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Biothiols, Taurine, and Lipid-Soluble Antioxidants in the Edible Pulp of Sicilian Cactus Pear (*Opuntia ficus-indica*) Fruits and Changes of Bioactive Juice Components upon Industrial Processing

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Biothiols, taurine, and flavonols, as well as tocopherols and carotenoids have been assessed in the edible pulp of Sicilian red (Sanguigna), yellow (Surfarina), and white (Muscaredda) cultivars of cactus pear. The yellow cultivar has the highest level of reduced glutathione (GSH, 8.1 ± 0.78 mg/100 g pulp), whereas the white cultivar showed the highest amount of cysteine (1.21 ± 0.12 mg/100 g pulp). Taurine accounted for 11.7 ± 1.0 mg/100 g in the yellow pulp, while lower levels were measured in the others. With the exception of kaempferol in the yellow cultivar (2.7 ± 0.2 μg/100 g pulp), the edible pulp of cactus pear was not a source of flavonols. Very low amounts of lipid-soluble antioxidant vitamins such as vitamin E and carotenoids were measured in all cultivars. As a consequence of industrial processing, a total loss of GSH and β-carotene and a net decrease of vitamin C and cysteine were revealed in the fruit juice, whereas betalains, taurine, and vitamin E appeared to be less susceptible to degradation.

KEYWORDS: biothiols; taurine; flavonols; tocopherols; carotenoids; cactus pear fruit; industrial juice.

INTRODUCTION

There is substantial agreement in considering dietary antioxidants, including vitamins and bioactive phytochemicals, useful against diseases originating in or aggravated by oxidative stress, such as cardiovascular diseases, cancer, and neurodegenerative disorders (*1*−*3*). The plant kingdom is a rich source of such molecules, with polyphenols being the most studied phytochemicals that are believed to provide antioxidative effects (*4*−*6*).

Recently, cactus pear has received considerable attention in the scientific community for its bioactive components, which might provide health benefits beyond basic nutrition. This plant is cultivated in Mexico, as well as in Italy, South Africa, and other countries, with different *Opuntia* species predominating in each country. Climate, region, and cultural practices may somewhat affect nutrient and mineral composition as well as amounts of bioactive components. Various research groups have reported on the antioxidant vitamins and phytochemicals of the fruits from different cultivars of *Opuntia ficus-indica* (L.) Mill. (*7*−*10*). Significant amounts of vitamin C are present (*8*−*10*). In addition, the fruits proved to be a rich source of betalain pigments, either betaxanthins or betacyanins (*9*−*14*), the redox properties and antioxidant activity of which have recently been demonstrated (*9*, *15*, *17*). Other compounds can be considered to provide more complete information about the antioxidant potential of the cactus pear fruits. In the present study, the presence of biothiols such as reduced glutathione (GSH), cysteine, and *n*-acetyl cysteine (NAC) in fruits from the Sicilian red (Sanguigna), orange-yellow (Surfarina), and white (Muscaredda) cultivars of cactus pear was investigated. Taurine, considered a cell-protective β-amino acid (*18*−*20*), with antioxidative effects (*21*, *22*), had been reported earlier in some *Opuntia ficus-indica* cultivars from Mexico and South Africa (*23*). It was therefore interesting to assess the taurine concentration in Sicilian cultivars.

Polyphenols such as isorhamnetin-3-rutinoside have been reported in a fresh juice including pulp and peel of fruits from the Sicilian cactus pear (*24*). In this study, the edible portion of the fruits from three Sicilian cultivars was investigated for the content of a number of flavonols. Finally, lipid-soluble antioxidants such as tocopherols and carotenoids were assessed.

Juice production is one of the most frequently utilized fruit and vegetable technology. Therefore, the effects of industrial processing to obtain juice on the antioxidant components of the cactus pear fruits were evaluated.

MATERIALS AND METHODS

Reagents and Chemicals. *N*-Acetylcysteine (NAC), cysteine, GSH, *N*-(1-pyrenyl)maleimide (NPM), *o*-phthalaldehyde, taurine, rutin, quercetin, kaempferol, isorhamnetin-3-rutinoside, ascorbic acid, α-, β-, and γ-tocopherol, and *trans*-α-, *trans*-, and *cis*-β-carotene were from Sigma Chemical Co. (St. Louis, MO). All-trans lycopene and phytofluene were from Extrasynthese (Cedex, France). All other chemicals and solvents

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were of the highest purity or high-performance HPLC grade (Merck, Darmstadt, Germany).

Plant Material and Sample Preparation. Cactus pear fruits were obtained from cactus plants grown in the region of San Cono (Sicily), collected at the beginning of October, November, and December, and processed within 72 h of collection. The cultivars investigated were the yellow (Surfarina), the red (Sanguigna), and the white cultivar (Muscaredda). Three different lots of four fruits, at comparable ripening stages, were analyzed for each cultivar. Fruits were manually peeled, and the pulp was weighed, finely chopped, and briefly homogenized with a tissue blender. The pulp was then strained through a gauze (0.6-mm mesh size) to eliminate the seeds, and the volume of the resulting juice was measured. The conversion factors for the cultivars were 0.86, 0.82, and 0.73 mL of filtered juice per gram of pulp for cv. yellow, cv. red, and cv. white, respectively. Pulp samples were portioned and stored at −80 °C, until analysis.

Industrial Juice. The fresh cactus pear fruit juice was a test product supplied by a local company (AGRES, srl, Carini, Palermo). Juice was obtained by processing whole fruits (95% yellow cultivar and 5% red cultivar) with a screw press. The resulting cloudy juice was piped in a juice refiner and then clarified by centrifugation. Pasteurization was carried out by a heat-evaporation process (80 °C, 20 s) using a counter-current heat exchange. The juice was not acidified before pasteurization. Finally, the juice was concentrated in a T.A.S.T.E. (thermally accelerated short-time evaporator), through 5 subsequent stages. The maximum operating temperature of the first effect did not exceed 76 °C, and total holding time in the concentration stage was less than 3 min. Following this procedure, the final juice was approximately 3-fold more concentrated than the juice before pasteurization. A 100-kg sample of fresh cactus pear fruits produced about 21 L of concentrated juice.

HPLC Procedures. A Gilson modular liquid chromatographic system (Gilson Inc., Middleton, WI), equipped with M 302 and 305 pumps, an injector model 77-25 (Rheodyne, Berkeley, CA) with a 100-μL injector loop, and a M 802c manometric module, was used. The chromatographic column was a 5-μm Spherisorb ODS2 column (250 × 4-mm i.d.) with a 5-μm Spherisorb ODS2 guard column (10 × 4 mm) (Supelco, Bellefonte, PA), except when specified. Detection was by a M 118 UV/visible detector or a M 122 fluorometer used along with the Gilson 712 HPLC System Controller Software.

Thiol Compounds. Thiol compounds were measured in fruit pulp and industrial juice after derivatization with NPM and fluorometric detection, according to Demirkol et al. (*25*). Briefly, samples (0.5 g) of either fruit pulp or industrial juice were mixed with 1.0-mL serine−borate buffer (100 mM Tris-HCl, 10 mM borate, 5 mM serine, and 1-diethylenetriaminepentacetic acid, pH 7) to prevent artifactual oxidation. The mixture was homogenized on ice for 2 min and then centrifuged at 2000*g* for 15 min at 4 °C. Aliquots of the supernatant (10−250 μL) were derivatized with 1.0 mM NPM in acetonitrile (1:4, v/v), and 25 μL was immediately submitted to analysis. The compounds were isocratically eluted with water/acetonitrile (30:70, v/v) containing 1 mL/L acetic acid and 1 mL/L *o*-phosphoric acid at 1 mL/min. Fluorometric detection was at an excitation wavelength of 330 nm and an emission wavelength of 375 nm.

Taurine. Samples of fruit pulp or industrial commercial juice were diluted with 5 mM phosphate saline buffer, pH 7.4 (PBS, 0.5 g/10 mL), homogenized, and briefly exposed to an ultrasonic bath. After centrifugation at 2000*g* for 15 min at 4 °C, samples were deproteinized with 20% sulfosalicylic acid (6:1, v/v) and clarified after centrifugation at 60000*g* for 30 min at 4 °C. Aliquots of the deproteinated supernatant were derivatized with an equal volume of a mixture of 25 mg of *o*-phthalaldehyde, 500 μL of methanol, 25 μL of β-mercaptoethanol, and 4.5 mL of 0.4 M potassium borate buffer, pH 10.4, and analyzed by HPLC according to Gonzalez-Quevedo et al. (*26*). Briefly, 100 μL of the sample was injected onto the chromatographic system eluted with increasing concentrations of acetonitrile in a 50 mM sodium phosphate buffer (pH 6.4) with 5% acetonitrile and 0.1% tetrahydrofuran at a flow rate of 1 mL/min. The main step gradient used was 0−10 min 95% and 10−55 min 70% of buffer solution. Fluorometric detection was at an excitation wavelength of 340 nm and an emission wavelength of 455 nm.

Flavonoids. Samples of fruit pulp were diluted with PBS, pH 7.4 (1.0 g/mL). Aliquots were analyzed by a 5-μm Spherisorb ODS2 column (15 × 0.46 cm), following Price et al. (*27*) with minor modifications. Separation was achieved with stepwise gradient elution. All gradient steps were performed linearly. Development was carried out in 30 min using the following gradient program: 85% mobile phase A (0.1% (v/v) trifluoracetic acid in water) and 15% mobile phase B (acetonitrile) at time zero and ramped to 65% A:35% B in 20 min; 0% A:100% B in an additional 10 min. The system was operated at a flow rate of 1.5 mL/min. Spectrophotometric revelation was at 365 nm. To determine conjugated flavonoids, aliquots of fruit pulp were heated at 90 °C for 2 h in 1.2 M HCl in 50% aqueous methanol (*28*), evaporated under reduced pressure, redissolved in PBS, and filtered prior to HPLC. Rutin, quercetin, kaempferol, and isorhamnetin-3-rutinoside were used as standards.

Ascorbic Acid. Evaluation of ascorbic acid concentration in the industrial juice was performed by reversed-phase HPLC, with spectrophotometric detection at 266 nm, as reported by Lazzarino et al. (*29*) with minor changes. These included length of the column (25 × 0.46 cm, particle size 5 μm) and isocratic elution with 10 mM/L KH_2PO_4 buffer, pH 7.0, containing 1% methanol and 10 mL/L tetrabutylammonium bromide, at 1.2 mL/min. The retention time of ascorbate was 5.3 min.

Tocopherols. Vitamin E was extracted from fruit pulp or industrial juice with dichloromethane (1:2, v/v). Aliquots were injected onto a column eluted with methanol at 1.0 mL/min (*30*). Detection was at 290 nm.

Carotenoids. Extracts with dichloromethane from fruit pulp and industrial juice (1:2, v/v) were analyzed on a HPLC column eluted with a mixture of acetonitrile/methanol/tetrahydrofuran (58.5:35:6.5, v/v/v) at a flow rate of 2.5 mL/min (*30*). Detection was at 450 nm.

Betalains. Pigments were extracted from the industrial juice with methanol (1:2, v/v). Methanol was evaporated (rotovaporator, 20 °C), and the aqueous phase was submitted to analysis on a HPLC column eluted with a 20-min linear gradient elution from solvent A (1% acetic acid in water) to 20% solvent B (1% acetic acid in acetonitrile) at a flow rate of 2 mL/min (*31*). Spectrophotometric detection was at 536 and 482 nm for betanin (12.0 min) and indicaxanthin (13.6 min), respectively. An automatic wavelength change after 12.5 min allowed the detection of both compounds in the same run.

All compounds were quantified by reference to standard curves constructed with 5−100 ng of each pure commercial compound, with the exception of betalains that were extracted and purified according Butera et al. (*9*). All procedures including extraction and analysis of fruit components were performed under dim red light to avoid artifactual photooxidation of lipids and to preserve light-sensitive vitamins.

RESULTS

Thiols are a type of mercaptans with redox-active sulfhydryl functional groups. The Sicilian cultivars of cactus pear fruits were investigated for their content of GSH and of its precursors cysteine and NAC. The highest GSH concentration was measured in the yellow cultivar, whereas the content of the red and white cultivars was about one-half. Very low amounts of cysteine were detected, without any substantial difference among the cultivars, while NAC was not detectable at all (Table 1). In our previous work (*9*), amounts of polyphenolics as low as 237 ± 20 ng/100 g fresh pulp were spectrophotometrically measured as quercetin equivalents in betalain-free methanol extracts from fruits of the red cultivar of Sicilian cactus pear. Present work investigated the presence of individual flavonols such as rutin, quercetin, kaempferol, and isorhamnetin-3-rutinoside in the edible fruit pulp by HPLC analysis. With the exception of small amounts of kaempferol in the yellow cultivar, none of the other flavonoids considered could be detected (Table 1). Acidic hydrolysis of fruit pulp samples prior to flavonoid analysis did not modify the quantitative or qualitative HPLC profiles. Antioxidative effects of taurine have recently been shown (*18*−*22*).

Table 1. Biothiols, Flavonoids, Taurine, and Lipid-Soluble Antioxidant Vitamins in Fruit Pulp of Sicilian Cactus Pear Cultivars[a,b]

compound	yellow cultivar (Sulfarina) at pH 5.5	red cultivar (Sanguigna) at pH 5.9	white cultivar (Muscaredda) at pH 5.6
biothiols (mg/100 g)	8.82 ± 0.8	4.17 ± 0.5	5.43 ± 0.5
GSH	8.1 ± 0.78	3.4 ± 0.5	4.22 ± 0.5
cysteine	0.72 ± 0.08	0.77 ± 0.1	1.21 ± 0.12
NAC	n.d.	n.d.	n.d.
flavonoids (μg/100 g)			
rutin	n.d.	n.d.	n.d.
quercetin	n.d.	n.d.	n.d.
kaempferol	2.7 ± 0.2	n.d.	n.d.
isorhamnetin-3-rutinoside	n.d.	n.d.	n.d.
taurine (mg/100 g)	11.7 ± 1.0	8.38 ± 0.75	8 ± 0.9
total vitamin E (μg/ 100 g)	115 ± 10	111.5 ± 0.5	114 ± 10
α-tocopherol	69 ± 5.9	68.5 ± 7.0	67 ± 5.9
δ-tocopherol	16 ± 1	14 ± 1	15 ± 1
γ-tocopherol	30 ± 3	29 ± 3	32 ± 3
carotenoids (μg/100 g)	1.48 ± 0.15	3.47 ± 0.3	1.45 ± 0.15
α-carotene	0.08 ± 0.01	0.16 ± 0.02	0.06 ± 0.01
trans-β-carotene	1.20 ± 0.11	3.01 ± 0.3	1.25 ± 0.10
cis-β-carotene	n.d.	n.d.	n.d.
all-trans lycopene	0.18 ± 0.03	0.27 ± 0.05	0.12 ± 0.01
phytofluene	0.02 ± 0.002	0.03 ± 0.004	0.02 ± 0.005

[a] Values are the mean ±SD of separate analyses carried out in duplicate with different preparations ($n = 3$). [b] n.d., not detectable.

Table 2. Antioxidant Content in an Industrial Juice of Sicilian Cactus Pear Fruit before and after Pasteurization and Concentration (final product)[a,b]

	industrial juice	
	before pasteurization 12.3 °Bx	final product 38.0 °Bx
vitamin E (μM)	3.95 ± 0.41	12.32 ± 1.10
β-carotene (μM)	28.68 ± 1.98	n.d.
vitamin C (mM)	1.75 ± 0.21	0.17 ± 0.02
indicaxanthin (mM)	0.26 ± 0.03	0.72 ± 0.07
betanin (μM)	21.7 ± 2.15	60.3 ± 5.08
taurine (mM)	0.61 ± 0.05	1.64 ± 0.15
GSH (mM)	0.24 ± 0.19	n.d.
cysteine (μM)	50.2 ± 4.1	10.2 ± 0.98

[a] Each value is the mean ±SD of two separate determinations carried out on the same preparation. [b] n.d., not detectable.

Analysis of three Sicilian cultivars of *Opuntia ficus-indica* fruits provided evidence of this compound in all cultivars, with the highest concentration in the yellow one (Table 1). Lipid-soluble antioxidant vitamins were assessed. Small amounts of vitamin E were found in all cultivars, without any significant variation. α-Tocopherol was the most abundant tocopherol in each case. Very modest amounts of carotenoids were measured in all cultivars, with the red cultivar showing the highest amount (Table 1).

The industrial processing of fruits to obtain juices include treatments that can affect the antioxidant content of the product. The amounts of biothiols, taurine, and lipid-soluble antioxidant vitamins, as well as of other characteristic antioxidant constituents such as vitamin C and betalain pigments, were assessed in a cactus pear fruit juice produced in Sicily before the pasteurization step, as well as at the end of processing. A total loss of GSH and β-carotene and a net decrease of the amount of vitamin C and cysteine were revealed in the final juice with respect to the juice before pasteurization (Table 2). On the contrary, when considering that the final product is approximately 3-fold more concentrated than before pasteurization (38.0 °Bx vs 12.3 °Bx), taurine, betalains, and vitamin E were almost unmodified, thus appearing less affected by the thermal treatment.

DISCUSSION

The fruits of cactus pear have recently raised attention for their nutritional and potential technological value (*32*−*34*). Beside nutrients such as sugars, vitamins, and minerals (*7*−*9*), they contain high amounts of betalains, natural pigments widely used as safe colorants for foods and cosmetics (*9*−*14*). In addition, recent studies focusing on the antioxidant potential of these fruits provided evidence of novel functional aspects (*9*, *10*). Betalains, the reducing and radical-scavenging activity of which have been demonstrated in various in-vitro and ex-vivo experiments (*9*, *15*−*17*, *35*), as well as vitamin C have appeared as important contributors to the total antioxidant activity of cactus pear fruits (*9*). This work reports that the fruit pulp from Sicilian cultivars contains other antioxidant molecules such as biothiols, which are fundamental to the cell antioxidative defense (*36*, *37*). GSH, cysteine, and NAC have recently been measured in a number of fruits (*25*). The yellow cultivar of the Sicilian cactus pear exhibits GSH amounts as high as 263.59 nmol/g edible pulp, which is higher than those in mango, strawberry, grapefruit, and papaya (*25*). The red and white cultivars were found to contain about one-half this concentration. While NAC was not found, cysteine levels, without any substantial difference among the cultivars, appeared higher than those reported for the above-mentioned fruits (*25*). Lipid-soluble compounds such as tocopherols and carotenoids have appeared in lower concentrations than other antioxidants of the cactus pear fruit. The total amount of vitamin E, with α-tocopherol being the most represented followed by γ- and δ-tocopherol, is in the range of that measured in most fruits and vegetables (*38*). On the other hand, the cactus pear does not appear to be a good source of carotenoids in comparison with yellow fruits such as mango and cantaloupe and with green leafy vegetables such as spinach and broccoli (*39*). Other authors (*40*) who have assessed tocopherols and β-carotene in the pulp oil of *Opuntia ficus-indica* purchased in Germany reported levels of total vitamin E and β-carotene higher than those in the present study. A number of reasons including cultivars, stage of ripeness, and extraction process may account for these differences.

Taurine, a semi-essential amino acid which is not included in proteins, is considered a cell protective compound. It may be involved in modulating the inflammatory response by complex formation with hypochlorous acid generated by neutrophyls (*18*−*20*). In addition, antioxidative effects have been shown, by inhibition of reactive oxygen species formation (*21*, *22*). In contrast to the majority of other fruits, high amounts of taurine have been reported in cactus pear cultivars from Mexico and South Africa (*23*). Our results show that the Sicilian cultivars of *Opuntia ficus-indica* contain taurine at a concentration lower than previously reported contents for American and African cultivars (*23*).

Flavonoids have recently been researched in fruits from the Sicilian cultivars of cactus pear. Isorhamnetin-3-rutinoside, amounting to 54.6 μg/mL, was reported for juice from whole fruits including the skin of red and yellow Sicilian cultivars, with very small amounts of rutin and kaempferol, and other flavonol glycosides, the amount of which were not quantified (*24*). Recently, spectrophotometric analysis from our group failed to reveal polyphenols in aqueous extracts free of betalains from the fresh fruit pulp of the yellow and white cultivars, whereas a small peak at 360 nm, which was interpreted as flavonols, was revealed in the red cultivar (*9*). The present HPLC

data show that very small amounts of kaempferol (2.7 μg/100 g edible fruit pulp) occur only in the pulp of the yellow fruit, whereas the other flavonols assessed, that is, quercetin, rutin, and isorhamnethin-3-rutinoside, are absent in all the Sicilian cultivars. It is therefore concluded that compounds other than flavonols had possibly caused interference in the spectral analysis previously performed. Compared with the predominating phytochemical components such as betalains, the range of which is 6−9 mg/100 g fruit pulp, depending on the cultivar (*9*, *10*), flavonols appear to represent only a minor component of the edible cactus pear fruits from Sicilian cultivars. In light of the antioxidative properties of polyphenols, and of the interest about their presence in vegetables and fruits, it seems important to mention that a previous study on *Opuntia ficus-indica* grown in Texas (*41*) reported that flavonoids amounted to 70 μg/g fruit fresh weight, although with some ambiguity with regard to the flavonoid content of fruit skin or pulp. On the other hand, other researchers have recently checked the fruit pulp of nine clones of *Opuntia ficus-indica* from California and of one clone of *Opuntia robusta* from South Africa for a number of phenolics; they did not find evidence of any flavonoid (*10*). The actual contribution of constituents of the fruit skin should be considered to rationalize the presence of flavonoids in the cactus pear fruit.

The current trend toward healthy diets makes the consumption of fruit juice a widespread alternative for fresh fruits. Highly reactive molecules such as free-radical scavengers and antioxidants may thereby be easily damaged during processing of food. Indeed, the industrially processed juice from whole fruit exhibited concentrations of cysteine and vitamin C 5- and 10-fold lower, respectively, than in the edible fruit pulp, whereas β-carotene and GSH were lost, which mainly appeared to be the result of thermal degradation during the pasteurization and concentration steps. Gurrieri et al. reported that exposure of a cactus pear juice for 2 min at the boiling temperature reduced greater than 50% of the vitamin C content (*42*). Therefore, though the industrial processing of the juice analyzed in the present study included less severe thermal treatments, the instability of vitamin C in fruit processing appears to be confirmed. On the contrary, vitamin E, which is less susceptible to heat (*43*), appeared to be preserved. Interestingly, betalains were not noticeably lost during processing, despite their thermal instability (*44*−*46*). Possibly, compared to the pure compounds, these molecules may be somewhat protected by matrix components in the juice. Regeneration of betacyanins by antioxidants during food processing has been reported (*47*, *48*). The contribution of skin components may also be considered.

Because of nutrients, vitamins, and mineral composition, cactus pear fruits have a nutritive value similar to that of other fruits. In addition, specific bioactive components can equip this fruit with a remarkable added value. Our recent in-vivo investigation in humans showed that consumption of fresh fruits from Sicilian cactus pear cultivars can decrease the body oxidative stress in healthy individuals (*49*) and that betanin and indicaxanthin are highly bioavailable (*50*). In light of all these data, the fruit of cactus pear seems to be considered as a functional food.

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