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# Evolution of Bioactive Components of Prickly Pear Juice (*Opuntia ficus indica*) and Cocktails with Orange juice

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### Abstract

The Prickly Pear cactus (*Opuntia ficus indica*) adapted to the conditions of arid areas where it offers to man and animals its nourishing and therapeutics properties The importance of this crop is related to its diversity of use and ecological role The juice obtained from the fruit pulp is characterised by a high pH 5.8 making its conservation difficult and its preservation requires thermal treatment at over 115 °C which is harmful for the bioactive constituents and color compared to orange juice, processed at temperatures below100 °C. The objective of this study aims to valuing this wealth of bioactive molecules of prickly pear and increase its consumption in the form of cocktails with orange juice. A stabilisation treatment by heat, weakly affects polyphenols rate which decreases from 10.5 % for cocktail of 30 %. The loss is 12 % in the prickly pear juice. However, the vitamin C content decreases from 25 to 29 % following thermal treatment at 85 °C for 30 min for the orange juice and prickly pear juice. During storage the loss of vitamin C is 25 % in the presence of 30 % pear juice; the loss in polyphenol is low. Microbiological quality control showed the effectiveness of pasteurisation at 85 °C. Valorisation in the form of natural cocktail juice is particularly interesting due to the contribution of bioactives compounds. It showed acceptable sensory quality.

Keywords: Opuntia ficus indica, cocktail, polyphenol, antioxidant ability, vitamin C,

### 1. Introduction

The Prickly Pear cactus (*Opuntia ficus indica*) called Nopal in the country of origin; Mexico, is a plant of CAM type (Crassulacean Acid Metabolism) adapted to the conditions of arid and semi-arid areas where it offers to man and animals its nourishing and therapeutics properties [1, 4].

The decrease of water resources and desertification accelerated by climate change can only encourage the countries concerned to engage in the development and operation of *Opuntia ficus indica* as an alternative system for production of fruits and cladodes as fodder and vegetables [5, 6].

The importance of this culture is related to its diversity of use and ecological role: protection of soil against erosion, and mainly fruit production [7, 8]. Modern medical research rediscovers the properties of this plant and its virtues. Indeed, the active molecules that compose it and act in interaction and synergy [9],

enabling it to fight effectively against some diseases [10, 11]. They prevent diseases as: ulcer, glycemia, cholesterol, allergies etc., and obesity [12, 13a, 13b, 14, 15]. Stem extract protects the liver and decreases the hepatoxicity induced by orga nophosphorus pesticide (chlorpyrifos) [16]. Other authors reported on the analgesic and anti-inflammatory effects of fruits and cladodes [17]. Their high content of pectin and dietary fibers facilitates intestinal transitand, and reduction of glycemia [18, 19].

Young cladodes also called «nopalitos» are generally consumed as fresh green vegetables, cooked, or as powder in Mexico and in the south of the United States [20-22] and used as a source of food additives [23-24].

Fruits are traditionally consumed fresh, but are also processed to various products (manufacture of jam, fermented drinks with low alcohol content, colonche) and juice extraction, clarification and concentration or reduction powders [25-28]. They are the subject of great



interest in recent years because of their food, medicinal and antioxidant properties [29].

These fruits are rich in vitamin C (31-38 mg/100g), betalains pigments, phenolic compounds, reducing sugars and minerals [30, 11, 31]. They are also characterised by low acidity and high soluble solids content features that make them susceptible to alteration and microbial growth [26]. Nutraceuticals fruit benefits arise from their antioxidant properties related to ascorbic acid, betalains including indicaxanthin and betaxanthin and their phenolic compounds including flavonoids [32]. Betalain pigments revealed recently as antioxidant possess a potential for capturing free radicals higher than vitamin C [5]. The prickly pears are a source of wealth of betalains. Their content is of 100 mg (indicaxanthin + betaxanthin) per kilogram of pulp [33]. Many researches have demonstrated the beneficial effects of phenolic compounds and betalain. The interaction of these molecules with the lipids in considered to be the basis of their beneficial activity [34,35]. Our research aims to valuing this wealth of bioactive molecules of prickly pear and increase its consumption by integration in the form of cocktails with orange juice. The behaviour of compounds was studied after evaluation of the new cocktail drink and its behavior under thermal treatment and storage and their sensory characteristics

### 2. Materials and Methods

### 2.1. Preparation of materials

The raw material used was prickly pear fruit (*Opuntia ficus-indica* inermis variety); collected at the end of maturity in the sub-coastal region of Blida at 400 m of altitude. Fruits were collected in September 2011 in order to have a balanced mixture of yellow and red-orange fruit. The juice is extracted from the pulp using a Robotic semi-professional brand juicer. It is kept at - 8 °C for 5 months prior to use.

The orange juice used in the cocktail com]es from the production line of the industrial unit "Vita Jus". This juice is composed of: 72.09 %, water, 27.72 % syrup (consisting of concentrated orange, pulp, sucrose, and water) and guar gum 0.04 % citric Acid 0.15 %.

Preparation of cocktail orange juice / pear juice was made to achieve pear juice proportions of 10 %, 20 % and 30 %(v/v) and a lower pH to 4.2 after addition

2.2. Chemical analyses and sensory tests

The dry matter (DM) content was determined after drying at 105 °C for 24 h and the mineral content by incineration in an oven at 550 °C [36].

The acidity is measured according to the standard method [36]. The results are expressed as percentage of citric acid.

Determination of total polyphenols was performed by the Folin-Ciocalteu reagent at 760 nm (according to Piga *et al.*,) [37]. The results are expressed in mg of gallic acid to 1 l of juice with respect to a reference range of gallic acid concentration. The carotenoid content was determined according to the method of Lichtenthaler [38] and the results were expressed in mg/l

Total pigments of raw juices studied were extracted with the mixture methanol - water: 80/20 (v/v). Following centrifugation, the supernatant containing fraction betalain is evaluated by spectrophotometry according to Beer-Lambert law at 484 and 535 nm. The molar extinction coefficients are  $6.5 \ 10^4 L \ mol^{-1} cm^{-1}$  for betanine (betaxanthine) and  $4.8 \ .10^4 L \ mol^{-1} cm^{-1}$  for indicaxanthin, according to Schwartz and Von Elbe [39, 40]

Content of ascorbic acid (vitamin C) was determined by the volumetric method according to AOAC (1990) by DCPIP (2, 6-dichlorophenol). The results are expressed in mg of ascorbic acid for 100 ml. Total sugars were measured according to the phenol sulphuric acid method Dubois, [41].

The determination of reducing sugars is carried out by the DNSA (dinitrosalicylic acid) method. To 1 g of sample is added to 50 ml of distilled water after homogenisation for 30 minutes and centrifugation at 200 rpm for 10 minutes. After filtration and preparation of dilutions  $10^{-1}$  to  $10^{-3}$ , 1 ml sample was introduced into a test tube, and then 2 ml of DNSA. After stirring and heating in a water bath shake for 7 minutes, we cold and addition 20 ml of distilled water; optical density is read at 540 nm against a reference blank. The results are expressed with respect to a glucose standard solution in g/100 g DM.

The antioxidant activity was determined according to the method described by Leitao *et al.* [42], 1 ml of DPPH (0.001 g of DPPH in 25 ml of Methanol) is added to 1.5 ml sample introduced into 1.5 ml of methanol. Control (blanc) is prepared under the same conditions by adding the DPPH (1.5 ml) in 2.5 ml methanol. After incubation for 30 minutes in the dark, Absorbance is read at 518 nm. The antioxidant activity (AA in %) of the sample is determined by: AA % =  $\frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$ 

A spectrophotometer of SHIMADZU.UV mini 1240 brand was used for all assays.

During thermal treatment, the processing time was 10, 20 and 30 min. at 85 °C it was choice to control pasteurization and its impact on bioactive constituents

Microbiological control was performed according to the AFNOR (Association Française De Normalisation) [43]. It concerned samples at 30 % juice prickly pear after thermal treatment of 10 min. duration.

The heat treatments to evaluate the evolution during storage of the bioactive constituents were carried out at a temperature of 85 °C during 10 to 30 min. for the different beverage.

Sensory tests: to assess the acceptability of the product and preference according to the dose of juice added, Sensory tests was performed by a jury of 25 people of different ages and sex and cultural level. The criteria studied were based on taste, appearance, acidity and smell

All the samples were analysed in triplicate and expressed as means  $\pm$  standard deviation (SD). The

Table 1:

Chemical and biochemical composition of fresh prickly pear juice and orange juice

Statistical analyses of data were performed with the STATICA 8 by PowerPoint software. The level of confidence required for significance was selected at  $P \le 0.05$ 

### 3. Results and Discussion:

The yield of juice from *Opuntia ficus indica* pulp was 59.63 %.

3.1. Composition of orange and prickly pear juice

The composition (Table 1) shows that the extract of prickly pear fruits is characterised by a pH of 5.8 which is comparable to fruit of Moroccan origin with 5.9, while Gurrieri *et al.* [30] found higher values of 6.4 - 6.5. The *Opuntia ficus-indica* juice is therefore characterised by a high pH and acidity about 0.06 % compared to other fruit juices such as orange, lemon and pineapple. These results are similar in Morocco, [31,44] with acidity of 0.02 %. This acidity and pH facilitate the alteration of juice and makes thermal treatment difficult, requiring temperature above 100°C which affects the color and components of the juice.

Parameters	Units	prickly pear juice	Reconstituted orange juice	
Dry weight	%	$11.2 \pm 0.1$	$10.27\pm0.8$	
Water	%	$88.8\pm0.1$	$89.73\pm0,08$	
pH	-	$5.85\pm0.01$	$3.8\pm0.01$	
Titratable Acidity	% citric acid	$0.06\pm0,\!006$	$0.8\pm0,02$	
Mineral matter	% DM	$7.04\pm0,\!41$	$5.06\pm0,\!07$	
Reducing sugars	mg/100g	5.08 ±0.08,	6.98 ±1.02	
carotenoids	mg/100 ml	$2.49\pm0.95$	$0.6\pm0.05$	
Total polyphenols	mg/100 ml	$56.7 \pm 1.83$	$29\pm1.69$	
Vitamin C	mg/100 ml	$37.7\pm2.75$	$50.7 \pm 1.45$	
Betalains	mg/1000 ml	$18.9 \pm 0,3$		
Antioxidant activity	%	$33.2 \pm 1.45$	$51.5 \pm 0.05$	

The content of minerals in the *Opuntia ficus indica juice* with 7.04 % of DM, is higher than that of the orange juice with 5, 06. Among these minerals according to Sawaya [45], calcium is the predominant followed by potassium (161 mg/100 g). The fruit of *Opuntia ficus-indica juice* is very high in sugars. The majority of which is represented by the reducing sugars with a content of 5.08 mg/100 ml. Same results were obtained by Kutti and Galloway [46]; Rodriguez *et al.* [47]. The prickly pear

juice is relatively rich in carotenoids with 2.49 mg/100 ml, while the orange juice it is 9mg that of carrot juice 11.78 mg/100 ml [5, 35, 48].

These results show that the juice of *Opuntia ficus-indica* is a major source of polyphenols (56.7 mg/100 ml), which is confirmed by other authors [49]. This level remains higher than that of the orange juice with 29 mg. [48], it can reach 37 mg/100 ml. The high content of ascorbic acid equivalent (vitamin C) with

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37,7 mg/100 ml prickly pear juice is comparable to those varieties of Sicilian and Moroccan of origin with content between 31 and 38 mg / 100 ml according (Gurrieri *et al.* [30], Maataoui [44]. That of orange juice is higher with more than 50 mg/100 ml (Park *et al..,*) [50] while the betalains of the prickly pear juice content is low compared with the results given by Khatabi [33].

Determination of the antioxidant activity of the extract of *Opuntia ficus-indica* fruit shows that the latter has an antioxidant activity (AA %) estimated at 33.19 % in DPPH equivalent, but reached 51.5 % in orange juice. This value is close to the Moroccan and Italian (Sicilia) varieties [39].

The wealth of both juices in bioactive compounds grants them a notable scavenging activity of free radicals, this activity is greater in the orange juice compared to that of prickly pear juice (33.19 %). This weak activity may be related to a long storage and effect on shelf-life of the fruits

The parameters: acidity and polyphenols, carotenoids and antioxidant power and the betalains add to fiber quality, indicates an interesting complementarity between the two juices, and therefore the possibility of exploitation for the formulation of a new rich and functional cocktail.

3.2. Evolution of bioactive compounds of prickly pear juice (Pp) and orange juice.

The heat treatment at 85 °C showed a significant evolution on bioactive constituents (Table 02).

Table 2:

Evolution of bioactive compounds of prickly pear juice and orange juice, during thermal treatment

	Polyphenols (mg/100 ml)		Vitamin C (mg/100 ml)		Carotenoids (mg/100 ml)	
Duration (min)	Prickly pear juice	Orange juice	Prickly Pear juice	Orange juice	Prickly pear juice	Orange juice
$T_0\!=\!0$	$56.7 \pm 1.83^{a}$	$29.03 \pm \! 1.69^{a}$	$37.5\pm2.75$ a	50.63±1.55 <sup>a</sup>	$2.53\pm0.10^{a}$	$0.6\pm0.05^{\ a}$
T <sub>1</sub> =10	55.10±1.70ª	27.32±1.17 <sup>ab</sup>	3575±0.95 ª	$47.8 \pm 1.33^{b}$	$2.41\pm0.16^{a}$	$0.57{\pm}0.02^{a}$
T <sub>2</sub> =20	53.29±1.14 <sup>ab</sup>	$24.42{\pm}\ 2.02^{ab}$	$33.8\pm3.18^{a}$	$46.08 {\pm}~ 0.90^{\text{bc}}$	$2.35\pm0.18^{a}$	$0.55{\pm}0.06^{a}$
T <sub>3</sub> =30	50,69±1.06 <sup>b</sup>	$25.06{\pm}0.86^{b}$	$28.6\pm2.10^{b}$	$44.85{\pm}0.83^{c}$	$2.1 \pm 0.18^{a}$	$0.51{\pm}0.07^{a}$
Probability	0.006	0.069	0.009	0.002	0.074	0.273

Polyphenols of orange juice are more sensitive to heat than those of prickly pear juice with a loss of up to 14 % for orange juice and 10 % prickly pear juice treated for 30 min. While vitamin C in prickly pear juice is more sensitive to heat treatment with a significant decrease (24 %) compared to that of orange juice which as a less important loss of 12 %. a difference that is explained by the effect of pH.

About carotenoids, heat treatment, show similarities in losses of the two juices: 14 % for the prickly pear juice and 16 % for orange juice.

3.3. Composition et evolution of components during thermal treatment

The composition of cocktails before treatment is reported in the table below (Table3).

Comparison of different values shows that in addition to fiber intake, betalains and minerals reported by different authors [10], cocktails at 30 % show that an optimum contribution is achieved for bioactive molecules; polyphenols, carotenoids, and a relative decrease in vitamin C, to this is added the contribution of betalains.

Juice pH is the contributing factor to the stability and preservation of constituents of juice and remains at an acceptable level 4, 12 at 30 % prickly pear juice addition and it can use a lower temperature than 100°C during sterilization. The antioxidant activity remains significant despite a low decrease in 7 %. This antioxidant capacity is due to polyphenols and betalains and the synergistic effect (table3)

Cocktail parameters	Orange juice	Cocktail at 10 %	Cocktail at 20 %	Cocktail at 30 %	Probability	Evolution (%)
polyphenols (mg/100 ml)	$29.33 \pm 0.66$	$31.77{\pm}0.32$	$35.59{\pm}0.95$	$39.1\pm0.11$	0.004	+ 24
Vitamin C (mg/100 ml)	$50.71 \pm 1.45$	49.50± 0,62	44.50± 0.8	39.76± 1.2	0,000	- 21
Carotenoids (mg/100 ml)	$0.6\pm0.05$	0.85± 0,08	$1.22\pm0.08$	$1.78 \pm 0.06$	0.000	+ 63
рН	3.73±0.03	3.83±0,03	3.94±0,04	4.11±0,05	0.001	+10
Antioxidant activity (% de DPPH)	$52.60 \pm 1.78$	$47.16\pm0.04$	$44.05{\pm}1.02$	$49.05{\pm}0{,}57$	0.000	-7

Table 3
Biochemical composition of cocktails: orange - prickly pear juice and antioxidant activity.

### 3.3.1. Evolution of the vitamin C content.

Following thermal treatment of different cocktails at 85 °C; a significate decrease in content of vitamin C is observed (p= 0.010) (fig.1). Demonstrating the fragility of this vitamin, but it is higher with 25 to 22 % for cocktail juice at 20 and 30 % prickly pear juice compared to 10 % and orange juice, but the loss is less significant for the prickly pear juice, this can be justified by prominence of polyphenols, carotenoids and by mucilage that protect this vitamin [37].

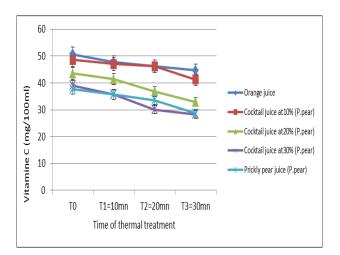


Figure 1: Evolution of the vitamin C content of different cocktails during heat treatment

### 3.3.1.1. Evolution of the polyphenol content

A relatively vigorous thermal treatment of the 3 cocktails and orange juice and prickly pear affect less significantly the polyphenol levels (p = 0.408), their decrease does not exceed 10 % for cocktail 30 %, but

achieves 18 % for orange juice alone. The loss is 12 % in prickly pear juice. Therefore, the two cocktails (20 %, 30 %) and prickly pear juice exhibit better resistance to thermal treatment. This resistance can be explained by the effect of pH and greater fragility of other antioxidants which confirms advanced hypothesis by Piga *et al.* [29, 37].

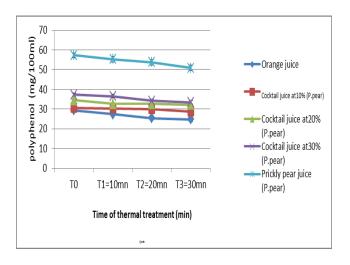


Figure 2: Evolution of content in polyphenol of different cocktails during thermal treatment

### 3.3.1.2. Evolution of the antioxidant activity of cocktails during thermal treatment.

The antioxidant activity decreases during thermal treatment (p = 0.010) which is observed with others fruits even after minimal treatment according Piga *et al.* [29].

The cocktail of prickly pear juice with 30 % has a high antioxidant activity compared to that of 10 % and 20 %, this is due to the presence of polyphenols,

carotenoids, betalains, the latter have a potential to capture free radicals higher than that of vitamin C [51]. Polyphenols and betalain pigments respond property's antioxidants benefic for the human health [cited in Khatabi (33)] The orange juice appears more stable than the 10 and 20 % of prickly pear juice cocktails, but at 30 %, the cocktail shows more stability because of the polyphenol richness This cocktail has a low variation indicating greater stability of this cocktail to heat (loss of 16 %) compared with cocktails (10 %, 20 %) where the loss is between 23 and 29 %, the loss in the orange juice is 18 %. The antioxidant activity of orange juice decreases rapidly after 10 min. of heat treatment due of the fragility of vitamin C.

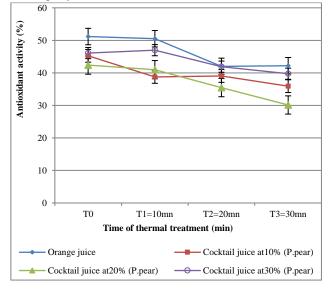


Figure 3: Evolution of the antioxidant activity of various cocktails during heat treatment

The behaviour observed following addition of 30 % prickly pear juice which is richer in polyphenols, betalains and mucilage can be explained by the synergies and interactions between molecules and the fact that polyphenols have anti-radical activity of 1.2 times higher than that of ascorbic acid; whereas pigments (betalains) have an activity of 1.5 times. [39] justify the anti-radical ability by the synergistic effect between the constituents of the juice. This may explain the changes observed in the case of cocktails.

## 3.3.1.3. Evolution of bioactive components during storage at 4 °C

### a) Vitamin C (ascorbic acid)

The results obtained show that the storage of cocktail and prickly pear juice at a temperature of 4 °C for 4 weeks affects their contents in vitamin C. (figure 4). Loss of the ascorbic acid from cocktail at 30 % of prickly pear juice is 25 % after 4 weeks of storage, but it is 40 and 38 % for cocktails at 10 and 20 % respectively. It seems that the prickly pear juice improves the stability of vitamin C during storage of the cocktail. The stabilizing or protective effect can be related to polyphenols and other constituents of the prickly pear juice. From the third week, a relative decrease of this dynamic is observed.

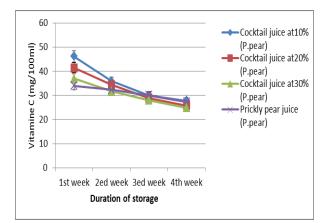


Figure 4: Evolution of the vitamin C content of different cocktails during storage at  $4^{\circ}C$ 

### b) Polyphenols

Conservation of cocktails for one month has led in a decrease of the polyphenols stability rate after the first week, especially for cocktails with proportions 10 and 20 % of prickly pear juice, this decease has reached 44 % and 45.13 % respectively. The loss was lower in the case of the juice at 30 %. It is 35 % (Figure 5). This is confirmed by Macheix *et al.* [52] who reported that the concentration of polyphenols decreases regularly during maturation and storage which was also observed by Piga *et al.* [37] after 6 days of storage under a foil and minimal treatment. This decrease in vitamin C and polyphenol content explains that the drop of the antioxidant activity.

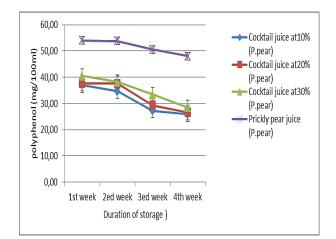


Figure 5: Evolution of content in polyphenol of different cocktails during storage

### 3.3.2. Quality assessment

### 3.3.2.1. Sensory quality

Sensory test showed that the taste was preferred by over 65 % for cocktails at 20 and 30 %, it was also noted that the colour was less appreciated by only 40 % of participants, while the smell was more appreciated for cocktails at 20 %, but the acidity was widely preferred for cocktails of 20 and 30 %. This degree of appreciation for a new drink is encouraging and may increase in view of the interest which is carried by the consumer for the prickly pear.

### 3.3.2.2. Microbiological quality

Microbiological analyses are performed on the cocktail juice (30 %) to verify the effectiveness of thermal treatment on the hygienic quality. after 10 min. of thermal treatment at 85 °C. It was observed Absence of contaminating germs (yeasts, mould, Total coliforms, total streptococci anaerobic sulphite reducers on the prickly pear juice as, this reveals the effectiveness of pasteurization at a pH below 4.12.

### 4. Conclusion

The results of our work on the composition of the juice of *Opuntia ficus indica* show a wealth in polyphenols. This juice differs from other juices by a high pH (5.85) and by a wealth in soluble sugars (especially reducing sugars), bioactive compounds dominated by: polyphenols (56.7 mg/100 ml), ascorbic acid, carotenoids and betalains.

These different compounds are responsible for the antioxidant ability. The importance of the antioxidant ability also indicates their medicinal and nutritional importance as reported by some authors [10].

An orange juice cocktail–prickly pear (about 30 %) with no additive, present acidity (pH 4.1) and an interesting composition in bioactive molecules wealth, while maintaining adequate consumer acceptability

The evolution of bioactive compounds of the *Opuntia ficus-indica* and cocktail made with the orange juice during thermal treatment at 85 °C and storage, shows that the cocktail has a better stability of the levels of vitamin C (ascorbic acid).

The results of the determination of antioxidant activity of the three cocktails (10; 20 and 30 % prickly pear juice) show that antioxidant activity, increases with the increase of prickly pear juice in cocktail.

The results of the sensory analyses of cocktails reveal acceptance of cocktails including 30 % of prickly by 65 % of subjects, but this can evolve over time. A limitation of the pH below 4.5 is necessary; this parameter is highly regarded among criteria of quality and is involved in the microbiological stability. It also explains the ease and efficiency of thermal treatment and a lower loss of polyphenols after 30 min. at 85°C

The quality of the cocktail which can be prepared from orange and fruit of *Opuntia ficus indica* (30% prickly pear juice) may constitute a possibility of revalorization at a low cost of two fruits present in the Mediterranean basin despite even if there is a loss of vitamin C which is compensated by betalains and polyphenols more diversified

Those of prickly pear juice rich in Isorhamnetin, derivatives are the dominant flavonol glycoside [53]. And in that of the orange juice, the major citrus flavanones are naringin and hesperidin [54].

As prospective, it is interesting to study the possibilities of enzymatic clarifications of juice to reduce the turbidity and for see the effect of use of clarified juice by microfiltration on the characteristics and acceptability. pH correction up to 3.8 - 3.9 can be considered and a level of prickly pear juice to 35 %. If this threshold is exceeded the color may decrease the acceptability.

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