Chemical Characterization of Sicilian Prickly Pear (*Opuntia ficus indica*) and Perspectives for the Storage of Its Juice

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In this work, Sicilian cultivars of prickly pear (*Opuntia ficus indica*) were partially characterized from a chemical point of view, and the possibility of long-term storage of their juice was investigated. The acidity of the prickly pear juice turned out to be very low (0.02%) and the pH very high (6.4−6.5) if compared with values found in other common fruit juices. In the perspective of processing and storage conditions according to Italian law, the acidity has been corrected by adding the proper amount of tartaric and/or phosphoric acid. The sugar content (mainly glucose and fructose) is very high (11−12%), and also L-ascorbic acid is present in considerable amount (31−38 mg/100 g). Among the transition metals, a high content of manganese(II) (1.7−2.9 ppm) and good amounts of iron(III) (0.6−1.2 ppm) and zinc(II) (0.3−0.4 ppm) were found. In particular, such ions appear to be present mainly in the thick skin of the fruit or "trapped" inside the pulp. Pectin methylesterase (PME) seems to be present in very small amount and/or is not highly active. Furthermore, PME activity decreases considerably after the necessary adjustment of the pH and the thermal treatment requested for long-term storage. After ∼2 months, none of the juices prepared was affected by noticeable sedimentation of the pulp. Finally, different samples of prickly pear juice were sensorially analyzed, employing descriptors such as color, aroma, viscosity, acidity, sweetness, and off-flavors. The results obtained can be considered very satisfactory, and the juice has been widely appreciated when compared with other products commonly available on the market such as pear and peach juices.

Keywords: *Prickly pear; Opuntia ficus indica; fruit juice; sensory analysis*

INTRODUCTION

The prickly pear cactus (*Opuntia ficus indica*) is a plant highly distributed in the Mediterranean area, Central and South America, and South Africa (Barbera and Inglese, 1993; Muñoz de Chàvez et al., 1995). In fact, owing to its crassulacean acid metabolism, this plant is characterized by a high potential of biomass production with very low water consumption (De Cortàzar and Nobel, 1992; Domìnguez-Lòpez, 1995). It is, therefore, extremely drought tolerant and grows abundantly under semiarid conditions. The content of proteins, carbohydrates, minerals, and vitamins (mostly vitamins A and C) in the fleshy stems (cladodes) is nutritionally significant, and in Mexico people eat them cooked as vegetables with meat or beans. Also, Mexican traditional medicine makes empirical use of nopal cladodes mainly for reducing serum cholesterol levels, regulating blood pressure, controlling gastric acidity, and treating several pathologies such as ulcer, fatigue, dyspnea, glaucoma, capillary fragility, liver conditions, rheumatic pain, and wounds (Muñoz de Chàvez et al., 1995; Domìnguez-Lòpez, 1995). Some new applications having greater scientific basis are being tried for the treatment of gastritis, hyperglycemia, arteriosclerosis, diabetes, and prostatic hypertrophy (Hegwood, 1990; Frati et al., 1990; Palevitch et al., 1993).

The nopal produces delicious juicy fruits containing a large number of hard seeds, and it is usually eaten raw after being peeled. The color of the pulp changes depending on the cultivar, from blood red to pale yellow. The very intense color of some cultivars makes the prickly pear fruit very attractive. This is due to the presence of water-soluble pigments such as betacyanins (purple-red) and betaxanthines (yellow-orange) (Piattelli et al., 1964; Piattelli and Minale, 1964; Forni et al., 1992) that can be isolated and used as natural food colorants (Barbera and Inglese, 1993; Odoux and Dominguez-Lopez, 1996). The fruit has also fairly high vitamin and sugar contents (which give it a very sweet taste) (Sawaya et al., 1983; Sepùlveda and Sàenz, 1990; Joubert, 1993; Muñoz de Chàvez et al., 1995).

There are also a number of traditional prickly pear products (Domìnguez-Lòpez, 1995; Sàenz, 1996). The juice can be either consumed as such, employed for ice cream preparations, or cooked and semidried to the consistency of toffee. Jams and syrups are also com-

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monly produced in South Africa. The fruit juice can be fermented, using appropriate yeast strains, either to produce ethanol or to make strong alcoholic beverages (Bustos, 1981; Retamal et al., 1987).

Finally, during the processing of a fruit juice for long-term storage, the purpose of a thermal treatment is to sterilize the product (from bacteria and fungi) as well as to inactivate endogenous enzymes such as pectin methylesterase (PME) (Castaldo et al., 1989; Giovane et al., 1990, 1996; Rillo et al., 1992). In fact, PME action results in the removal of methoxy groups from pectin and leads to its precipitation as calcium pectate. Such separation of the pulp from the serum, generally referred to as "cloud loss", is an unattractive feature in fruit and vegetable products. Unfortunately, because of the thermal resistance of this enzyme (Laratta et al., 1995a,b), the thermal treatment often requires very high temperatures (105−115 °C). Such drastic treatment generally causes undesirable effects known as "off-flavors" or "cooked-flavors".

In this work we have studied some biochemical parameters of the prickly pear fruit from Sicilian cultivars and examined some perspectives for the long-term storage of its juice. In particular, we have determined total acidity, sugar, and L-ascorbic acid (vitamin C) contents, transition metal ions, and PME activity. We have also investigated how some of these parameters are affected by a thermal treatment of pasteurization. Finally, we have carried out a sensory analysis on prickly pear juice samples using descriptors such as color, aroma, viscosity, acidity, sweetness, off-flavors, and overall acceptability in comparison with other fruit juices commonly available on the market.

MATERIALS AND METHODS

Preparation of Juice. All experiments were carried out on prickly pear fruits purchased at a local market. Only fruits at similar ripening stages were selected for further processing and analysis. Three different lots of fruit for each cultivar were juiced separately. The fruits were peeled and blended. The pulp and juice were separated from the seeds by filtration. The seeds were then washed abundantly with water to remove the pulp attached and dried at 60 °C for 24 h. Juice samples were stored frozen at −20 °C. All measurements were carried out in triplicate with samples from different lots of fruit.

Acidity Determination. The total acidity was determined on 50 g aliquots of juice by recording the volume of 0.1 N NaOH (Merck) necessary to take the sample to pH 8.1 (which was measured potentiometrically) (Tateo, 1978). Experiments were carried out on juices from the three cultivars as well as on their relative skins. In this last case, after the samples were carefully blended to homogeneity, they were diluted 2-fold and then titrated with 0.1 N NaOH as described above.

Sugar Analysis. The sugar analysis was performed by HPLC using a WAT 44355 column (Waters high-performance carbohydrate column) equipped with a refractive index detector (Waters 410). The stationary phase was made of spherical silica beads (4 μm, Nova-Pak) with trifunctional aminopropylsilane bound to it. An acetonitrile (Merck) and water solution (75:25 v/v) was employed as mobile phase and the elution carried out in isocratic mode. All samples were injected after having been properly diluted and filtered at 0.45 μm. The assay was run on samples from the three cultivars as well as on standard sugars purchased from Sigma-Aldrich Co.

Total Nitrogen Determination. The total nitrogen content was measured using the Kjeldahl method. An exact amount of sample was weighed and mineralized in a glass tube after the addition of concentrated sulfuric acid, a small amount of selenium as catalyzer, and copper(II) oxide. At firsr, the glass tube was heated to ∼200 °C and, after the addition of a solution containing 40% hydrogen peroxide, to ∼400 °C until a clear light blue solution was obtained [due to the formation of copper(II) complexes]. After cooling, a few milliliters of water was added to the solution that was then placed into the Kjeldahl apparatus (Kjeltec Auto Analyzer 1030 distiller) for the determination of the nitrogen content. NaOH (0.01 N) was added in excess, and the total nitrogen present was distilled as ammonia. The excess of NaOH was then back-titrated with 0.01 N HCl in the presence of a mixed indicator (bromocresol green and methyl red).

L-Ascorbic Acid Determination. L-Ascorbic acid (vitamin C) was determined by HPLC, employing a column packed with a polystyrene/divinylbenzene resin (PLRP-S 100 Å) and a UV detector at $\lambda = 220$ nm (Waters 484). A 0.2 M monobasic monohydrate sodium phosphate $NaH_2PO_4 \cdot H_2O$ (Merck) solution, taken to pH 2.14 with concentrated HCl (36% w/v), was employed as mobile phase. All of the samples were injected after having been properly diluted and filtered at 0.45 μm, and the elution was carried out in isocratic mode. The same standard, purchased from Sigma-Aldrich Co., was employed. The assay was performed on the juices from the three cultivars before and after thermal treatment.

Determination of Transition Metal Ions. The prickly pear juice samples were first filtered on a Büchner to remove the pulp and then filtered at 0.45 μm, and the pigments were removed on activated charcoal (Sigma-Aldrich Co.). The samples were then boiled for 5−10 min and filtered again. The content of transition metal ions was determined by anionic chromatography (Haddad and Jackson, 1990) on the juices from the yellow and white cultivars. The resin is obtained through an electrostatic aggregation process between the "core" of a sulfonic resin and a "rubber" layer containing quaternary ammonium ions. The instrument employed was the DX-100 ion chromatograph (Dionex) equipped with an IonPac CS5A (Dionex) column, a 375 μL knitted reaction coil, and a UV−vis detector. A solution containing 7 mM pyridine-2,6-dicarboxylic acid (H_2PDCA) (Sigma-Aldrich Co.), 66 mM potassium hydroxide, 74 mM formic acid, and 5.6 mM potassium sulfate was used as mobile phase. The addition of H_2PDCA as chelating agent yields the formation of anionic 1:1 or 1:2 metal/ligand complexes. Once separated, the metal ions were detected through the formation of their mixed complexes with 0.5 mM 4-(2-pyridylazo)resorcinol (PAR) in 1 M 2-dimethylaminoethanol, 0.5 M ammonium hydroxide, and 0.3 M sodium bicarbonate (Sigma-Aldrich Co.), and their absorbance at 500−540 nm was recorded.

Spectrophotometric and Electron Paramagnetic Resonance (EPR) Measurements. All UV−vis measurements were recorded on a Hewlett-Packard HP 8452 A diode-array spectrophotometer using 1 cm path quartz cuvettes. EPR spectra were recorded on a conventional X-band (9.5 GHz) spectrometer (Bruker ER 200 D) equipped with a low-temperature control unit and driven by the ESP 3220 data system. 1,1-Diphenyl-2-picrylhydrazyl ($g = 2.0036$) was used to calibrate the klystron frequency, and the magnetic field scan was controlled by a Bruker ER 035 M gaussmeter. Room temperature EPR spectra were obtained using a Bruker quartz flat cell. For the measurements at 150 K, a cold nitrogen stream, controlled by the ER 4111 VT system, was allowed to flow inside the cavity. For experiments at 77 K, the samples were frozen directly in liquid nitrogen.

PME Activity. The assay for PME activity was performed according to the method of Hagerman and Austin (1986). As substrate was used a 0.2% w/v citrus pectin (Sigma-Aldrich Co.) solution, containing 100 mM NaCl and 0.001% w/v bromothymol blue (Sigma-Aldrich Co.), pH 7.5. The measurements were performed on 3 mL of substrate by following the decrease in absorbance at 616 nm upon the addition of the proper amount of standard PME (from orange peel, Sigma-Aldrich Co.) or fruit juice.

Prickly pear juice samples were prepared as follows: a 10 g aliquot of each sample was homogenized and treated with an 8.8% w/v NaCl solution. After 2−3 h of stirring at 4 °C, the samples were centrifuged (6000 rpm, 15 min, 4 °C), and the surnatants were resuspended in 8.8% w/v NaCl for 2−3 h

and centrifuged again. All surnatants were then collected together and assayed.

To calibrate the assay, galacturonic acid should be employed, which is the acid produced by the PME action. However, because weak acids with similar pK_a values yield the same color change, acetic acid was employed. The linear portion of the curve was best fitted and used to convert the change in absorbance per minute (Δ_{abs}/min) into micromoles of H^+ per minute, where 1 μmol of H^+ produced per minute corresponds to 1 PME enzymatic unit. The assay was performed on the standard PME as well as on the fruit juices from the three cultivars before and after thermal treatment.

PME activity was also assayed on the prickly pear juices as a function of temperature and pH. As far as temperature dependence, the samples were incubated at 30, 60, and 90 °C for various intervals up to a total time of 120 min. All samples were then cooled to 25 °C for the activity measurement. For pH dependence, the samples were incubated at pH 3, 4, and 5 for various intervals up to a total time of 5 h. The pH of all samples was then corrected to 7.5 to run the assay.

Pasteurization. Prior to thermal treatment, all prickly pear juices were acidified to pH 4.5 using tartaric and/or phosphoric acid (Carlo Erba). Then, 400 mL of juice was poured into a 1000 mL Erlenmeyer flask and heated to boiling temperature with a 75 MW microwave oven (Mullin, 1995). The juices were kept at 100 °C for variable times (from 2 to 4 min) and transferred into autoclaved glass jars in sterile environment. The jars were then quickly sealed, cooled in an iced water bath, and stored at room temperature. Such pasteurization was performed on prickly pear juices from the three cultivars, which were assayed for sugar content, L-ascorbic acid content, and PME residual activity as a function of pasteurization conditions. Clearly, such thermal treatment does not pretend to simulate an industrial pasteurization protocol, and it is surely far more drastic compared to a process that would be used for a commercial product. Nevertheless, it allowed us to qualitatively investigate the effects of temperature on several physicochemical parameters of the prickly pear juice and to gain information that could be extremely useful in the eventual optimization of pasteurization conditions for applications on a larger scale. Furthermore, such treatment did not cause major undesirable effects such as significative alterations in the color of the juice and/or "off-flavors" or "cooked-flavors".

Sensory Analysis. A preference test was carried out employing untrained students at the University of Catania, Italy. Such a test was performed on prickly pear juice from the red cultivar in comparison with commercially available pear and peach juices. Instead, a ranking test was carried out on prickly pear juice from the yellow cultivar with a trained sensory panel (Amerine et al., 1965). The judges were 41 students, 24 females and 17 males, aged between 20 and 25 from the College of Agriculture, University of Catania. In a preliminary session tha aim of the work was explained, and the recommended behavior before and during the test was described. The ranking test was carried out on yellow prickly pear juice, pear juice, and peach juice using seven descriptors: color, aroma, viscosity, acidity, sweetness, off-flavors, and overall acceptability. The descriptive terms were accompanied by numerical scores rated on a scale from 1 to 9 (Lanza et al., 1995). The three samples were presented to assessors in a random order. Statistical analysis of sensory data was carried out using the SPSS version 8.0 (SPSS Inc., 1998). One-way analysis of variance was used to evaluate the data scattering around means. Duncan's multiple-range test was used to evaluate the significant differences among mean scores ($p > 0.05$). Cross-validated discriminant analysis was successively applied to the various parameters in order to classify the fruit juices into separate groups (Lanza et al., 1998).

RESULTS AND DISCUSSION

In this work we have employed prickly pear fruits from a second late bloom, induced by the manual removal of the first flowers by the end of June. This allows ripe fruits of considerably higher quality, that is, of larger size, with very juicy pulp, and containing a lower number of seeds, to be harvested late in the season (between September and November). The color of the pulp changes depending on the cultivar, from purple-red (red cultivar) to yellow-orange (yellow cultivar), to pale yellow (white cultivar). More than 90% of the Italian prickly pear production is of the yellow cultivar; the red and the white ones are less abundant, respectively around 7−8 and 2−3%. The composition of the fruits examined is reported in Table 1. The edible part is ∼60% of the fruit; the thick skin together with hundreds of small and hard seeds accounts for over one-third of the total weight.

Table 1. General Composition of Sicilian Prickly Pear Fruit

cultivar	skin (%)	juice and pulp (%)	seeds (%)
red	35.7 ± 1.0	61.4 ± 1.0	2.9 ± 0.3
yellow	39.1 ± 1.4	58.1 ± 1.5	2.8 ± 0.3
white	37.6 ± 0.9	58.9 ± 0.9	3.5 ± 0.4

Chemical Characterization. The total acidity was determined on prickly pear juice and skin for the three cultivars. The results obtained are reported in Table 2, expressed as w/w percentage of monohydrate citric acid. The total acid content in our prickly pear juice is of the order of 0.02%, which is very low in comparison with the acidity of other fruit juices such as pear (0.3%), orange (0.8%), apple (0.9%), peach (0.9%), strawberry (0.9%), pineapple (1.1%), raspberry (1.8%), plum (2.2%), and apricot (2.4%) (Belitz and Grosch, 1999; Tateo, 1978). Because of the extremely low content of organic acids, the pH of the prickly pear juice turns out to be very high, with values of the order of 6.4−6.5 commonly measured. On the other hand, the acid content of the peels of all three cultivars was found to be of the order of 0.12%, that is, much higher compared to that of the edible part of the fruit. As a consequence, the pH measured was considerably lower, in the range of 5.0−5.3. This information could be particularly interesting from an industrial point of view if also the skins would need to be blended during the large scale processing of a commercial product. Finally, the results obtained are in good agreement with literature data from Mexican and Chilean cultivars (Sawaya et al., 1983; Sepùlveda and Sàenz, 1990; Joubert, 1993).

The sugar analyses have been performed by HPLC using a chromatographic adsorption process in which silanolic −OH groups are functionalized with propylamino groups. Our results, reported in Table 2, show the presence of good amounts of glucose and fructose in all three cultivars under investigation. The values of glucose measured varied in the range of 6.0−6.4%, and the values of fructose encountered ranged between 5.4 and 6.0%. Also, a slightly higher content of both sugars was found in the yellow and white cultivars, consistent with these fruits tasting on average sweeter than red fruits. On the other hand, the data obtained show no detectable presence of sucrose. However, even though we do not have any evidence that sucrose may in fact be present in the fruit at some point during fruit development and/or ripening, we suggest that its absence could be due to the activity of the enzyme β-fructofuranosidase-fructohydrolase or invertase. Such an enzyme would, in fact, hydrolyze eventual sucrose present in equal amounts of glucose and fructose. β-Fructofuranosidase-fructohydrolase is indeed com-

Table 2. Chemical Composition of Red, Yellow, and White Cultivars of Sicilian Prickly Pear Fruit

	skin		juice					
cultivar	pH	acidity (%)	pH	acidity (%)	glucose (%)	fructose (%)	total N (mg/100 g)	ascorbic acid (mg/100 g)
red	5.06 ± 0.11	0.11 ± 0.01	6.40 ± 0.14	0.02 ± 0.01	6.0 ± 0.3	5.4 ± 0.2	52.8 ± 0.8	31 ± 3
yellow	5.27 ± 0.09	0.12 ± 0.01	6.44 ± 0.12	0.02 ± 0.01	6.2 ± 0.3	6.0 ± 0.4	53.9 ± 0.7	38 ± 4
white	5.14 ± 0.07	0.14 ± 0.02	6.48 ± 0.09	0.02 ± 0.01	6.4 ± 0.2	5.7 ± 0.3	52.8 ± 1.2	33 ± 3

monly present in many vegetable tissues, and it has been shown that prickly pear cultivars can contain either alkaline or acid invertases (depending on the pH required for maximum activity) (Ouelhazi et al., 1992; Kuti and Galloway, 1994). HPLC data also allowed us to confirm, as could be expected, that the sugar content is not affected by a thermal treatment of pasteurization (data not shown). Finally, our results are in good agreement with data reported in the literature (Sawaya et al., 1983; Sepùlveda and Sàenz, 1990; Joubert, 1993; Domìnguez-Lòpez, 1995) for other cultivars studied, and the total sugar content is similar or higher when compared with that of other common fruit juices such as pineapple (12.3%), apple (11.1%), pear (9.8%), peach (8.5%), plum (7.8%), orange (7.0%), apricot (6.1%), strawberry (5.7%), and raspberry (4.5%) (Belitz and Grosch, 1999; Cappelli and Vannucchi, 1990).

For the nitrogen content analysis the Kjeldahl method was employed and the data obtained are shown in Table 2. Such measurements yielded values around 53 mg/100 g well within the range of literature data reported for other cultivars, for which values as low as 34 mg/100 g and as high as 131 mg/100 g have been observed (Sawaya et al., 1983; Sepùlveda and Sàenz, 1990; Muñoz de Chàvez et al., 1995). Finally, the amount of nitrogen present in prickly pear results on average comparable to that found in other common fruits such as apple (32 mg/100 g), pear (48 mg/100 g), apricot (64 mg/100 g), pineapple (80 mg/100 g), plum (80 mg/100 g), orange (112 mg/100 g), peach (128 mg/100 g), and strawberry (144 mg/100 g) (Belitz and Grosch, 1999; Cappelli and Vannucchi, 1990).

In Table 2 are reported the results obtained for the HPLC determination of ascorbic acid (vitamin C) in prickly pear juice. Interestingly, vitamin C is present in significant amounts in all three cultivars, and values in the range of 31−38 mg/100 g were commonly measured. In particular, the yellow cultivar seems to contain the largest amount of ascorbic acid, averaging 38 mg/100 g. Even considering some variability observed in the measurements, these differences might be considered significative. Therefore, it is possible that the biosynthetic pathway of the ascorbic acid is intrinsically slightly more "efficient" in the yellow cultivar cactus pear. This result is particularly interesting, considering that this cultivar is currently the most attractive from a commercial point of view and itself accounts for ∼90% of prickly pear cultivation in Italy. Moreover, the ascorbic acid content in prickly pear on average turns out to be higher than in most common fruits such as plum (3 mg/100 g), pear (4 mg/100 g), apple (6 mg/100 g), peach (7 mg/100 g), apricot (9 mg/100 g), banana (20 mg/g), and pineapple (25 mg/100 g) and only slightly lower in than other fruits such as orange (50 mg/100 g) or strawberry (60 mg/100 g) (Belitz and Grosch, 1999; Cappelli and Vannucchi, 1990). Finally, HPLC experiments on the samples from the three cultivars after thermal treatment have confirmed, as could be expected, that the ascorbic acid present is easily degraded at high temperatures. In fact, >50% of the vitamin C content was already lost after 2 min at boiling temperature and, in the case of the white sample, was completely destroyed (data not shown). However, it is necessary to recall that our pasteurization treatment is very drastic, and during a large scale industrial processing of the juice, much lower temperatures and/or shorter times would be employed. This would clearly be a great advantage for both the nutritional and organoleptic characteristics of the juice.

Table 3. Determination of Transition Metal Ion Content in the Yellow and White Cultivars of Prickly Pear Fruit

	yellow cultivar		white cultivar	
cation	retention time (min)	amount (ppm)	retention time (min)	amount (ppm)
Mn^{2+}	10.44	1.7 ± 0.3	10.40	2.9 ± 0.3
Zn^{2+}	7.50	0.3 ± 0.1	7.51	0.4 ± 0.2
Fe^{3+}	5.11	0.6 ± 0.2	5.07	1.2 ± 0.3

The transition metal ions have been separated as negatively charged complexes by anionic chromatography on a proper stationary phase (Haddad and Jackson, 1990). Such analysis could not be carried out reliably on the red cultivar because of the high concentration of betacyanin pigments, which gives a deep purple-red color to the pulp of the fruit. These pigments could not be fully removed and caused some interference during the detection of the different metal ions complexed with PAR in the visible region of the spectrum at 500−540 nm. As shown in Table 3, where the retention times and the relative amounts (parts per million) are reported, the metal ions found are mainly Mn^{2+}, Zn^{2+}, and Fe^{3+}. In particular, very high is the level of manganese, which averaged 1.7 and 2.9 ppm for the yellow and white cultivars, respectively. This observation turns out to be particularly important if we consider that manganese-(II), together with other trace elements, is well-known to be an essential nutrient because of the multiple roles it plays in vivo (Fraùsto da Silva and Williams, 1991). In particular, manganese seems to be involved in the biosynthesis of mucopolyliposaccharides and in the metabolism of bone tissues. In fact, from studies on rats where osteoporosis was artificially induced, it seems that manganese deficiencies could have a key role in such pathologies. Moreover, manganese is also an activator of various enzymatic systems such as phosphatases, kinases, or glycosyltransferases and is found as cofactor of several metalloenzymes such as pyruvate-carboxylase and superoxydismutase. Significative amounts of Zn^{2+} and Fe^{3+} of the order of 0.3−0.4 and 0.6−1.2 ppm, respectively, were also found, in particular in the white fruits, which turned out to be the richest in mineral content. Finally, it is worth mentioning that the metal content in prickly pear fruit can be quite variable, most likely depending on the chemical characteristics of the soil. In fact, prickly pear cactus can be cultivated in a whole variety of different soils, such as volcanic (at the base of Mount Etna), sandy, and clayey.

EPR Characterization. EPR experiments were carried out on several samples of *filtered* juice and *unfil-*

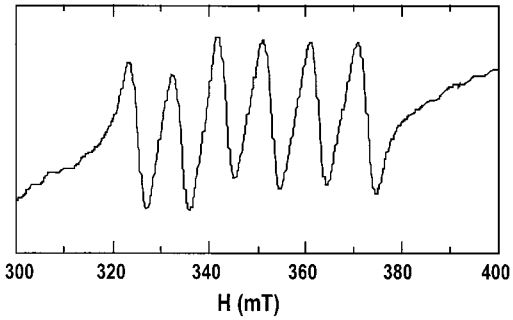

Figure 1. Room temperature EPR spectrum recorded on blended prickly pear pulp and skin of yellow cultivar. Instrumental settings: modulation frequency = 100 kHz; modulation amplitude = 9.73 G; time constant = 328 ms; sweep time = 3 min; receiver gain = 1×10^5; microwave power = 40 mW; microwave frequency = 9.777 GHz.

tered juice, that is, juice containing pulp fibers, as well as on the peel or skin of all three cultivars of prickly pear. Such measurements confirmed the presence of significative amounts of paramagnetic metal ions such as manganese(II) and iron(III). Mn^{2+} is characterized by long spin−lattice relaxation times, and it is therefore possible to observe well-resolved spectra with a characteristic six-line pattern ($2I + 1$) (Figure 1) even at room temperature (Kuska and Rogers, 1971). The magnetic parameters are close to those of the free hexaquaion (as typically observed), but the isotropic hyperfine coupling constants are slightly higher [g_{iso} = 2.000(5); A_{iso} = 0.0092(3) cm^{-1}] (Goodman and Raynor, 1970), suggesting interaction with coordinating ligands. At 150 K a band at g_{eff} = 4.290(5) (Pilbrow, 1990) can be observed, characteristic of high-spin iron (III) complexes with rhombical distortions. Around $g = 2$ also the typical six lines of manganese become evident, but the pattern is complicated by the overlapping of parallel and perpendicular transitions with slightly different values of g and hyperfine coupling constants. At 77 K, together with the other peaks previously described, a new iron(III) signal appears at $g \approx 9$ split in two lines resonating at slightly different magnetic fields [g_{eff} = 9.52(4); g_{eff} = 9.10(5)] (Pilbrow, 1990). However, such signals are not due to the simple free iron(III) aquaion, which is usually characterized by a single broad band.

Therefore, EPR measurements allow us to conclude that Mn^{2+} and Fe^{3+} are not found as free ions but are bound to complex ligands or biological matrices. We hypothesize that such ions could function as cofactors of cell wall proteins or have some structural role in the assembly of cell wall biopolymers. Finally, very intense Mn^{2+} and Fe^{3+} EPR signals were evident in the unfiltered juice as well as in the skin samples, strongly suggesting that these two metal ions are most likely either "trapped" inside pulp fibers or localized at the interface between the edible part of the fruit and the skin.

PME Activity. PME (Castaldo et al., 1989; Giovane et al., 1990; Rillo et al., 1992) is an enzyme bound to the cell wall by electrostatic interactions and is involved both in the growth of the cell wall and in its biodegradation during the ripening of the fruits. Pectin is a polymer made of galacturonic acid units and of its corresponding methyl ester units, bound together through α 1→4 glycosidic linkages. PME acts by producing pectins with a low degree of methylation, which are then enzymatically cleaved by polygalacturonase (PG). Therefore, the synergic effect of these two enzymes plays a major role in the softening of the fruit during ripening.

As a consequence, the control of PME enzymatic activity becomes of primary importance in all biotechnological processes focused on the processing and storage of fruit and vegetable juices (Giovane et al., 1995, 1996). In fact, when the PME, naturally present in the juice, acts to demethylate the pectins, a separation of the pulp from the juice, commonly referred to as "cloud loss", is observed. This sedimentation of the solid phase is due to the formation of free carboxyl groups that react with the calcium(II) ions present in the juice. The resulting calcium pectate is insoluble and precipitates. Obviously, such cloud loss is an undesirable feature of all fruit juice and vegetable products.

PME activity was assayed by following the enzymatic hydrolysis of methyl ester groups of the pectin:

$$\text{pectin}-COOCH_3 + H_2O \rightarrow \text{pectin}-COO^- + H^+ + CH_3OH$$

A continuous spectrophotometric method developed by Hagerman and Austin turns out to be convenient, sensitive, and specific (Hagerman and Austin, 1986; Giovane et al., 1996). PME activity is detected by recording the decrease in absorbance at 620 nm of the bromothymol blue. This dye is particularly appropriate to follow the reaction because it changes color between pH 7.6 (blue) and pH 6.2 (yellow) and the enzyme has its maximum activity between pH 7.0 and 7.2. Moreover, this dye does not have any effect on PME activity (Hagerman and Austin, 1986).

The PME activity measured in the prickly pear juice was ∼1.1 units/g, quite low compared with other fruits, in which it usually ranges between 2.5 units/g (apple) and 224 units/g (tangerine) (Giovane et al., 1990; Rillo et al., 1992; Castaldo et al., 1989). Therefore, this enzyme either must be present in low amount or, alternatively it must be not very efficient in the degradation of the pectin. We have also measured the enzyme activity as function of pH and temperature. As shown in Figure 2A, after only 60 min, the activity decreases by 78% at pH 4 and by 83% at pH 3. As far as thermal stability is concerned, Figure 2B shows that at 90 °C, after only 1 min, the activity decreases by 93.3%. The assay was also carried out on prickly pear samples from the three cultivars at pH 4.5 before and after pasteurization. The results are reported in Table 4 and demonstrate how the thermal treatment contributes to considerably inactivate the enzyme, by ∼80% after 2 min and by ∼90% after 4 min.

These results are particularly interesting if we consider that, for the preparation of a juice for commercial purposes, Italian law requires the pH to be <4.5. Therefore, an industrial pasteurization protocol of a prickly pear juice should require relatively low temperatures and/or short times, which would yield a fruit juice with better nutritional and organoleptic qualities.

Sensory Analysis. A test of preference on purple-red prickly pear juice, in comparison with similar commercial products (pear and peach juices), was carried out with untrained students at the University of Catania, Italy (employed as models of generic consumers or customers) to evaluate the possibility of employing the juice from such a cultivar for further investigation and eventually for the production of a juice for commercial use. The results, elaborated by frequencies,

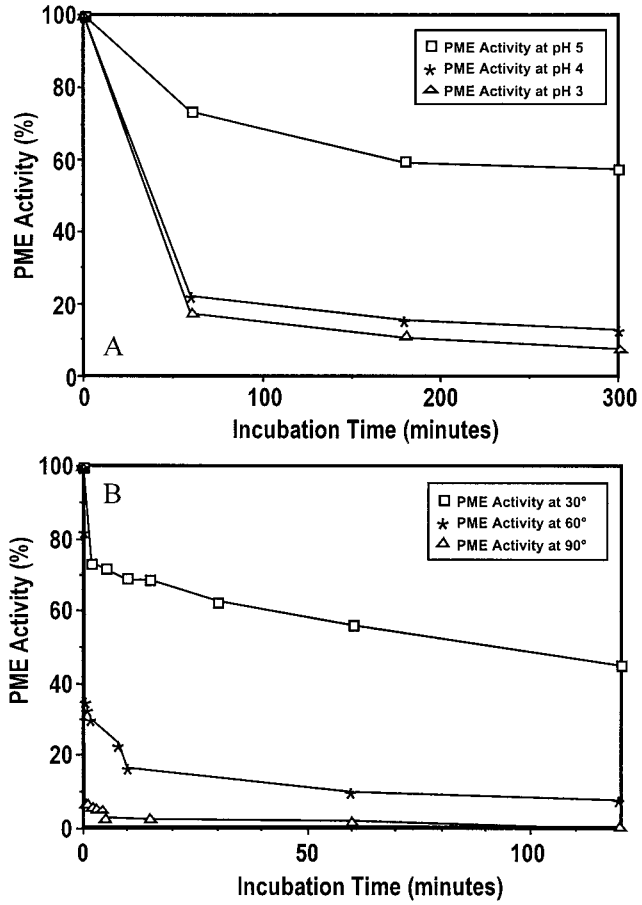

Figure 2. PME activity versus time (A) for different values of the pH and (B) for different values of the temperature.

Table 4. Effect of Thermal Treatment on PME Activity (Units per Gram) in Prickly Pear Juice

	heating time (100 °C)		
cultivar	0 min	2 min	4 min
red	1.07 ± 0.02	0.24 ± 0.03	0.08 ± 0.02
yellow	1.12 ± 0.03	0.11 ± 0.03	0.11 ± 0.02
white	1.02 ± 0.03	0.13 ± 0.01	0.12 ± 0.03

showed that 65.9% of the judges preferred pear and peach juices to red prickly pear juice, 31.7% selected this product as second, and only 2.4% as first. As the judges remarked, the insufficient acceptability of this juice was correlated above all with the much too intense color (which was often mistaken for artificial), the insipid taste, and the presence of some vegetable aroma.

As a consequence, we turned our attention to the yellow cultivar. Moreover, in the perspective of a long-term storage of the juice (Italian regulations require pH <4.5), the acidity was corrected using tartaric and/or phosphoric acid and some sucrose was added (to bring the total sugar content to 15%) in order to restore the original typical taste and flavor, which were partially lost because of the addition of the high amounts of acid necessary for pH correction. A ranking test was then carried out employing a trained panel of 41 students at the College of Agriculture, University of Catania, Italy. This test was performed on the "corrected" juice from yellow prickly pear in comparison with commercially available pear and peach juices. In fact, such products are widely available and consumed in Italy and, therefore, are to be considered acceptable compared to a new product such a prickly pear juice. The descriptors used

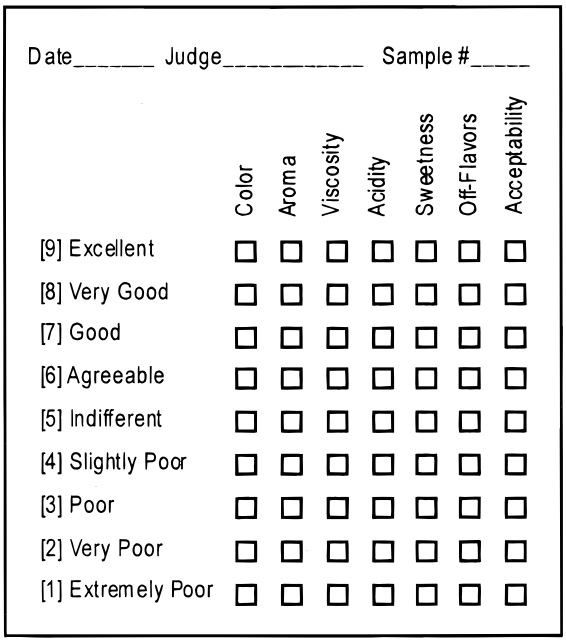

Figure 3. Schematic form used for the ranking test. Assessors were asked to evaluate three "unknown" fruit juice samples and to express their judgment by checking the most appropriate box for each descriptor.

were color, aroma, viscosity, acidity, sweetness, off-flavors, and overall acceptability. As shown in Figure 3, the descriptive terms were accompanied by numerical scores to aid in judging. The scores were rated on a scale from extremely poor (corresponding to 1) to excellent (corresponding to 9) (Lanza et al., 1995, 1999).

One-way analysis of variance was used to evaluate the data scattering around means. Duncan's multiple-range test was used to evaluate the significant differences among mean scores, and the results obtained are reported in Table 5. Because no significant differences in acidity and sweetness between pear and prickly pear were recorded, such juices were located in the same subset for these descriptors. On the other hand, for the other descriptors (color, aroma, viscosity, off-flavors, and acceptability) the prickly pear juice was classified into a separate subset that showed significant differences versus the pear/peach subset with average scores, respectively, of 6.10 (prickly pear), 7.07 (pear), and 7.18 (peach).

Discriminant analysis with cross-validation (CV) was performed (Lanza et al., 1998). CV reduces bias because, at each step, the estimated model has no knowledge of the data point to classify; that is, each case is classified by the functions derived from all cases other than that case. The data point is, in fact, a "future" observation. As reported in Table 6, the level of predictive probability in the differentiation of products showed that 63.4% of prickly pear samples were correctly classified, whereas 36.6% were wrongly classified (9.8% as pear juice and 26.8% as peach juice); 61.0% of pear juice samples were correctly classified, whereas 39.0% were wrongly classified (7.3% as prickly pear and 31.7% as peach). Peach juice was correctly classified in 56.1% of cases versus 43.9% wrongly classified (17.1% as prickly pear and 26.8% as pear). Evidently, the peach juice is characterized by some features similar to the prickly pear juice. In Table 7 is shown the matrix used for the determination of canonical discriminant functions. Variables are ordered by absolute size of correlation within the same

Table 5. Sensory Analysis on Prickly Pear Juice, Pear Juice, and Peach Juice

	color		aroma		viscosity		acidity		sweetness		off-flavors		acceptability	
juice	set 1	set 2	set 1	set 2	set 1	set 2	set 1	set 2	set 1	set 2	set 1	set 2	set 1	set 2
prickly pear	6.66		5.80		6.29		6.56		6.59		4.71		6.15	
pear		7.29		7.85		7.68	6.95		6.92			4.98		7.85
peach		7.54		7.80		7.59	7.02			7.71		5.00		7.59

Table 6. Cross-Validated Classification Results

	predicted group membership (%)		
juice	prickly pear	pear	peach
prickly pear	63.4	9.8	26.8
pear	7.3	61.0	31.7
peach	17.1	26.8	56.1

Table 7. Structure Matrix of Canonical Discriminant Functions

	function	
descriptor	1	2
aroma	0.762[a]	0.440
acceptability	0.557[a]	0.085
viscosity	0.543[a]	0.276
off-flavors	0.314[a]	0.276
sweetness	0.148	0.742[a]
color	0.274	0.438[a]
acidity	0.125	0.143[a]

[a] Largest absolute correlation between each variable and any discriminant function.

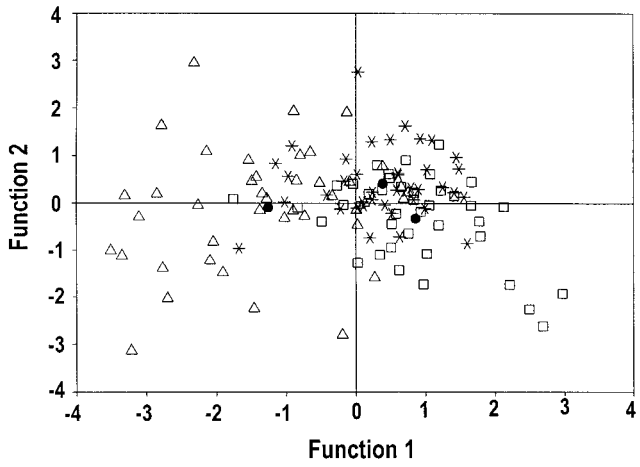

Figure 4. Canonical discriminant functions: (△) prickly pear juice; (□) pear juice; (∗) peach juice; (●) group centroids.

function. The results obtained are shown in Figure 4, where the three samples appear with greater spread along the first function (aroma, viscosity, and acceptability) and less along the second function (sweetness, color, and acidity).

The discriminant analysis based on the sensory data has been very valuable to determine that, although yellow prickly pear juice scored lower with respect to standard juices used for comparison (which are generally considered very good), they can all be positioned in the same group. Therefore, we strongly believe that optimizing processing conditions and by means of various technological treatments it would be possible to produce a prickly pear juice with a score even closer to that of the highly distributed peach and pear juices.

CONCLUSIONS

This work has shown that prickly pear juice, especially from the yellow cultivar, can be a very interesting candidate for the processing of new commercial products. In particular, the main results discussed can be summarized as follows. The acidity of the prickly pear juice is generally very low (0.02%) and, consequently, the pH very high (6.4−6.5) if compared with the values commonly found in other fruit juices. In the perspective of a long-term storage of the prickly pear juice according to Italian law (pH <4.5), the acidity has to be corrected by adding either phosphoric tartaric and/or phosphoric acid. The sugar content (mainly glucose and fructose) is quite high (11−12%). The ascorbic acid (vitamin C) is present in significative amounts (31−38 mg/100 g), which is quite interesting given its antioxidant properties. As in other fruits, the nitrogen content represents only a small percentage. Among the transition metals we have found a high content of manganese(II) and discrete amounts of iron(III) and zinc(II). In particular, EPR experiments suggested that such metals are either trapped inside pulp fibers or present at the interface between the fruit and the skin. The PME enzyme seems to be present in small amounts and/or is barely active. After >2 months, none of the prickly pear samples prepared was affected by significative clarification of the juice.

The eventual variability in the measurements is inevitably due to the different factors that influence the physical and chemical parameters of the fruit such as ripening state, kind of cultivation, climatic conditions, and composition of the soil. Therefore, particular attention needs to be given to the selection of the fruits in an attempt to keep these factors as uniform as possible.

Prickly pear juice, high in energy and rich in vitamins and minerals, could be either consumed as such or employed to prepare new beverages with improved taste and/or nutritional properties. For example, given its sweetness and its low acidic content, as well as its strong flavor and color, it could be blended to “correct” other fruit juices characterized by high acidity and/or lacking in attractive color or intense flavors. The preparation of new alcoholic and nonalcoholic drinks based on the prickly pear could greatly extend the distribution and marketing of this delicious fruit (De Cortàzar and Nobel, 1992; Barbera and Inglese, 1993; Muñoz de Chàvez et al., 1995; Domìnguez-Lòpez, 1995).

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LITERATURE CITED

Amerine, M. A.; Pangborn, R. M.; Roessler, E. B. *Principles of Sensory Evaluation of Food*; Academic Press: New York, 1965.

Barbera, G.; Inglese, P. *La Coltura del Ficodindia*; Edagricole: Bologna, Italy, 1993.
Belitz, H. D.; Grosch, W. *Food Chemistry*; Springer-Verlag: Berlin, Germany, 1999.
Bustos, O. E. Alcoholic Beverage from Chilean *Opuntia Ficus Indica*. *Am. J. Enol. Vitic.* **1981**, *32*, 228−229.
Cappelli, P.; Vannucchi, V. *Chimica degli Alimenti—Conservazione e Trasformazioni*; Zanichelli: Bologna, Italy, 1990.
Castaldo, D.; Quagliuolo, L.; Servillo, L.; Balestrieri, C.; Giovane, A. Isolation and Caracterization of Pectin Methylesterase from Apple Fruit. *J. Food Sci.* **1989**, *54*, 653−655.
De Cortàzar, V. G.; Nobel, P. S. Biomass and Fruit Production for the Prickly Pear Cactus, *Opuntia Ficus Indica*. *J. Am. Soc. Hortic. Sci.* **1992**, *117*, 558−562.
Domìnguez-Lòpez, A. Review: Use of the Fruits and Stems of the Prickly Pear Cactus (*Opuntia* spp.) into Human Food. *Food Sci. Technol. Int.* **1995**, *1*, 65−74.
Forni, E.; Polesello, A.; Montefiori, D.; Maestrelli, A. High-Performance Liquid Chromatographic Analysis of the Pigments of Blood-Red Prickly Pear (*Opuntia Ficus Indica*). *J. Chromatogr.* **1992**, *593*, 177−183.
Frati, A. C.; Jimenez, E.; Ariza, C. R. Hypoglycemic Effect of *Opuntia Ficus Indica* in Non Insulin-Dependent Diabetes Mellitus Patients. *Phytother. Res.* **1990**, *4*, 195−197.
Fraùsto da Silva, J. J. R.; Williams, R. J. P. *The Biological Chemistry of the Elements—The Inorganic Chemistry of Life*; Clarendon Press: Oxford, U.K., 1991.
Giovane, A.; Quagliuolo, L.; Castaldo, D.; Servillo, L.; Balestrieri, C. Pectin Methylesterase from *Actinidia chinensis* Fruits. *Phytochemistry* **1990**, *29*, 2821−2823.
Giovane, A.; Balestrieri, C.; Quagliuolo, D.; Servillo, L. A Glycoprotein Inhibitor of Pectin Methylesterase in Kiwi Fruit. Purification by Affinity Chromatography and Evidence of a Ripening-Related Precursor. *Eur. J. Biochem.* **1995**, *233*, 926−929.
Giovane, A.; Laratta, B.; Loiudice, R.; Quagliuolo, L.; Castaldo, D.; Servillo, L. Determination of Residual Pectin Methylesterase Activity in Food Products. *Biotechnol. Appl. Biochem.* **1996**, *23*, 181−184.
Goodman, B. A.; Raynor, J. B. Electron Spin Resonance of Transition Metal Complexes. In *Advances in Inorganic Chemistry and Radiochemistry*; Emelius, H. J., Sharpe, A. G., Eds.; Academic Press: New York, 1970; Vol. 13, pp 135−362.
Haddad, P. R.; Jackson, P. E. Ion Chromatography Principles and Applications. In *Journal Chromatography Library*; Elsevier Science Publishers: Amsterdam, The Netherlands, 1990; Vol. 46.
Hagerman, A. E.; Austin, P. J. Continuous Spectrophotometric Assay for Plant Pectin Methyl Esterase. *J. Agric. Food Chem.* **1986**, *34*, 440−444.
Hegwood, D. A. Human Health Discoveries with *Opuntia* sp. (Prickly Pear). *HortScience* **1990**, *25*, 1515−1516.
Joubert, E. Processing of the Fruit of Five Prickly Pear Cultivars Grown in South Africa. *Int. J. Food Sci. Technol.* **1993**, *28*, 377−387.
Kuska, H. A.; Rogers, M. T. Electron Spin Resonance of Transition Metal Complex Ions. In *Coordination Chemistry*; Martell, A. E., Ed.; Van Nostrand Reinhold: New York, 1971; Vol. 1, pp 186−263.
Kuti, J. O.; Galloway, C. M. Sugar Composition and Invertase Activity in Prickly Pear Fruit. *J. Food Sci.* **1994**, *59*, 387−388, 393.
Lanza, C. M.; Pagliarini, E.; Tomaselli, F. Sensory and Chemical Evaluation of Frozen Blood-Orange Juice. *Agric. Mediterranea* **1995**, *125*, 421−426.
Lanza, C. M.; Russo, C.; Tomaselli, F. Relationship between Geographical Origin and Fatty Acid Composition of Extra-Virgin Olive Oils Produced in Three Areas of Eastern Sicily. *Ital. J. Food Sci.* **1998**, *10*, 359−366.
Lanza, C. M.; Pagliarini, E.; Lanza, G. Study of the Shelf-Life of Cured *cv* Tarocco Oranges by Sensory and Physico-Chemical Parameters. *J. Sci. Food Agric.* **2000**, *80*, 241−246.
Laratta, B.; Loiudice, R.; Giovane, A.; Quagliuolo, L.; Servillo, L.; Castaldo, D. Thermostability of Three Pectinesterase Isoenzymes in Tomato Fruit. *Food Chem.* **1995a**, *52*, 415−418.
Laratta, B.; Fasanaro, G.; De Sio, F.; Castaldo, D.; Palmieri, A.; Giovane, A.; Servillo, L. Thermal Inactivation of Pectin Methylesterase in Tomato Puree: Implications on Cloud Stability. *Process Biochem.* **1995b**, *30*, 251−259.
Mullin, J. Microwave Processing. In *New Methods of Food Preservation*; Gould, G. W., Ed.; Blackie Academic and Professional: Glasgow, U.K., 1995; pp 112−134.
Muñoz de Chàvez, M.; Chàvez, A.; Valles, V.; Roldan, J. A. The Nopal: A Plant of Mainfold Qualities. In *World Review of Nutrition and Dietetics*; Simopoulos, A. P., Ed.; Karger: Basel, Switzerland, 1995; Vol. 77, pp 109−134.
Odoux, E.; Dominguez-Lopez, A. Le Figuier de Barbarie: une Source Industrialle de Bétalaïnes? *Fruits* **1996**, *51*, 61−78.
Ouelhazi, N. K.; Ghrir, R.; Diep Le, K. H.; Lederer, F. Invertase from *Opuntia Ficus-Indica* Fruits. *Phytochemistry* **1992**, *31*, 59−61.
Palevitch, D.; Earon, G.; Levin, I. Treatment of Benign Prostatic Hypertrophy with *Opuntia Ficus-Indica* (L.) Miller. *J. Herbs, Spices Med. Plants* **1993**, *2*, 45−49.
Piattelli, M.; Minale, L. Pigments of Centrospermae I. Betacyanins from *Phyllocactus Hybridus Hort.* and *Opuntia Ficus-Indica* Mill. *Phytochemistry* **1964**, *3*, 307−311.
Piattelli, M.; Minale, L.; Prota, G. Isolation, Structure and Absolute Configuration of Indicaxanthin. *Tetrahedron* **1964**, *20*, 2325−2329.
Pilbrow, J. R. *Transition Ion Electron Paramagnetic Resonance*; Clarendon Press: Oxford, U.K., 1990.
Retamal, N.; Duràn, J. M.; Fernàndez, J. Ethanol Production by Fermentation of Fruits and Cladodes of Prickly Pear Cactus (*Opuntia Ficus Indica* (L.) Miller). *J. Sci. Food Agric.* **1987**, *40*, 213−218.
Rillo, L.; Castaldo, D.; Giovane, A.; Servillo, L.; Balestrieri, C.; Quagliuolo, L. Purification and Properties of Pectin Methyl Esterase from Mandarin Orange Fruit. *J. Agric. Food Chem.* **1992**, *40*, 591−593.
Sàenz, C. Use of Prickly Pear Fruit. *Intermed. Technol. Food Chain* **1996**, *18*, 10−11.
Sawaya, W. N.; Khatchadourian, H. A.; Safi, W. M.; Al Muhammad, H. M. Chemical Characterization of Prickly Pear Pulp, *Opuntia Ficus Indica*, and the Manufacturing of Prickly Pear Jam. *J. Food Technol.* **1983**, *18*, 183−193.
Sepùlveda, E.; Sàenz, C. Caracteristicas Quimicas y Fisicas de Pulpa de Tuna (*Opuntia Ficus Indica*). *Rev. Agroquim. Tecnol. Aliment.* **1990**, *30*, 551−555.
Tateo, F. *Analisi dei Prodotti Alimentari*; Chiriotti Editori: Pinerolo (TO), Italy, 1978.

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