides is not allowed as the fruit, both peel and flesh, is eaten raw. Therefore, alternatives to fungicides are required. Postharvest hot water dip (HWD) treatments have been developed as a nonchemical method of disinfection of fresh harvested fruits and vegetables and are an effective way to reduce decay development in several citrus fruits (*7, 8*) including kumquats (*9–11*). Commercial application of HWD as a single means of decay control in kumquat was implemented by Ben-Yehoshua et al. (*11*) to facilitate kumquat exports by ship rather than by air.

Although the decay-reducing effect of HWD on fruit is well documented, much less is known about its impact on nutraceuticals and the functional properties of fruit (*12*), and no published reports can be found in the literature on kumquats.

The present study was thus designed to investigate the effect of a standard hot water treatment on various phytochemicals in kumquats that represent the added nutraceutical value of the fruit (*13*).

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MATERIALS AND METHODS

Plant Material. The investigation was carried out on kumquat [*Fortunella japonica* Lour. Swingle] cv. Ovale, grown in an experimental orchard located in central western Sardinia (Italy), receiving standard horticultural practices. Fruits were randomly harvested in March, when commercially mature (total soluble solids content/titratable acidity ratio = 5.24 ± 0.27) and delivered to the laboratory immediately after harvest.

Treatments and Storage Conditions. Medium-size fruits free from defects were selected, placed into boxes (100 fruits per box), and grouped into two treatment groups of three boxes each (replications). The fruits of the first group were untreated (control fruit), whereas fruits of the second group were subjected to a standard treatment, water dipping at 50 °C for 2 min, for extending the postharvest life of kumquat fruit (*9, 10*). Dip treatment was performed as described previously (*14*). After treatments, fruits were allowed to dry at room temperature and stored for 21 days at 17 °C and ca. 80% relative humidity (simulated shelf-life conditions) (*10*). All analyses were performed following treatments and at the end of storage.

Chemical Analyses of Juice. Titratable acidity (TA) of juice was determined using a potentiometric titrator (Metrom 720 SM Tritino, Swiss); 5 mL of juice diluted in 45 mL of distilled water was titrated with 0.1 N NaOH to pH 8.1. The results were calculated as percentage of anhydrous citric acid. The percentage of total soluble solids (TSS) was measured by a digital refractometer (PR-101, Atago, Japan).

Chemical Analysis of Whole Fruit. All chemicals used were of analytical reagent grade and purchased from Aldrich, Acros (Milan, Italy), Fluka (Buchs, Switzerland), Carlo Erba (Milan, Italy), and Extrasynthese (Genay, France),

Carbohydrates, Flavonoids, Total Phenols, and Antioxidant Activity. Three replicates of 20 fruits each were halved to remove the seeds, homogenized, and used for analysis. A 1 g sample was diluted in 10 mL of aqueous methanol (1:1 v/v). The sample was stirred for 1 h and centrifuged at 13876*g* for 15 min, and the supernatant was collected for analysis prior to filtration with a 0.45 μm Teflon membrane filter.

Carbohydrates were analyzed according to the method of Yang and Ming-Yu (*15*). A LaChrom Merck-Hitachi liquid chromatograph (Hitachi Ltd., Tokyo, Japan) consisting of a D-7000 system manager, an L-7100 pump, and an L-7200 autosampler coupled with an evaporative light scattering detector (ELSD SEDEX 60LT, France), was used. A prevail carbohydrate ES column (250 mm × 4.6 mm, 5 μm, Alltech Italy srl, Milan, Italy) with a guard column (7.5 × 4.6 mm i.d.) thermostated at 30 °C was employed. The isocratic mobile phase was a mixture of CH_3CN/ultrapure water (75:25, v/v) with a flow rate of 1 mL/min. The ELSD detector was set as follows: drift tube temperature, 45 °C; nebulizer gas (air) pressure, 2.5 bar.

Flavonoids were determined according to the method of Mouly et al. (*16*). The samples (100 μL) were analyzed using a LaChrom-Merck-Hitachi liquid chromatograph (Hitachi Ltd., Tokyo, Japan) consisting of a D-7000 system mananger, an L-7100 pump, an L-7200 autosampler, and an L-7455 photodiode detector (DAD). A C_{18} Alltima column (250 mm × 4.6 mm, 5 μm, Alltech Italia), equipped with a precolumn (7.5 × 4.6 mm i.d.) thermostated at 30 °C was employed. The solvent gradient was performed by varying the proportion of solvent A [H_2O/CH_3CO_2H (97:3, v/v)] to solvent B (CH_3CN) as follows: initial condition was 5% B; at 15 min, 20% B; at 20 min, 25% B; at 30 min, 80% B; at 40 min, 100% B; at 50 min, 5% B, with a flow rate of 1 mL/min. The DAD detector was set at λ 280 and 360 nm. Flavonoids were identified by matching the retention times and their UV spectral characteristics against those of standards. Their quantification was calculated according to external standard method curves of standard compounds. Stock solutions of standard were prepared in methanol.

Total phenolic content was analyzed according to the Folin−Ciocalteu colorimetric method (*17*). Total phenols were expressed as gallic acid equivalent.

Antioxidant activity was assessed using the free radical DPPH (*18*) using a 100 μL sample. The mixture containing 3 mL of a methanol solution of 0.16 mM DPPH was allowed to react for 15 min in a cuvette. The inhibition percentage of the absorbance at 515 nm of DPPH solution added with sample was calculated using the following equation: inhibition % = $(Abs_{t=0} - Abs_{t=15min})/Abs_{t=0} \times 100$.

Essential Oil Composition. Three replicates of 20 fruits each were hydrodistilled simultaneously for 1 h in a Clevenger-type apparatus (*19*). The essential oils were recovered directly using a micropipet from above the distillate without the addition of any solvent. The essential oils were stored with anhydrous sodium sulfate in dark vials at 4 °C. Solutions of 1% (v/v) oils were prepared in *n*-hexane before GC analysis. A Varian CP 3800 gas chromatograph (Varian, Inc., Palo Alto, CA) coupled with a Saturn 2000 ITMS detector, a Varian CP 7800 autosampler, a split−splitless injector, and a MS ChemStation was used. The column was a fused silica capillary DB-5MS (5% phenylmethylpolysyloxane, 30 m × 0.25 mm; film thickness = 0.25 μm) (J&W Scientific Fisons, Folsom, CA). Injector, trap, manifold, and transfer line temperatures were set at 150, 170, 100, and 200 °C, respectively. The oven temperature was programmed as follows: from 60 to 180 °C (3 °C/min) and isothermally held for 15 min. Helium was used as carrier gas at 1 mL/min; 1 μL of each sample was injected in the split mode (1:20). MS conditions were as follows: ionization mode EI from 50 to 450 amu. The essential oil components were identified by comparison of their relative retention times with those of standard references or by comparison of their retention index (RI) relative to the series of *n*-hydrocarbons. Computer matching against a commercial library (*20, 21*) and 'homemade' library mass spectra made from pure standards and components extracted from known oils, as well as MS literature data, was also used for the identification. The KI calculated were in agreement with that reported by Adams (*20*). Individual essential oil components were expressed as percentages by peak area normalization measurement.

Ascorbic Acid, Dehydroascorbic Acid, Tocopherols, and Carotenoids. Twenty fruits per replication were halved to remove the seeds, frozen at −20 °C, lyophilized with a freeze-dryer (model Modulyo 4 K, Edwards High Vacuum International, West Sussex, U.K.), finely ground (<1 μm) with a centrifugal ball mill (Retsh model S1, Germany), and stored at −20 °C until analysis. The content of moisture was determined by difference of weight before and after freeze-drying.

Ascorbic acid (AA) and dehydroascorbic acid (DHA) were analyzed according to the method of Ting and Rouseff (*22*). A 500 mg aliquot of lyophilized sample and 100 mg of *o*-phenylenediamine (OPD) were transferred to a 50 mL volumetric flask and diluted to volume with methanol. The flask was sonicated for 3 min and allowed to stand for 1 h at 4 °C in the dark. A fraction of the resulting mixture was then filtered through a 0.45 μm Teflon membrane filter and injected into the HPLC chromatograph for the analysis. An Agilent 1110 series liquid chromatograph (Agilent Technologies, Waldbronn, Germany), consisting of a G1322A degasser, a G1311A quaternary pump, a G1313A autosampler, and a DAD UV 6000 LP (Spectra System Termo Quest, Milan, Italy), was used. The sample (100 μL) was analyzed using a Waters Spherisorb 5 μm ODS 2 column (250 × 4.6 mm, 5 μm) (Waters Spherisorb, S.p.A., Milan, Italy). The isocratic mobile phase was prepared by mixing 100 mL of trimethylcetylammonium bromide (0.05 M) solution in methanol to 900 mL of an aqueous solution of potassium dihydrogen phosphate (0.1 M). The flow rate was 1 mL/min, and the detector was set at λ 265 and 348 nm for AA and DHA, respectively.

Tocopherols were analyzed according to the method of Pirisi et al. (*23*). One hundred milligrams of lyophilized sample was weighed in a glass tube and extracted two times with 5 mL of dichloromethane by sonication for 2 min. The extracts were combined, 4 mL was evaporated to dryness, and the residue was dissolved in 1 mL of mobile phase and filtered through a 0.45 μm Teflon membrane filter. A LaChrom-Merck-Hitachi liquid chromatograph (Hitachi Ltd.) consisting of a D-7000 System Manager, an L-7100 pump, an L7200 autosampler, and an L-7485 fluorescence detector and a Waters Spherisorb 5 μm ODS 2 column (250 × 4.6 mm, 5 μm) were employed. The isocratic mobile phase was a mixture methanol/acetonitrile (50:50, v/v) with a flow rate of 1 mL/min. The fluorescence detector was set at λ 290 nm (excitation) and 330 nm (emission). Quantitative determinations were performed according to the standard addition method by measuring peak height versus concentration. The calibration graphs were constructed by injecting standard solutions prepared in matrix at five concentration

Table 1. Influence of Hot Water Dipping (HWD, 2 min at 50 °C) and Storage Conditions on Titratable Acidity (TA), Soluble Solids Content (SSC), SSC:TA Ratio, and Glucose, Fructose, Sucrose, and Total Sugars Concentrations in Kumquat Fruit

	treatments			
	0 days[a]		21 days at 17 °C	
variable	control	HWD	control	HWD
TA	3.00 a	3.06 a	2.88 a	2.85 a
SSC (°Brix)	15.70 ab	14.93 b	15.86 a	16.36 a
SSC:TA ratio	5.23 ab	4.87 b	5.52 ab	5.75 a
glucose (g/100 g)	2.68 a	2.88 a	2.61 ab	2.27 b
fructose (g/100 g)	2.44 ab	2.64 a	2.41 ab	2.21 b
sucrose (g/100 g)	5.65 ab	5.64 ab	5.74 a	5.29 b
total sugars (g/100 g)	10.79 a	11.17 a	10.78 a	9.78 b

[a] Time 0 = following treatment. In each row, mean separation by LSD test, $P \leq 0.05$.

levels. Good linearities were achieved in the range of 0.1–5 mg/kg, with correlation coefficients between 0.998 and 0.999. The decision limits and detection capability were calculated as described in Council Directive 96/23/EC of the European Commission and were, respectively, 0.09 and 0.15 mg/kg for α-tocopherol and 0.03 and 0.05 mg/kg for β-tocopherol.

Carotenoids. A 500 mg lyophilized sample was weighed in a glass tube and extracted two times with 5 mL of *n*-hexane/chloroform (50:50, v/v) containing 0.1% butyl hydroxytoluene (BHT) by sonication for 3 min. The extracts were combined, 1 mL was evaporated to dryness, and the residue was dissolved in 1 mL of *n*-hexane and filtered through a 0.45 μm Teflon membrane filter. An Agilent 1110 series liquid chromatograph (Agilent Technologies), consisting of a G1322A degasser, a G1311A quaternary pump, a G1313A autosampler, and a diode array detector UV 6000 LP (Spectra System Termo Quest, Milan, Italy) and a Waters Spherisorb S5 NH_2 column (4.6 × 250 mm) were employed. The flow rate was 1 mL/min. *n*-Hexane with 0.1% BHT (w/v) was employed as mobile phase A and tetrahydrofuran (THF) as mobile phase B. The gradient run was as follows: 100% A (1 min), at $t = 10$ min 50% A/50% B, and held for 5 min. Carotenoids were detected by absorbance at $\lambda = 450$ nm. Quantitative determinations were performed according to the standard addition method measuring peak height versus concentration. Good linearities were achieved in the range of 0.1–5 mg/kg, with correlation coefficients between 0.9995 and 0.9998. The decision limits and detection capability were as follows: β-carotene, 0.010 and 0.016 mg/kg; β-cryptoxanthin, 0.008 and 0.013 mg/kg; zeaxanthin, 0.011 and 0.017 mg/kg; and lutein, 0.036 and 0.058 mg/kg.

Statistical Analysis. Statistical analysis (a unifactorial complete randomized block design) (*24*) was performed by Statgraphics software (Manugistics, version 5 Professional, 2000) statistical program. Mean comparisons were performed by the Fisher's least significant difference test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Results of this study have shown that titratable acidity, soluble solids content, maturity index, glucose, fructose, sucrose, and total sugars were not significantly affected by HWD (**Table 1**). After storage, higher levels of glucose and total sugars were recorded in HWD fruit.

Investigations on lemons (*25*) have shown that heat treatment at 55 °C for 5 min significantly increased the sucrose content of the flavedo, whereas glucose and fructose contents decreased slightly and the raffinose content was unaffected. On the other hand, glucose and fructose levels remained significantly higher in fruit dipped in water at 48 °C for 4 min than in control pomegranate (arils) during storage (*26*). Thus, the influence of heat treatments on carbohydrate levels is dependent on fruit species, treatment type, and storage conditions. Although the principal biologically active form of vitamin C is L-ascorbic acid (AA), its oxidated compound, L-dehydroascorbic acid (DHA), is also active (*27*). In many horticultural crops DHA represents <10% of total vitamin C and usually increases during storage (*28*). Generally, AA content exhibits a gradual decline in horticultural crops as storage temperature or duration increases, although some chilling-sensitive crops show more losses in vitamin C when they are stored at low temperature (*29*). Present data on kumquat (**Table 2**) reveal that DHA contributed approximately 10% of total vitamin C. Differences in AA and DHA and total vitamin C due to treatment and storage conditions were not significant. Mean values of AA and DHA were, respectively, lower and within the range reported for other citrus fruit (*30*).

Table 2. Influence of Hot Water Dipping (HWD, 2 min at 50 °C) and Storage Conditions on the Concentrations (Milligrams per 100 g of Edible Portion) of Ascorbic Acid (AA), Dehydroascorbic Acid (DHA), AA + DHA, α- and γ-Tocopherol, Total Vitamin E, β-Carotene, β-Cryptoxanthin, Lutein, Zeaxanthin, and Total Carotenoids in Kumquat Fruit

	treatments			
	0 days[a]		21 days at 17 °C	
compound	control	HWD	control	HWD
AA	18.19 a	19.66 a	18.03 a	17.59 a
DHA	1.81 a	2.11 a	2.04 a	1.94 a
AA + DHA	20.00 a	21.77 a	20.06 a	19.53 a
α-tocopherol	1.05 b	1.03 b	1.66 a	1.53 a
γ-tocopherol	0.14 a	0.17 a	0.15 a	0.14 a
total vitamin E	1.19 b	1.20 b	1.81 a	1.67 a
β-carotene	0.33 ab	0.23 bc	0.40 a	0.20 c
β-cryptoxanthin	0.26 c	0.41 a	0.39 a	0.34 b
lutein	0.44 a	0.34 b	0.20 b	0.22 b
zeaxanthin	0.24 ab	0.33 a	0.19 b	0.15 b
total carotenoids	1.27 a	1.31 a	1.18 a	0.90 a

[a] Time 0 = following treatment. In each row, mean separation by LSD test, $P \leq 0.05$.

Most fruits and vegetables contain low to moderate levels of vitamin E (*31–33*). However, due to the abundance of plant-derived foods in our diets, they provide a significant and consistent source of vitamin E (*34*). The first attempt of isolation and identification of α-tocopherol in citrus (orange flavedo) was performed by Newall and Ting (*35*). Sawamura et al. (*36*) investigated the relationships between tocopherol levels and changes in the flavedo of citrus fruit and the occurrence of rind spot disorder.

Our results (**Table 2**) show that vitamin E in kumquat was represented by α-tocopherol and, to a lesser extent, γ-tocopherol. HWD did not affect the levels of tocopherols, although significantly higher levels of α-tocopherol were detected after storage. Investigations in vegetables, fruits, and berries have revealed that α-tocopherol levels remained fairly stable following freezing, canning, and marmalade and jam making (*37*). Higher vitamin E levels were present in boiled broccoli and blanched and boiled spinach, compared to raw products, whereas in other vegetables little changes in vitamin E content between raw and heat-processed crops have been recorded (*38*).

Carotenoids were represented by β-carotene, β-cryptoxanthin, lutein, and zeaxanthin, in agreement with previous studies (*39*). Of these, only β-carotene and β-cryptoxanthin are known to have provitamin A activity (*40*). With respect to β-carotene, β-cryptoxanthin has lower vitamin A activity but notably higher antitumor promoter activity (*41*). The results of Wang et al. (*39*) show that β-cryptoxanthin and lutein were found to be, respectively, the major and minor carotenoids present in kumquat fruit cultivated in Taiwan. Our study (**Table 2**) found an opposite trend, with β-cryptoxanthin and lutein being the

Table 3. Influence of Hot Water Dipping (HWD, 2 min at 50 °C) and Storage Conditions on the Relative Percentage of Essential Oil Components of Flavedo 'Ovale' Kumquat Fruit

	treatments			
	0 days[a]		21 days at 17 °C	
essential oil component	control	HWD	control	HWD
α-thujene	0.82 a	1.00 a	0.97 a	0.86 a
sabinene	0.12 a	0.12 a	0.13 a	0.11 a
β-pinene	0.02 a	0.02 a	0.02 a	0.03 a
myrcene	2.86 a	3.99 a	3.79 a	3.80 a
α-phellandrene	0.05 b	0.07 b	0.81 a	0.67 a
α-terpinene	0.01 b	0.04 b	0.13 a	0.13 a
p-cymene	0.41 a	0.68 a	0.70 a	0.51 a
limonene	91.51 a	89.28 a	89.30 a	88.79 a
trans-ocimene	0.01 c	0.03 bc	0.05 a	0.04 ab
γ-terpinene	0.68 a	0.45 a	0.12 a	0.14 a
terpinolene	0.08 b	0.06 b	0.31 a	0.31 a
p-cymenene	0.18 b	0.27 ab	0.36 a	0.31 a
linalol	0.57 a	0.49 a	0.45 a	0.60 a
cis-limonene oxide	0.05	nd[b]	nd	nd
cis-*p*-menth-2,8-dien-1-ol	0.02 a	0.02 a	0.02 a	0.01 a
p-mentha-1,5-dien-1-ol	0.06 b	0.18 a	0.10 ab	0.12 ab
terpinen-4-ol	0.07 b	0.11 ab	0.10 ab	0.14 a
p-cymen-8-ol	0.04 b	0.06 ab	0.09 a	0.09 a
α-terpineol	0.38 a	0.70 a	0.38 a	0.46 a
octyl acetate	0.09 a	0.11 a	0.10 a	0.14 a
trans-carveol	0.10 c	0.13 c	0.33 b	0.48 a
cis-carveol	0.01 b	0.02 b	0.06 a	0.07 a
carvone	0.23 a	0.24 a	0.15 a	0.21 a
unknown	0.12 a	0.11 a	n.d.	n.d.
α-copaene	0.02 a	0.03 a	0.02 a	0.02 a
β-caryophyllene	0.01 a	0.02 a	0.01 a	0.01 a
cis-muurola-4 (14,5-diene)	1.55 a	1.35 ab	1.01 ab	0.99 b
γ-gujunene	0.01 b	0.01 b	0.17 a	0.01 b
bicyclogermacrene	0.05 a	0.06 a	0.07 a	0.04 b
7-epi-α-selinene	0.08 a	0.12 a	0.11 a	0.07 a
germacrene B	0.03 a	0.03 a	0.02 a	0.03 a

[a] Time 0 = following treatment. In each row, mean separation by LSD test, $P \leq 0.05$. [b] nd, not detected.

minor and major carotenoids present in the edible part of the fruit. Such a difference may be ascribed to various factors, including the diverse cultivar, geographic site of production, stage of maturity, and postharvest handling (*40, 42*).

HWD increased the concentrations of β-cryptoxanthin and decreased lutein with respect to control fruit but did not significantly affect β-carotene and zeaxanthin. After storage, as a result of some changes in the individual carotenoids, lower levels of total carotenoids were recorded in HWD fruit in comparison to their initial values. According to the estimated retinol equivalent (RE) content (*43*), our findings confirm that kumquat fruit is a rich source of vitamin A (>140 RE). Lutein and zeaxanthin are vitamin A inactive but have been associated with lowered risk of macular degeneration and cataract (*44*).

In kumquat fruit essential oil is not a product of commercial interest but represents an added value because the fruit is eaten raw, including the rind, where most essential oil is present. Monoterpenes are non-nutritive dietary phytochemicals present in the essential oils of citrus fruits and other plants. A number of these dietary monoterpenes have antitumor activity. For example, *d*-limonene, which is a major component of citrus peel oils, and other related monoterpenes have chemopreventive and chemotherapeutic activity against various forms of tumors when ingested during the initiation phase (*45*). The present results show that the yield of essential oils averaged 0.18 ± 0.02%, differences due to treatment and storage conditions being nonsignificant. Thirty-one compounds were identified in kumquat essential oils (**Table 3**). Among them, limonene was the most abundant compound (~91.51%), as in other citrus fruit. The major compounds in addition to limonene were myrcene (~2.9%), cis-muurola-4(14,5-diene (~1.6%), α-thujene (~0.8%), and linalol (0.7%). The other compounds occurred only in small amounts (<0.4%).

Table 4. Influence of Hot Water Dipping (HWD, 2 min at 50 °C) and Storage Conditions on the Concentrations of Flavonoids (Milligrams per 100 g), Total Phenols (Milligrams per 100 mL of Gallic Acid) and Antioxidant Activity (Percent DPPH Inhibition) in Kumquat Fruit

	treatments			
	0 days[a]		21 days at 17 °C	
compound	control	HWD	control	HWD
narirutin	107.00 b	130.10 a	107.10 b	98.10 b
rhoifolin	36.66 ab	40.21 a	33.00 b	33.04 b
total flavonoids	143.70 b	170.31 a	140.08 b	131.20 b
total phenols	290.60 a	271.80 b	265.50 bc	248.81 c
antioxidant activity	12.60 a	11.84 ab	11.16 b	8.99 c

[a] Time 0 = following treatment. In each row mean separation by LSD test, $P \leq 0.05$.

All citrus essential oils have radical scavenging activity the efficacy of which seems to depend on the levels of such compounds as γ-terpinene, terpinolene, neral, and geranial, all of notable activity (*46*). The present data revealed that kumquat essential oils contained γ-terpinene (0.68%) and terpinolene (0.08%), whereas neral and geranial were not detected.

On the whole, there were few changes following HWD in the relative composition of essential oil with respect to control except for the significant increase in *p*-mentha-1,5-dien-1-ol. As a result of storage, some differences in minor compounds were recorded in comparison to their initial values. After storage, compounds such as *p*-cymene and *p*-cymen-8-ol, known to be responsible for off-flavors (*47*), increased in control fruit. The presence of off-flavors was not perceived in the present study either after treatments or after storage, presumably because of the low levels of these compounds.

Many studies have dealt with the use and properties of flavonoids (*48, 49*). Other investigations have attempted to apply flavonoid composition in the classification and discrimination of citrus (*50, 51*) and provided detailed information on flavonoid composition in fruit tissue (flavedo, albedo, and segment epidermis) of *Citrus* species. In 'Ovale' and 'Meiwa' kumquat (*Fortunella crassifolia* Swingle), for example, the flavonoids were represented by narirutin, followed in abundance by neoponcirin and poncirin and, to a much lesser extent, by rhoifolin, naringin, and hesperidin (*52*). Our findings (**Table 4**) showed that flavonoid composition in raw kumquat was represented only by narirutin, the most abundant component (107 mg/100 g), and rhoifolin (~37 mg/100 g); no detectable levels of other flavonoid compounds were found. It is reported that certain environmental and growing factors (e.g., photosynthetic photon flux, crop load) may have a role in the accumulation of such antioxidant compounds as AA, β-cryptoxanthin, and β-carotene, thus affecting antioxidant activity in 'Meiwa' kumquat (*53*). The present data show that HWD significantly increased the levels of narirutin and total flavonoids with respect to control fruit. After storage, the levels of narirutin and total flavonoids in control fruit remained fairly unchanged but decreased significantly in HWD fruit. Neither treatments nor storage conditions significantly affected rhoifolin content.

Proper heat treatments have been reported to enhance the antioxidant capacity of citrus fruit, due to the increase in total cinnamic and benzoics in the free fraction (*54*). Studies on pomegranates (*Punica granatum* L. cv. Mollar de Elche) (*26*)

revealed that fruit dipping in hot water at 45 °C for 4 min increased the total antioxidant activity in arils with respect to control, due to the higher levels of total phenolics and, to a lesser extent, ascorbic acid and anthocyanin content. We found no treatment-dependent differences in antioxidant activity before storage, whereas storage conditions significantly increased total phenol concentrations of fruits (**Table 4**). It has been reported that various abiotic stresses may affect the levels of nutraceuticals in crop tissues, and a stress, or combined stresses, may be applied to selectively increase health-promoting phytochemicals and avoid the accumulation of undesirable compounds (*13, 55, 56*). The present study on kumquat fruit revealed that the impact of HWD on levels of nutraceuticals was generally low and that beneficial or adverse effects produced by treatments (increases or decreases of individual nutraceuticals) may be challenged by storage conditions.

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