

Colour Stability of Wild Cactus Pear Juice

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Abstract—Prickly pear (*Opuntia spp*) fruit has received renewed interest since it contains a betalain pigment that has an attractive purple colour for the production of juice. Prickly pear juice was prepared by homogenizing the fruit and treating the pulp with 48 g of pectinase from *Aspergillus niger*. Titratable acidity was determined by diluting 10 ml prickly pear juice with 90 ml deionized water and titrating to pH 8.2 with 0.1 N NaOH. Brix was measured using a refractometer and ascorbic acid content assayed spectrophotometrically. Colour variation was determined colorimetrically (Hunter L.a.b.). Hunter L.a.b. analysis showed that the red purple colour of prickly pear juice had been affected by juice treatments. This was indicated by low light values of colour difference meter (CDML*), hue, CDMa* and CDMb* values. It was observed that non-treated prickly pear juice had a high (colour difference meter of light) CDML* of 3.9 compared to juice treatments (range 3.29 to 2.14). The CDML* significantly ($p < 0.05$) decreased as the juice was preserved. Spectrophotometric colour analysis showed that browning was low in all treated prickly juice samples as indicated by high values at 540 nm and low values at 476 nm (browning index). The brightness of prickly pear had been affected by acidification compared to other juice treatments. This study presents evidence that processing has a positive effect on the colour quality attribute that offers a clear advantage for the production of red-purple prickly pear juice.

Keywords—Colour, Hunter L.a.b, Prickly pear juice, processing, physicochemical.

I. INTRODUCTION

Cactus pear (*Opuntia spp*), commonly known as prickly pear, is a wild fruit that grows under arid conditions [1].

Prickly pear fruit contains betalain pigments, the natural colourants that give it an attractive purple colour which has good potential in juice production. The fruit also contains red-violet betacyanins and yellow betaxanthins [2]-[5]. One of the most frequently utilized fruit technologies is juice production. Juices are much appreciated for their nutritive value, sensory properties and modern technologies and Good Manufacturing Practice (GMP) allow the production of juice colour that closely approximates the raw fruit from which they are derived [1]. Colour is one of the most important quality parameters of juice products that strictly relates to consumer perception [6]. Consumers demand high quality products and

greatly appreciate the fresh appearance of minimally processed food [7]-[8]. Colour is critically appraised by consumers and often forms the basis for their selection or rejection of juice [9]. Colour is basically specified by the geometry and spectral distributions of three elements: the light source, the reflectivity of the sample and the visual sensitivity of observer. Each of these elements was defined by the Commission Internationale de l'Eclairage (CIE). The definition was aimed at stimulating the human colour perception based on a 2° field of view, a set of primaries (red, green, blue), and colour-matching function [10].

Colour is considered a fundamental physical property of foods products since it has been widely demonstrated that it correlates well with other physical, chemical and sensorial indicators of product quality. In fact, colour plays a major role in the assessment of external quality in food industries, and is used for process design and control [11]-[12] and food engineering research [6], [13]. Juice colour is affected by natural enzymes, oxidation of ascorbic acid and the Maillard reaction, which depends on the content of reducing sugars, proteins, and temperature [14]. Food colour can be assessed by colorimeters and spectrophotometers which usually provide readings in XYZ, RGB and L*a*b* color space [15]. XYZ is a device independent colour system created by CIE in 1931. CIE XYZ is based on direct measurements of the human eye, and serves as the basis from which many other colour spaces are defined [10], [15]. RGB is a universally accepted colour system for colour representation in television and video sets, CRT displays and many capture devices using red, green and blue primaries. It is not a perceptually uniform colour space, i.e., differences between colours in the 3D RGB space do not correspond to colour differences as perceived by humans. [15] described L*a*b*, the international standard for colour measurement developed by CIE in 1976, as a perceptually uniform and device-independent colour space providing consistent colour regardless of the input or output device (i.e., camera, scanner, monitor, and printer). Tristimulus colorimeters apply the principle of using filters in combination with a light source and a detector to spectrally emulate the standard observer functions of the eye, given direct evaluations of X, Y, and Z. Spectrophotometers give wavelength-by-wavelength analyses of the light reflecting or light transmitting properties of objects throughout the visible range. The areas under the resulting curves can be converted into X, Y, and Z [10], [15]. Processing and storage of food products have promoted several investigations into nutritional and colour degradation caused by processing variables, including light or oxygen exposure, enzyme treatment, sugar adjustment, acidification, heat treatment, processing time, and low

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temperature storage [16], [17] The current study determined the effects of processing variables such as enzyme treatment, sugar adjustment, acidification, heat treatment, low temperature storage, and freezing and thawing on prickly pear juice colour quality.

II. MATERIALS AND METHODS

2.1 Prickly Pear Processing

Algeria prickly pear fruit is not readily available on the market, but was found in abundance at Matoks, a village in the Limpopo Province of South Africa. The researchers harvested mature ripe fruits in Matoks village. Prickly pear fruits were dethorned by removing the glochids, sweeping them on grass and rinsing with cool tap water before packaging in plastic bags for transportation to the Food Science laboratory. The fruits were carefully selected and sorted using criteria of homogeneity in terms of red purple colour, maturity and ripeness. Fruits that were low in quality (defective, damaged and had an intense dark purple colour which was an indication of over ripeness) were removed. Cleaning of the fruit involved dethorning for a second time under running tap water followed by a cold water rinse to reduce the field heat, and rubbing the fruit surface with a tablecloth to remove the hair thorns. The prickly pear was stored in a cold room (7°C) for a maximum of two days before juice extraction.

2.2 Juice Making Process

The juice processing method was adopted from a previously published protocol [18]. Juice extraction was performed two days after the prickly pear was crushed using a blender set to a speed of 500 rpm to produce prickly pear pulp from which samples were taken for laboratory analyses. To another 1200 g sample of prickly pear pulp was added 48 g pectinase from *Aspergillus niger* and the mixture incubated for 1 hour in a water bath at 50°C in order to increase the juice yield, reduce the processing time, improve the extraction of some components (aroma, colour) and to obtain the partial or total liquefaction of plant tissues. Prickly pear pulp was diluted with water to increase the liquefaction of the pulp that would facilitate passing through 80- to 10-micron sieves. The final juice was collected in the receiver container and transferred to packets. Prickly pear juice samples of 100 ml were analyzed for soluble sugar, packaged in 250 ml sterilized bottles and kept in a cold room for further analysis. Brix-adjustment was done by adding refined white sugar to prickly pear juice until 17°Brix. Adjusted prickly pear juice samples of 100 ml were analyzed for pH and filled in 250 ml Schott bottles which were then sterilized by submerging in a boiling water bath. After equilibrating to room temperature, the bottles were tightly closed and kept in a cold room (7°C) for one day for other analyses. Acidification was achieved by adjusting the prickly pear juice pH from 3.8 to 3.4 with citric acid. Acidified prickly pear juice samples were analyzed for sugar content and filled in 250 ml sterilized bottles that were tightly closed and

kept in a cold room. The remaining prickly pear juice was transferred to 500 ml sterile bottles and tightly closed to prevent oxidation of the samples. Heat-treatment was achieved by submerging the 500 ml samples of prickly pear juice in a water bath at 72°C for 10 minutes. Aliquots (125 ml) of the prickly pear juice were transferred to three 250 ml sterilized bottles. The separate aliquots were kept in a cold room (7°C), a refrigerator (4°C) and a freezer (-5°C), respectively. Thawing of prickly pear juice was achieved by using a refrigerator thawing cycle method 24 hours before the analyses. Prickly pear juice samples stored at different temperatures were used in the analyses.

2.3 Physicochemical Analysis

2.3.1 Titratable Acidity and pH Determination

The titratable acidity expressed to citric acid was assessed by standard procedures. In brief, the titratable acidity was expressed, as milliliters of 0.5 M NaOH required to raise the pH of 100 g of substance to pH 6.3. The pH meter was standardized and calibrated with pH 7.00 and 4.00 standard solutions. The pH of 50 ml prickly pear juice contained in a 250 ml beaker was determined as follows. Hand burettes (25 ml) were checked (particularly the operation of the tap) and filled with 0.5 M NaOH. The pH was measured using a glass electrode connected to a standard pH-Meter PHM82 (Radiometer, Copenhagen, Denmark). The test sample was heated to the requisite sample temperature at which the pH meter was standardized. Samples were accurately weighed into a 100 ml beaker with the aid of the 10-ml pipette. The rinsed electrode was inserted into the sample and the pH recorded to the nearest 0.01 pH unit while stirring. NaOH was added in aliquots of about 1.5 ml until pH 5.6 was reached, and then in smaller increments of about 0.15 ml until pH 6.3 was attained. The volume (ml) was recorded to the nearest 0.1 ml of 0.5 M NaOH required to raise the pH of the sample to 6.3.

2.3.2 Determination of Soluble Solids

Five drops of prickly pear juice sample was placed on a refractometer plate and covered with a plate and the light turned on. The refractometer viewer interface was adjusted so that it lined up with the X-shape on the viewer screen. Readings for soluble solids values were then recorded.

2.3.3 Determination of Ascorbic Acid

The amount of ascorbic acid present in prickly pear juice was measured by spectrophotometry [19] It uses the principle that L-ascorbic acid reduces the tetrazolium salt MTT [3-(4, 5-dimethylthiazolyl)-2, 5-diphenyl tetrazolium bromide] in the presence of the electron carrier PMS (5-methylphenazium methyl sulfate) to a formazan product, which was determined by its absorbance at 578 nm. This gives the total reducing substance in the sample. The analysis was carried out directly on prickly pear juice. The above reaction was measured in a stopped UV grade cuvette of 10 mm path length. Solution was

conveniently dispensed using an automatic pipette. MTT in citric acid buffer pH 3 (1 ml) was mixed by inversion with 1.5 ml distilled water and 0.1 ml of sample and the absorbance (A1) read after 6 min. After reading the samples at 6 min, 0.10 ml PMS solution was added and mixed and allowed to stand for 20 min at ambient temperature (recommended range 20-25°C) and the absorbance (A2) read. The absorbance difference was calculated as A2-A1.

2.3.4 Colour Evaluation

2.3.4.1 Colorimetric Colour Analysis

Approximately 20 ml of each sample was dispensed into separate petri dishes. These samples were then analyzed for colour variation. Hunter a, b and L parameters of prickly pear juice were determined with a colorimeter. The net color difference (ΔE) was calculated from the A, B and L parameters, using the equation:

$$\Delta E = \sqrt{\Delta a^2 + \Delta b^2 + \Delta L^2} \quad (1)$$

Where $\Delta a = a - a_0$, $\Delta b = b - b_0$ and $\Delta L = L - L_0$; subscript '0' indicates initial colour. Colour was determined three times on triplicate prickly pear samples. The hue angle difference (ΔH) was also calculated as described by [23]:

$$\Delta H = 2\sqrt{C_0 C_1} \sin \left[\frac{\Delta h}{2} \right] \quad (2)$$

with

$$h = \arctan \frac{b}{a} \quad (3)$$

$$\Delta h = h_0 - h_1 \quad (4)$$

$$C = \sqrt{a^2 + b^2} \quad (5)$$

where subscript '0' indicates initial colour and subscript '1' colour at a selected time during processing.

2.3.4.1 Spectrophotometric Colour Analysis

Samples of prickly pear juice were shaken vigorously to resuspend the pulp particles and immediately placed into transparent plastic cuvettes (475 mm×350 mm×10 mm) to take the colour readings in a dim ambient illumination to avoid possible interferences from other external light sources. Furthermore, the cuvettes containing the samples were placed inside a box lined with homogeneous grey cardboard, to which the external illumination source was attached. The zoom, to which the probe was attached, was placed in a straight line from the sample, specifically 50 cm apart. Concerning the geometry of presentation, 45°C incident illumination was used. The blank reference measurements were made with the cuvette

filled with distilled water against a reference pressed plate. The colour of the juice was assessed against a white background and a black foreground. The browning and brightening index of prickly pear juice was recorded at wavelengths of 476 nm and 540 nm. The browning index was calculated from the equation:

Browning index = Values of 476 nm divided by the values of 540 nm, Brightening index = Values of 476 nm + values of 540 nm

III. RESULTS

3.1 Physicochemical Analysis

The results of pH and titratable acidity of prickly pear juices are presented in Table 1. Prickly pear pulp had a higher pH value of 3.9 compared to enzyme treated pulp and the other juice treatments. No differences were observed among enzyme treated pulp, non treated juice and Brix adjusted juice. A significant ($P < 0.05$) decrease was observed after acidification, due to the addition of citric acid to prickly pear juice which lowers the pH.

TABLE I
 PHYSICO-CHEMICAL PARAMETERS OF PRICKLY PEAR JUICES

Treatments	pH	TA	SS (°Brix)	SS:AR	AA (mg)
Standard Ranges of PPP	5.3-7.1	0.05- 0.18	12-17	NI	1-40
Prickly Pear Pulp	3.88 ^a	0.47 ^g	3.68 ^e	7.72 ^e	29.82 ^a
Enzyme-Treated Pulp	3.73 ^d	0.58 ^d	4.28 ^d	7.31 ^f	30.19 ^a
Non-treated Juice	3.79 ^c	0.48 ^f	3.38 ^f	6.92 ^g	29.13 ^b
Brix -adjusted Juice	3.82 ^b	0.48 ^f	15.23 ^c	31.19 ^a	29.12 ^b
Acidified Juice	3.02 ^g	0.72 ^b	15.84 ^b	21.75 ^d	28.94 ^b
Heat-Treated Juice	3.05 ^f	0.74 ^a	16.21 ^a	21.72 ^d	27.98 ^c
Refrigerated Juice	3.09 ^e	0.67 ^c	15.94 ^b	23.56 ^c	27.69 ^c
Frozen-Thawed Juice	3.05 ^f	0.57 ^e	15.83 ^b	27.72 ^b	28.03 ^c

PPP: prickly pear pulp; NI: no information; TA: titratable acidity; AA: ascorbic acid; SS: soluble solids; SS:AR: sugar acid ratio; Non-treated prickly pear juice sample is the standard. Superscripts within columns and treatments with the same letter are not significantly different at $p < 0.05$.

Refrigerated and frozen-thawed prickly pear juice samples showed no significant difference in pH at $p < 0.05$. Acidification, heat-treatment, refrigeration and freezing had no effect on the pH of the juice. Enzyme treated pulp had high value of titratable acidity compared to prickly pear pulp. This shows that the addition of *Aspergillus niger* pectinase to juice had increased titratable acidity. A decrease in titratable acidity was observed after sieving, when the juice was produced. This indicates the effect of processing on the chemical quality of prickly pear juice. An increase in acidity was observed as the proportion of citric acid introduced to juice decreased after heat treatment. Despite the variation in total titratable acidity

of the products, values were relatively low, from 0.5 to 0.73, showing that all prickly pear juice treatments could be recommended for consumption.

3.2 Total Soluble Sugar and Ascorbic Acid Content

Total soluble sugar of the prickly pear samples was measured by refractometry. The refractometer works on the principle of light refraction through liquids as light passes from air into a liquid it slows down. The relationship between refractive index and the amount of dry substance forms the basis of the Brix scale, which is a measure of the number of gram of sugar present per 100 g of aqueous sugar solution. The critical angle of refraction changes with concentration soluble solids (SS) refers mostly to molecules that are truly soluble in aqueous solution. A convenient approximation here was to assume that a 1 °Brix reading by a refractometer equates with a 1 g sugar level in the prickly pear juice. Soluble solids content varied from 3.6 to 16 oBrix (Table 1). Non-treated prickly pear juice had a lower SS and soluble solids to acid ratio (SS:acid ratio) than any of the juice treatment applied. The soluble solids and acid ratio of non-treated juice and treated prickly pear juice varied mainly as a function of the amount of sugar added. These parameters were similar to most juices produced from common fruits such as apple and strawberry. The presence of citric acid reduced SS:acid ratio balance values. It is logic that the SS content drops when other dissolved components are added. Prickly pear pulp to which was added 8% (w/v) of pectinase from *Aspergillus niger* and incubated for 1 hour in water bath at 50°C had a higher ascorbic acid value compared to non-treated prickly pear pulp, signifying the purpose of introducing enzyme to the pulp to improve the ascorbic acid content, colour and aroma of the products. Non-treated prickly pear juice had a lower ascorbic acid value compared to enzyme treated pulp, but higher than treated juice samples, suggesting that juice processing and preservation had decreased the amount of ascorbic acid content of prickly pear juice.

3.3 Colour Analysis

3.3.1 Hunter L.a.b. Analysis

The colour of a product is one of the most important properties that influence the consumer's response to it. Therefore, one of the objectives of this study was to determine the colour change of prickly pear juices during processing and preservation. The principle of the Hunter L.a.b. analysis of colour and spectrophotometry measures the amount of light reflected by the sample compared to light reflected from a standard template. The purple standard template was used for processed and preserved prickly pear juice since Algeria prickly pear is purple. The results of Hunter L.a.b. analysis are shown in Table II.

TABLE II
 HUNTER LAB COLOUR PRICKLY PEAR JUICE
 COLOUR DIFFERENCE METER

Treatments	L*	a*	b*	Hue
Non-Treated	3.95 ^a	3.87 ^a	0.09 ^a	1.33 ^a
Brix-Adjusted	3.29 ^b	3.67 ^c	-0.42 ^b	-6.52 ^c
Acidified	2.58 ^c	3.78 ^b	-0.08 ^c	-1.21 ^b
Heat-Treated	2.51 ^d	2.82 ^e	-0.81 ^b	-1.22 ^b
Refrigerated	2.16 ^c	3.47 ^d	-0.47 ^b	-7.7 ^d
Frozen-Thawed	2.14 ^e	3.67 ^c	-0.82 ^b	-12.59 ^e
Standard Error	0.015	0.019	0.123	0.039

Superscripts within columns and treatments with the same letters are not significantly difference at p<0.05. A negative value of CDMb* means blue and positive value denotes a yellow colour. Non-treated prickly pear juice is regarded as the standard reference.

The analysis showed that non-treated prickly pear juice had a high CDML* (colour difference meter of light) of 3.9 compared to treated juices (range: 3.29 to 2.14). The CDML* significantly (p<0.05) decreased as the juice was preserved. Since CDML* is a measure of the light of colour from a light to a dark axis [27](Hunter 1985), the juice treatment thus applied decreased the CDML*value of prickly pear juices as they became darker. Frozen-thawed juice had the darkest reddish purple colour compared to the other juice treatments. This indicates that freezing had a significant effect on the colour of prickly pear juice. The objective of analyzing the CDMa* and CDMb* was to investigate the effects of processing and preservation on colour saturation of prickly pear juices. Non-treated prickly pear juice had the highest CDMa* (3.87) compared to treated prickly pear juices. Brix-adjusted and frozen-thawed prickly pear juices had similar CDMa* values of 3.67 (Table II). Acidified prickly pear juice had a higher CDMa* compared to the other juice treatments. Thus, juice treatment has an effect on reducing the red colour of prickly pear juices. Although the CDMa* values varied, all the prickly pear juices had the recognizable red purple colour. All treated prickly pear juices had negative CDMb* values whereas non-treated prickly pear juice had a positive value. The treated prickly pear juices had CDMb* values ranging between -0.08 and -0.82. This indicates that the non-treated prickly pear juice contained the yellow betalain pigments and treated prickly pear juices have the yellow pigment of betalain changed to a blue colour.

3.3.2. Spectrophotometer Colour Analysis

The objective of colour analysis by spectrophotometry was to investigate the effect of processing and preservation on the brightness and browning of prickly pear juice. The relevant results are presented in Table III. Acidified prickly pear juice had the highest absorbance reading at both 540 nm and 476 nm and also the highest brightness index compared to other juice treatments and non-treated prickly pear juice. Heat-treated prickly pear had the highest browning index, implying that it has the ability to inhibit the browning of prickly pear juice. The red purple colour of prickly pear did not differ significantly between juice treatments, i.e., the colour was constant for all treatments. Since the red purple colour characteristics of prickly pear remained fixed, it can be

concluded that the red-purple colour of prickly pear obtained presented a higher stability to pH changes and to the thermal treatments. This offered a clear advantage of the red-purple prickly pear for the production of juice, and prickly pear can be also regarded as a good source of betalain pigments. Table III. Effects of Processing and Preservation on the Browning and Brightness Index of Prickly Pear Juice

TABLE III

BROWNING AND BRIGHTNESS INDEX OF PRICKLY PEAR JUICE

Treatments	540 nm	476 nm	Browning Index 476 nm/540 nm	Brightness Index 476 nm + 540 nm
Non-Treated	3.26 ^b	3.23 ^a	0.99 ^{ab}	6.50 ^b
Brix-Adjusted	3.29 ^a	3.21 ^{ab}	0.98 ^b	6.50 ^b
Acidified	3.31 ^a	3.24 ^a	0.98 ^b	6.55 ^a
Heat-Treated	3.17 ^c	3.17 ^{bc}	1.00 ^a	6.34 ^c
Refrigerated	3.12 ^d	2.96 ^d	0.94 ^c	6.08 ^d
Frozen- Thawed	3.17 ^c	3.16 ^c	0.99 ^{ab}	6.32 ^b
Standard Error	0.006	0.015	0.006	0.012

Superscripts within column and treatments with the same letter are not significantly different at $p < 0.05$.

IV. DISCUSSION

This study showed that juice treatments affected the light and bright red purple colour of prickly pear. Light and bright colours are due to betalain pigments which were not only of major importance in maintaining colour stability throughout processing, but also because they give the juice an attractive colour. Colour differences that occur were probably due to some changes in the betalain pigments and development of furfural and hydroxyl-methylfurfural compounds [21]. The colour of prickly pear juice was altered by the methods of preservation used. Heat-treated juice sample was darker (low CDML* value). This could be due to the Maillard reaction, since reducing sugar and amino acids are required in this reaction. °Brix-adjustment generally have a positive effect on sweet taste and sensory acceptance of the juice, but initiated little browning caused by the Maillard reaction, which could also limit the storage temperature of prickly pear juice. The ascorbic acid content and acidification of the juice protected the natural colour and exhibited anti-browning effects that are consistent with previous studies [21], [22]. The red hue of the heat-treated juice also changed as indicated by low CDMA* and CDMb* values compared to non-treated and acidified juice samples. Similar results were reported for prickly pear juice blends [23], [24]. The pigments responsible for the red purple colour were highly affected by juice treatments as shown by the decrease in spectrophotometric absorbance values at wavelengths 540 nm and 476 nm. This result was also confirmed by the decrease in Hunter L.a.b. CDMb* with negative values which suggested that the blue colour predominates the yellow colour. This observation corroborates similar findings on prickly pear pigment stability, although in sensory evaluations no significant differences in acceptability of visual colour of prickly pear juices could be demonstrated [2], [24]. The colour stability was not a problem

in prickly pear juices because the recognizable red purple colour was retained. Browning is due to the action of polyphenolases which occur naturally in the fruit tissue. These enzymes catalyze the oxidation of phenols, also naturally present in the fruit, to form compounds called quinones. The melanoidins which constitute the brown pigment were low in treated prickly pear juice samples as indicated by their high browning indices. Browning was not a particular problem since colour stability is greater and browning occurs to a lesser degree in low pH or in acidic medium [25], [26]. This could be due to the ascorbic acid content of prickly pear juice, because ascorbic acid inhibits the browning reaction by reducing the quinones back to the original phenol compounds. The little browning that occurred in the prickly pear juice could be due to the presence of oxygen which enhances the conversion of phenols to quinones [27], [28].

V. CONCLUSION

Colour stability of prickly pear juice was examined in this study. Processing the juice with enzyme had positive effects on juice extraction and colour modification. The study also found that both acidification and heat treatment improves the brightness index of prickly pear juice. Moreover, the addition of sugar to the juice for enhancing sweetness affected the brightness and turned the juice into a dark red purple colour. The quality studies showed that most of juice processing variables led to better colour retention and a lower degree of browning. Colour changes had no significant effects on the juice. Combined methods and applications of newer processing technologies on the effect of colour are recommended for future work in this field.

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