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Evaluation of different methods for the production of juice concentrates and fruit powders from cactus pear

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Abstract

The present work aimed both at improvement and extension of a previously developed process for the production of cactus pear juice. The total yield was increased by 10% through processing whole instead of peeled fruits and by further optimisation of pulp enzymation. As an alternative to HTST pasteurisation, cross-flow microfiltration was applied for non-thermal cactus pear juice preservation. Juice concentrates and fruit powders were produced by rotary evaporation and freeze drying at laboratory scale and compared to products obtained at pilot plant-scale applying a three-stage column evaporator and spray drying, respectively. To monitor process-related quality changes, the resulting products were characterised in terms of colour and selected quality parameters. For both juice concentrates and fruit powders, initial colour characteristics were retained. In addition to betanin isomerisation, C_{11}-epimerisation of proline-betaxanthin was demonstrated to be a valuable indicator of the respective heat treatment applied. Whereas no adverse reactions were observed during juice production, non-enzymatic browning and HMF formation were found after concentration at pilot plant-scale and freeze drying, respectively.
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Industrial relevance: Although cactus pear meets all requirements for food colouring purposes, no attempt has been made so far to exploit this potential. The feasibility of processing cactus pear juice into concentrates and fruit powders was demonstrated in this study. In contrast to single-strength juice, concentrates and powders are easier to handle during transportation and storage and also open further fields of application that may promote cactus pear fruit processing at industrial scale in the future.

1. Introduction

To meet customers' expectations of appealing and appetising products, food colouring is a prerequisite to compensate process-related losses to improve overall appearance of food (Newsome, 1986; Schnitter, 2002). However, the use of synthetic dyes worries consumers through the association of E-numbers with insalubrious chemical substances (Dietz, 2004). On the other hand, not requiring E-number declaration, water- or oil-based plant extracts are receiving growing interest in the progressing replacement of synthetic dyes (Duhard, Garnier, & Mégard, 1997; Stich, Kloos, & Hoeck, 1999; Stintzing & Carle, 2004) with forecasted annual growth rates of 4.5% on the European and US markets until 2009 (Anderson,

2003). Moreover, colouring foodstuffs may substantially profit from the fast growing functional food market, especially in the dairy and beverage sector (Menrad, 2003), because the abundance of fruit or vegetable ingredients offer health-promoting and nutritional benefits (Pszczola, 2003). The latter attributes have been highlighted for cactus fruits only recently (Butera et al., 2002; Feugang, Konarski, Zou, Stintzing, & Zou, 2006; Galati et al., 2003; Stintzing, Schieber, & Carle, 2001; Stintzing, Schieber, & Carle, 2003).

Although red beet preparations commonly applied for colouring dairy products exhibit both technological and sensorial disadvantages such as peatiness, nitrate accumulation and risk of microbial carry-over (Stintzing et al., 2001), cactus pear as a promising source of water-soluble betalains has been neglected so far. In a previous study, customised hues covering the entire spectrum from bright yellow to purplish-blue were successfully produced by blending purple and

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yellow cactus fruit juices (Moßhammer, Stintzing, & Carle, 2005a). Therefore, in continuation of an earlier study introducing a process for cactus pear juice manufacture (Moßhammer, Stintzing, & Carle, 2005b) the production of concentrated fruit juices displaying higher tinctorial strength as well as lower storage and transportation costs (Cassano, Jiao, & Drioli, 2004) should be developed. Furthermore, to open further fields of application, this work aimed at the production of coloured fruit powders which may boost the nutritional and visual quality of colouring of fruit and cereal bars or instant products (Nickerson, 1999). In addition, powdered products have a longer shelf life when handled properly (Glicksmann, 1969; Revie & Thomas, 1972).

Therefore, the present study aimed at the development of a process for the production of both juice concentrates and powders from cactus pear at laboratory and pilot plant-scale, respectively. Since betalains are regarded as thermolabile compounds (Stintzing & Carle, 2004), alternative processes for juice concentration and preservation, including cross-flow microfiltration and freeze drying, should be considered.

Besides pigment decay, further reactions affecting visual appearance may occur upon thermal exposure (Barreiro, Milano, & Sandoval, 1997; Suh, Noh, Kang, Kim, & Lee, 2003) such as non-enzymatic browning through Maillard reactions or ascorbic acid oxidation (Liao & Seib, 1988). Hence, quantitative and qualitative colour changes in terms of CIE $L*C*h°$, total colour differences $\Delta E*$, betalain contents, overall pigment retentions and browning indices were monitored. Finally, further relevant quality parameters such as haze, glucose and fructose together with organic acids, proline, formol numbers, and 5-hydroxymethylfurfural (HMF) should be determined.

2. Materials and methods

2.1. Plant material

Opuntia ficus-indica fruits of the cultivar 'Gialla' were purchased from Sicily (Italy) in 2003 and 2004. Whereas fruits from 2003 were frozen at −26 °C prior to processing, fruits from 2004 were processed without previous freezing.

2.2. Solvents and reagents

All reagents and solvents except 5-hydroxymethylfurfural (Fluka, Buchs, Switzerland), Fructozym MA-X-Press (Erbslöh, Geisenheim, Germany), Rohapect B1L (AB Enzymes, Darmstadt, Germany) and maltodextrin (18–20 DE, EGESIE, Nuremberg, Germany) were purchased from VWR (Darmstadt, Germany) and were of analytical or HPLC grade. Deionised water was used throughout.

2.3. Microfiltration of cactus pear juices at pilot plant-scale

Through improvement of a previously established process (Moßhammer et al., 2005b), cactus pears were processed into juice as follows: For concentration and spray drying experi-

ments, cactus pears from 2003 (170 kg) were thawed prior to processing. Freeze drying experiments were conducted with fresh cactus pears from 2004 (130 kg) (Fig. 1). Whole fruits were crushed in a grinding mill (Bucher, Niederweningen, Switzerland). The obtained mash was strained through a finisher (Type PAP 0533; mesh sizes: 1.5 mm and 0.4 mm, rotational speed: 400 rpm; Bertuzzi Food Technology, Brugherio, Italy) to remove seeds, peel (pericarp) and mesocarp fibers. Further clarification steps such as separation, acidification, enzymatic hydrolysis of the pectin-like substances and chamber filtration were carried out as described by Moßhammer et al. (2005b). Since Rohapect B1L (AB Enzymes, Darmstadt, Germany) exhibited improved hydrocolloid degradation in laboratory experiments (data not shown), this enzyme preparation was used at a dosage of 350 ppm instead of Fructozym MA-X-Press (Erbslöh, Geisenheim, Germany). To avoid heat load, juice after chamber filtration was further processed using a pilot plant-scale microfiltration plant (Pall Seitz Schenk, Waldstetten, Germany) fitted with a 0.2 μm ceramic membrane (Membralox P19-60, Pall Seitz Schenk, Waldstetten, Germany) at a flow rate of 22.5 L/h. The cold-sterile prepared single strength juice with 13% TSS was rapidly cooled and frozen at −26 °C until further processing.

2.4. Juice concentration

2.4.1. Concentration at laboratory scale

Microfiltered juice (1.5 kg) was concentrated in a rotary evaporator (Laborota 4000, Heidolph Instruments, Schwabach, Germany) to 33±1% TSS and 65±1% TSS at 25 °C and frozen at −26 °C until analyses.

2.4.2. Concentration at pilot plant-scale

Concentration was carried out using a three-stage column evaporator (Type PAF 53 S, Unipektin, Zürich, Switzerland). Fifty kilograms of the aseptically filtered juice were passed three times through the three-stage evaporator at 60–80 °C applying a vacuum of 200 mbar and a feed rate of 50 L/h. The obtained semi-concentrates (33±1% TSS) and the final concentrates (65± 1% TSS) were rapidly cooled to 0 °C and frozen at −26 °C prior to spray drying and analytical characterisation. The yield of each concentration step was calculated as follows: [Apparent yield [%]=(100/m_{JC})×$m_{Concentrate}$], [Yield of respective processing step [%]=(100/m_{JC})×($m_{Concentrate}$×(TSS$_{Concentrate}$/TSS$_{JC}$))], where m_{JC} is the mass prior to concentration and $m_{Concentrate}$ is the resulting mass after concentration, respectively.

2.5. Drying

2.5.1. Freeze drying

One-hundred and five grams of a mixture containing 5 parts of microfiltered juice and 1 part of maltodextrin (18–20 DE) were dried in a laboratory scale freeze dryer (Lyovac GT2, Finn-Aqua, Munich) at 25 °C until constant weight (72 h). The resulting powder was sealed airtight and stored at 5 °C until analyses.
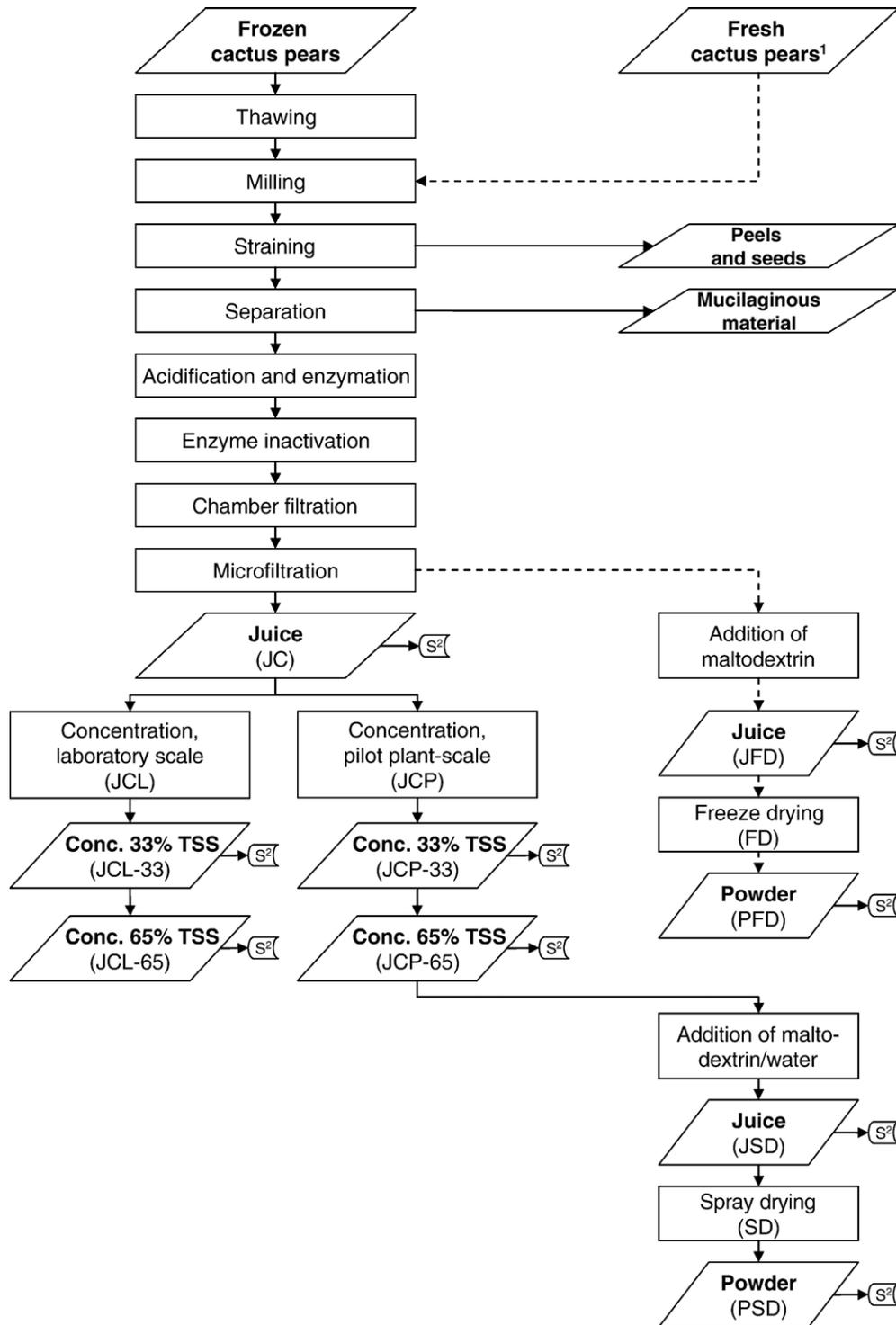
Fig. 1. Production scheme and sampling of different cactus pear juice products (juice, concentrate, powder). [1]Fresh cactus pears were exclusively used for the production of freeze dried powder (dashed line). [2]S = Sample.

2.5.2. Spray drying

Since cactus pear juice is rich in both fructose and glucose, the addition of a carrier was a prerequisite to avoid clogging. Because maltodextrin was found to be best for spray drying of betacyanin pigments from *Amaranthus* (Cai & Corke, 2000), this carrier was used for encapsulation. Five hundred grams of a mixture containing 1 part of con- centrate (JCP-65) and 1.5 parts of maltodextrin pre-dissolved in 1 part of H_2O were dried using a laboratory scale spray dryer (Büchi 190 Mini spray dryer, Büchi Labortechnik, Flawil, Switzerland) with optimised temperatures of 165 °C and 90 °C for inlet and outlet, respectively. The obtained powders were sealed airtight and stored at 5 °C until analyses.

For process evaluation, samples of each processing step were taken and assigned to the three categories "concentrated (C)", "freeze dried (FD)" and "spray dried (SD)" with specific reference samples for each group (Table 1).

2.6. Determination of moisture content

Moisture content was determined using an electronic moisture analyser (MA40-000V2, Sartorius, Göttingen, Germany) registering mass loss until constant weight.

2.7. Determination of chemical parameters

Glucose and fructose were determined using enzyme test kits (R-Biopharm, Darmstadt, Germany). Formol numbers and proline concentrations were assessed according to IFU methods no. 30 and no. 49, respectively (IFU, 1983, 1984). Hydroxymethylfurfural (HMF), vitamin C (ascorbic acid plus dehydroascorbic acid) and organic acids (malic acid, citric acid, oxalic acid) analyses were performed with an HPLC system (Merck, Darmstadt, Germany) equipped with an auto sampler L-7200, an interface module D-7000, a pump L-7100, a column oven Jetstream Plus, and a UV detector L-7400. Separation was achieved at 25 °C applying an analytical scale Aminex HPX-87H column (300 × 7.8 mm i.d.; Bio-Rad, Munich, Germany) fitted with a C_{18}-ODS security guard column (4 mm × 3.0 mm i.d.; Phenomenex, Torrance, CA, USA). Aqueous trifluoroacetic acid (0.1%, v/v), at a flow rate of 0.4 mL/min was used as solvent. Quantification was performed at 210 nm for vitamin C and organic acids and at 280 nm for HMF by external standard determination, respectively.

Each sample was adjusted to pH 7.25 ± 0.75 with NaOH (10%, v/v) prior to analyses. Due to coelution of ascorbic and malic acids (R_t = 15.6 min) and determination of both ascorbic

and dehydroascorbic acids, three different runs for each sample were required: Either 0.1 mL water (H_2O sample) or 0.1 mL dithiothreitol solution (1 mg/L, dithiothreitol sample) or 0.1 mL 5% H_2O_2 solution (H_2O_2 sample) were added, respectively, to aliquots of a 1 mL sample. Oxalic acid (R_t = 10.9 min), citric acid (R_t = 12.9 min) as well as HMF (R_t = 55.0 min) were assessed by analysing the water sample (H_2O sample). After complete oxidation of ascorbic acid, the H_2O_2 sample was used for malic acid determination. Ascorbic and dehydroascorbic acids were quantified as follows: [Peak area$_{Ascorbic\ acid}$ = (Peak area$_{Ascorbic\ acid + malic\ acid}$, H_2O sample) − (Peak area$_{Malic\ acid}$, H_2O_2 sample)]. [Peak area$_{Dehydroascorbic\ acid}$ = (Peak area$_{Ascorbic\ acid + malic\ acid}$, dithiothreitol sample) − (Peak area$_{Ascorbic\ acid + malic\ acid}$, H_2O sample)]. Total vitamin C was calculated by summing up dehydroascorbic and ascorbic acid contents.

2.8. Haze and non-enzymatic browning

The amount of haze was determined according to Wong and Stanton (1989) by subtracting the absorbance of deionised water from the absorbance of the product samples at 700 nm.

Non-enzymatic browning was monitored spectrophotometrically according to Billaud, Brun-Mérimée, Louarme, and Nicolas (2004) at 340, 350, 360 and 420 nm, respectively.

2.9. Spectrophotometric betalain quantification and colour analyses

CIE $L^*C^*h°$ determinations and pigment quantification were performed using a UV–Vis spectrometer (Perkin-Elmer, Überlingen, Germany) equipped with UVWinLab V 2.85.04 and Wincol V2.05 colour softwares (Perkin-Elmer Instruments, Norwalk, CT, USA) after diluting the juice samples with McIlvaine buffer (pH 6.5) to obtain an absorption value of 1.0 ± 0.1. Colour properties of fruit powders were assessed using a Chroma-Meter CR-300 for solid samples (Minolta, Osaka, Japan).

The betalain content (BLC) was calculated as described earlier (Stintzing et al., 2005): [BLC [mg/L] = (A × DF × MW × 1000)/(ε × 1)], where A is the absorption value at the absorption maximum corrected by the absorption at 650 nm, DF is the dilution factor and 1 is the pathlength (1 cm) of the cuvette. For quantification of betacyanins (Bc) and betaxanthins (Bx) constituting the major betalains in cactus pear juice (Stintzing et al., 2005), the molecular weights (MW) and molar extinction coefficients (ε) of betanin (MW = 550 g/mol; ε = 60,000 L mol^{-1} cm^{-1} in H_2O; λ = 538 nm) and indicaxanthin (MW = 308 g/mol; ε = 48,000 L mol^{-1} cm^{-1} in H_2O; λ = 480 nm) were applied, respectively.

For colour assessment, Bx/Bc ratios were calculated by dividing betaxanthin by betacyanin contents. Additionally, chroma [$C^* = (a^{*2} + b^{*2})^{0.5}$], hue angle [$h° = \arctan(b^*/a^*)$] and total colour difference [$\Delta E^* = (\Delta L^{*2} + \Delta C^{*2} + \Delta H^{*2})^{0.5}$] with [$\Delta L^* = L^*_{processed\ sample} − L^*_{reference\ sample}$], [$\Delta C^* = C^*_{processed\ sample} − C^*_{reference\ sample}$] and [$\Delta H^* = 2\sin((h°_{processed\ sample} − h°_{reference\ sample})/2) × (C^*_{processed\ sample} × C^*_{reference\ sample})^{0.5}$] were determined using illuminant D_{65} and

Table 1
Sample description

Process	Reference sample	Sample preparation
Concentration		
JC	–	–
JCL-33	JC	Dilution with H_2O to 13.0 ± 0.1% TSS [a]
JCL-65	JC	Dilution with H_2O to 13.0 ± 0.1% TSS [a]
JCP-33	JC	Dilution with H_2O to 13.0 ± 0.1% TSS [a]
JCP-65	JC	Dilution with H_2O to 13.0 ± 0.1% TSS [a]
Freeze Drying		
JFD	–	–
PFD	JFD	Grinding with a laboratory mill A10 (Janke and Kunkel, Staufen i. Br., Germany), dissolving in H_2O, adjusting to 26.9 ± 0.1% TSS [b]
Spray Drying		
JSD	–	Addition of 2 parts H_2O
PSD	JSD	Dissolving in H_2O, adjusting to 61.3 ± 0.1% TSS [c], addition of 2 parts H_2O

[a] Standardisation to the total soluble solids content of JC (13.0% TSS).
[b] Standardisation to the total soluble solids content of JFD (26.9% TSS).
[c] Standardisation to the total soluble solids content of JSD (61.3% TSS).

10° observer angle based on visible spectra monitoring from 380–780 nm, respectively (Gonnet, 1998; Stintzing et al., 2003).

2.10. Determination of betalain retention by HPLC-MS/MS

HPLC analyses were performed according to Kugler, Stintzing, and Carle (2004) using a Merck Hitachi LaChrom Elite HPLC system (Merck, Darmstadt, Germany) equipped with an L-2130 pump, an L-2200 autosampler, a Jetstream column oven and an L-2450 diode-array detector. Mobile phase A consisted of 1% (v/v) formic acid in water, and aqueous MeCN (80:20 MeCN/H$_2$O, v/v) was used as B. Peak separation was achieved on an analytical scale Atlantis dC$_{18}$ column (250×4.6 mm i.d.; Waters, Wexford, Ireland) fitted with a C$_{18}$-ODS security guard column (Phenomenex, Torrance, CA, USA) at a flow rate of 1 mL/min at 30 °C. Starting isocratically with 100% A for 2 min, a linear gradient was followed from 0 to 20% B in 60 min and then from 20% to 100% B in 5 min. Betaxanthins and betacyanins were monitored at 470 and 538 nm, respectively.

Using identical chromatographic conditions, LC-MS analyses were performed on a 1100 HPLC series system (Agilent, Waldbronn, Germany) equipped with ChemStation software, a G1322A degasser model, a G1312A binary gradient pump model, a G1329/1330A autosampler model, a G1316A column-oven model, and a G1315A diode array detector model. The HPLC system was connected in series with a Bruker (Bremen, Germany) model Esquire 3000+ ion trap mass spectrometer fitted with an electrospray ionisation source operating in the positive mode. Nitrogen was used as dry gas at a flow rate of 12 mL/min and a pressure of 70 psi. The nebuliser temperature was set to 365 °C. Using helium as the collision gas (1.2×10^{-8} bar), collision-induced dissociation spectra were obtained with a fragmentation amplitude of 1.2 V (MS/MS).

All processing variants (2.4.–2.5.) were analysed in duplicate. For each sample, moisture and colour characteristics were assessed in triplicate. All other chemical quality parameters were assessed in duplicate, except vitamin C, organic acids and HMF which were determined once after verifying accuracy (RSD±1.0%) in duplicate.

3. Results and discussion

To improve both feed and juice yield, optimisation of the crushing step (Moßhammer et al., 2005b) was found to be a prerequisite. For this purpose, laborious and lossy carborundum peeling was replaced by crushing the whole fruits with a grinding mill (Fig. 1). Peels and seeds were subsequently separated with a finisher to prevent discolouration from chlorophyll degradation. As recently demonstrated for highly thermolabile juices, cold sterilisation by membrane technologies represents a gentle alternative to common heat preservation treatments (Cassano et al., 2004; Matta, Moretti, & Cabral, 2004; Vaillant et al., 2005; Vaillant et al., 1999). Therefore, the replacement of the pasteurisation step by cross-flow microfiltration appeared to be promising for further

improvement of a recently developed process (Moßhammer et al., 2005b).

Juice concentrates and fruit powders were produced under mild temperature conditions at laboratory scale using a rotary evaporator and a freeze dryer, respectively, and compared to products obtained at pilot plant-scale by evaporation in a three-stage column evaporator and by spray drying, respectively.

3.1. Microfiltration of cactus pear juice at pilot plant-scale

Through modification of a previously developed process (Moßhammer et al., 2005b), an improvement of the overall yield from 37% to 47% was achieved mainly by grinding fresh fruits instead of processing carborundum peeled frozen fruits. Seeds, mesocarp fibres and peel were removed by a finisher. Furthermore, increasing the product feed from 20 to 170 kg may also contribute to yield improvement. Both in view of energy costs and easier handling of milling compared to carborundum peeling in a previous work (Moßhammer et al., 2005b), processing fresh fruits presents an interesting option in the production of cactus pear juice. Due to the stickiness and viscosity of the pre-clarified juice, further enzymation experiments as performed by Moßhammer et al. (2005b) appeared to be crucial. Since Rohapect B1L (AB Enzymes, Darmstadt, Germany) is recommended to improve the filtration flux in manufacturing concentrates from tropical fruits, this enzyme preparation was compared to Fructozym MA-X-Press (Erbslöh, Geisenheim, Germany) selected previously (Moßhammer et al., 2005b). As a result, Rohapect B1L at a dosage of 350 ppm proved to be most suitable for hydrocolloid degradation. Moreover, pigment retention should be increased by replacing pasteurisation with cold-sterile cross-flow filtration.

3.2. Characteristics of concentrated and powdered cactus pear juice products

Processing yields and selected quality parameters of the concentrated cactus juice products obtained are compiled in Table 2.

3.3. Chemical parameters

3.3.1. Organic acids and sugars

Since virtually no changes in organic acid contents were detected upon processing (data not shown), ester formation from sugars and acids (Tarrach, Bochmann, & Herrmann, 1987; Tarrach & Herrmann, 1987) does not occur during cactus juice processing. Therefore, only data for microfiltered juice (JC) are exemplarily given which amounted to 45 mg/L, 6.7 g/L and 1.6 g/L for oxalic acid, citric acid (after acidification with citric acid to pH=4) and malic acid, respectively.

For both concentration by evaporation and freeze drying, glucose and fructose contents slightly increased during processing without changing the natural glucose/fructose ratio (Table 3). In the same line, spray drying did not provoke substantial concentration changes. However, through addition of maltodextrin containing approximately 10% glucose

Table 2
Product yields and physical characteristics of concentrated cactus fruit juice products

Product	Mass prior to concentration [a]	Mass after concentration [a]	Apparent yield; Yield-of concentration step [a, b]	Colour characteristics	TSS [a, c] Moisture content [d]
Concentrate, laboratory scale (JCL)	1.5±0.0 kg	0.3±0.0 kg	20.0±0.0%; 100.0±0.0%;	– [e]	65.5±0.2% [c]
Concentrate, pilot plant-scale (JCP)	50.6±0.0 kg	8.1±0.2 kg	16.0±0.4%; 81.0±1.7%	– [e]	65.3±0.1% [c]
Powder, freeze-dried (PFD)	105.7±0.4 g	32.5±0.2 g	30.7±0.1%; – [f]	$L^*=90.6\pm0.2$ [g] $C^*=46.5\pm0.7$ [g] $h°=93.5\pm0.2$ [g]	7.0±0.3% [d]
Powder, spray-dried (PSD)	500.0±0.0 g	48.4±2.4 g	9.7±0.5%; – [f]	$L^*=92.9\pm0.2$ [g] $C^*=35.6\pm0.1$ [g] $h°=97.7\pm0.4$ [g]	6.9±0.8% [d]

[a] Mean values of duplicate measurements±standard deviation.
[b] Calculated as described in Section 2.4.1. Concentration at pilot plant-scale.
[c] TSS=Total soluble solids [%].
[d] Mean values of triplicate measurements±standard deviation. Moisture content [%].
[e] See Fig. 4.
[f] Not applicable.
[g] Solid samples. Rediluted powder characteristics are shown in Fig. 4.

(supplier's note), the natural glucose/fructose ratio of 1.2 (JC) was increased to 1.4 and 1.6 for JFD and JSD, respectively. Since precise adjustment of the samples to the TSS value of the reference samples was difficult, slight fluctuations of both glucose and fructose contents were found. In general, however, the glucose/fructose ratios remained unaffected during all other processing steps.

3.3.2. Vitamin C

Whereas vitamin C decay during concentration amounting to 85% for JCL-33 and 90% for JCL-65 were unexpectedly high for concentrates produced at laboratory scale, losses for pilot plant-scale concentrates were considerably lower, with 3% for JCP-33 and 15% for JCP-65, respectively. The higher vitamin C loss in the former case may be ascribed to longer heat exposure: The concentration step in the rotary evaporator took approximately 3.5 h for JCL-33 and 6 h for JCL-65, while the dwell time for the concentrates in the three stage evaporator was only a few minutes. Freeze drying entailed a vitamin C loss of only

10% and was the most gentle process. In contrast, spray drying caused a vitamin C decrease of 50–55% (Table 3).

3.3.3. Amino acids

Amino acid contents were monitored by determination of formol numbers and proline quotients (Table 3). Variation of formol numbers was negligible for all processing steps monitored indicating unchanged amino acid concentrations. In contrast, proline quotients slightly decreased during concentration suggesting proline release by degradation of the main betalain proline-betaxanthin (Moßhammer et al., 2005b), particularly in spray- and freeze-dried products.

3.3.4. 5-Hydroxymethylfural

Due to the general acceptance as an indicator of heat treatment resulting from glucose at low pH values (Steber & Klostermeyer, 1987), 5-hydroxymethylfural (HMF) contents were determined to evaluate the impact of concentration, spray or freeze drying on the product quality. Since HMF contents

Table 3
Selected quality parameters of different cactus fruit juice products [a]

Process	Glucose [b] [mg/L]	Fructose [b] [mg/L]	Glucose/Fructose	Vitamin C [b] [mg/L]	Formol number [b] [mL 1 M NaOH/L]	Proline quotient [c]	HMF [mg/L] [b]
Concentration							
JC	55.1 (±0.3)	44.8 (±1.0)	1.2	73.8 (±1.8)	14.2 (±0.1)	15.8	0.7 (±0.0)
JCL-33	59.3 (±1.4)	48.3 (±0.9)	1.2	11.5 (±1.7)	14.8 (±0.1)	15.3	0.8 (±0.0)
JCL-65	59.5 (±2.7)	49.0 (±1.7)	1.2	6.9 (±1.2)	14.6 (±0.1)	14.9	0.8 (±0.0)
JCP-33	61.9 (±2.3)	51.4 (±0.8)	1.2	71.7 (±1.3)	14.4 (±0.0)	14.7	1.6 (±0.0)
JCP-65	58.6 (±3.0)	50.2 (±0.4)	1.2	63.0 (±1.2)	14.5 (±0.0)	15.0	1.7 (±0.0)
Freeze drying							
JFD	57.5 (±0.9)	41.5 (±1.9)	1.4	13.2 (±1.4)	11.5 (±0.0)	13.4	1.5 (±0.1)
PFD	60.8 (±1.5)	44.0 (±1.9)	1.4	11.9 (±0.2)	11.6 (±0.0)	12.1	1.9 (±0.0)
Spray drying							
JSD	36.1 (±0.1)	22.8 (±0.3)	1.6	18.4 (±0.2)	7.2 (±0.0)	14.7	1.5 (±0.2)
PSD	35.2 (±0.5)	23.2 (±0.2)	1.5	8.9 (±0.7)	6.9 (±0.0)	15.1	2.0 (±0.3)

[a] Sample names are given in Fig. 1.
[b] Mean values of duplicate measurements±standard deviation.
[c] Proline quotient=Formol number [mL 1 M NaOH/L]/Proline [g/L].

Table 4
Browning characteristics and haze of different cactus fruit juice products

Process	Absorbance index [a]				Haze [b]
	340 nm	350 nm	360 nm	420 nm	700 nm
Concentration					
JC	8.865 (±0.164)	7.281 (±0.148)	5.933 (±0.145)	2.644 (±0.119)	0.009 (±0.000)
JCL-33	9.077 (±0.336)	7.413 (±0.275)	6.014 (±0.230)	2.548 (±0.102)	0.023 (±0.005)
JCL-65	7.830 (±0.187)	6.410 (±0.202)	5.207 (±0.200)	2.211 (±0.139)	0.042 (±0.001)
JCP-33	9.346 (±0.012)	7.700 (±0.043)	6.280 (±0.069)	2.566 (±0.058)	0.068 (±0.005)
JCP-65	9.770 (±0.060)	8.112 (±0.019)	6.660 (±0.058)	2.593 (±0.038)	0.117 (±0.010)
Freeze drying					
JFD	6.788 (±0.153)	5.536 (±0.134)	4.465 (±0.114)	2.323 (±0.067)	0.022 (±0.004)
PFD	8.745 (±0.221)	7.263 (±0.219)	5.940 (±0.205)	3.067 (±0.138)	0.122 (±0.110)
Spray drying					
JSD	5.426 (±0.301)	4.571 (±0.310)	3.525 (±0.304)	1.649 (±0.197)	0.107 (±0.005)
PSD	4.869 (±0.058)	3.979 (±0.041)	3.235 (±0.030)	1.266 (±0.197)	1.649 (±0.094)

[a] Absorbance index = absorbance at λ times dilution factor (Billaud et al., 2004). Mean values of triplicate measurements ± standard deviation.
[b] Mean values of duplicate measurements ± standard deviation.

were below 2 mg/L in all samples, staying far below the limit of 20 mg/L established for fruit juices (A.I.J.N., 1996), cactus pear processing is easily feasible with common techniques for fruit juice manufacture. Only minor HMF formation was found in both concentrates obtained by evaporation (JCP) and freeze drying (PFD) at pilot plant-scale (Table 3).

3.4. Haze and non-enzymatic browning

Displaying absorption values of ≤0.1 at 700 nm, samples obtained after dilution of concentrates (both JCL and JCP) and from freeze-dried powders after reconstitution were absolutely clear. In contrast, spray-dried samples (PSD) showed a visible turbidity resulting in a significant absorbance at 700 nm (Table 4). This may be due to the high amount of maltodextrin in spray-dried cactus juice powder which was associated with an inferior solubility compared to freeze-dried preparations.

Cactus pear is rich in glucose, fructose and amino acids, especially proline (Stintzing, Schieber, & Carle, 1999; Stintzing et al., 2003; Moßhammer et al., 2005b), fulfilling the prerequisites for non-enzymatic browning during processing. However, in a former study (Moßhammer et al., 2005b)

browning of cactus pear juice was negligible even after pasteurisation which was ascribed to the predominance of proline exhibiting low Maillard potential.

Interestingly, absorbance indices at 420 nm only slightly increased for freeze-dried samples, whereas no increments were observed for both concentrates (JCL and JCP) and spray-dried powders, respectively. Since advanced Maillard reaction products (MRPs) are monitored at wavelengths between 420 and 450 nm (Billaud et al., 2004), and slight absorbance changes were exclusively found for freeze-dried powders at 420 nm, indices for soluble pre-melanoidins (340–360 nm) were used for closer inspection of browning reactions during processing. Both JCL and JCP concentrates and PSD powders showed a slight decrease of absorbance while values increased for FD powders after reconstitution indicative of pre-melanoidin formation.

3.5. Betalain contents

3.5.1. Total betalain contents

Betaxanthin (Bx) and betacyanin (Bc) contents, Bx/Bc ratios, proline/Bx ratios, pigment retentions as well as proline-Bx/isoproline-Bx and betanin/isobetanin ratios were determined

Table 5
Pigment characteristics of different cactus fruit juice products

Process	Total betalain content [a] [mg/L]	Betaxanthin content (Bx) [a] [mg/L]	Betacyanin content (Bc) [a] [mg/L]	Bx/Bc	Proline [mg/L] [a]	Proline/Bx
Concentration						
JC	38.6 (±0.5)	34.5 (±0.3)	4.1 (±0.1)	8.4	900.3 (±9.1)	26.1
JCL-33	38.6 (±0.1)	34.5 (±0.0)	4.1 (±0.0)	8.4	969.0 (±2.0)	28.1
JCL-65	38.5 (±0.0)	34.4 (±0.0)	4.1 (±0.0)	8.4	979.8 (±9.2)	28.5
JCP-33	32.3 (±0.6)	28.5 (±0.5)	3.8 (±0.1)	7.5	976.3 (±14.4)	34.3
JCP-65	31.5 (±1.0)	27.6 (±0.9)	3.9 (±0.1)	7.1	963.5 (±13.3)	34.9
Freeze drying						
JFD	40.8 (±0.3)	34.5 (±0.3)	4.3 (±0.1)	8.0	859.2 (±54.5)	24.9
PFD	40.6 (±0.5)	34.5 (±0.3)	4.1 (±0.0)	8.4	857.6 (±1.3)	24.9
Spray drying						
JSD	16.8 (±0.5)	14.4 (±0.4)	2.4 (±0.3)	6.0	511.4 (±21.1)	35.5
PSD	15.2 (±0.5)	13.4 (±0.3)	1.8 (±0.3)	7.4	455.9 (±2.3)	34.0

[a] Mean values of duplicate measurements ± standard deviation by spectrophotometric determinations.

Table 6
Retention of major betalain pigments from cactus pear upon different concentration conditions assessed by HPLC-DAD measurements at $\lambda=470$ nm

Process	Histidine-Bx retention [%] [a] ($R_t=10.4$ min)	Glutamine-Bx retention [%] [a] ($R_t=16.3$ min)	GABA[b]-Bx retention [%] [a] ($R_t=29.2$ min)	Isoproline-Bx retention [%] [a] ($R_t=29.9$ min)	Proline-Bx retention [%] [a] ($R_t=31.2$ min)	Betanin retention [%] [a] ($R_t=36.8$ min)	Isobetanin retention [%] [a] ($R_t=40.7$ min)
Concentration							
JC	100.0	100.0	100.0	100.0	100.0	100.0	100.0
JCL-33	71.2 (±3.7)	80.5 (±2.6)	86.1 (±1.4)	103.1 (±1.1)	98.1 (±0.4)	95.5 (±0.4)	110.1 (±2.4)
JCL-65	69.4 (±4.4)	71.2 (±3.5)	85.2 (±4.7)	94.1 (±1.5)	96.9 (±0.5)	91.4 (±1.9)	118.4 (±1.1)
JCP-33	51.5 (±3.6)	60.2 (±2.9)	84.7 (±1.8)	120.9 (±4.7)	83.9 (±0.0)	77.3 (±1.2)	114.8 (±3.3)
JCP-65	27.5 (±1.4)	45.7 (±2.8)	83.5 (±0.4)	195.6 (±4.0)	71.1 (±2.8)	55.7 (±3.1)	176.9 (±1.4)
Freeze Drying							
JFD	100.0	100.0	100.0	100.0	100.0	100.0	100.0
PFD	95.4 (±1.8)	81.2 (±2.2)	79.3 (±0.0)	92.9 (±0.3)	93.6 (±1.4)	96.5 (±2.4)	101.9 (±2.2)
Spray Drying							
JSD	100.0	100.0	100.0	100.0	100.0	100.0	100.0
PSD	85.2 (±5.5)	125.7 (±4.3)	95.0 (±5.4)	98.4 (±1.6)	95.7 (±2.1)	96.8 (±3.7)	98.2 (±3.7)

[a] Mean values of duplicate measurements±standard deviation. Bx=Betaxanthin.
[b] γ-aminobutyric acid.

to monitor the impact on betalain stability during concentration, spray drying and freeze drying, respectively (Tables 5–7; Figs. 2 and 3).

Both total Bx (34.5 mg/L) and Bc contents (4.1 mg/L) of microfiltered juice (JC) were in the same range as the "pasteurised juice" in a former study (Bx content=28.8 mg/L, Bc content=3.0 mg/L; Moßhammer et al., 2005b). Degradation of the predominant proline-betaxanthin (Fig. 2) caused proline release with subsequent increase of the proline/Bx ratio. Due to the lower proline/Bx ratio of JC compared to heat-treated juice (proline/Bx=43.1; Moßhammer et al., 2005b), microfiltration was found to be superior to pasteurisation with respect to pigment retention.

During concentration at laboratory scale virtually no changes for Bx or Bc contents and Bx/Bc ratios were observed. However, both proline contents and proline/Bx ratios slightly increased indicating proline-betaxanthin decay. This finding corroborates with Moßhammer et al. (2005b) who claimed proline contents a very sensitive indicator for betaxanthin degradation. Upon pilot plant-scale concentration, declining Bx (relative Bx loss ~15%) and Bc (relative Bc loss ~ 6%) contents together with a decrease of the Bx/Bc ratio revealed a higher pigment decay. The superior heat stability of betacyanins is in agreement with previous studies (Herbach, Stintzing, & Carle, 2004b; Moßhammer et al., 2005b). Additionally, after processing into concentrates, proline contents and proline/Bx ratios, respectively, exhibited higher increments compared to concentrates produced at laboratory scale (Table 5).

After freeze drying, betalain and proline contents together with proline/Bx ratios were virtually unchanged. For spray-dried samples, minor decrements of both yellow and red

betalains were found accompanied by decreasing proline contents and proline/Bx ratios.

3.5.2. Individual betalain patterns

Pigment retentions of the major betaxanthins and betacyanins of cactus pear (Tables 6 and 7; Fig. 2) were determined by HPLC to get an insight into the thermal stability of individual compounds.

Although isoproline-Bx (isoindicaxanthin) has been assumed not to occur in nature (Terradas & Wyler, 1991), isoproline-Bx was detected by LC-MS in all samples including freshly squeezed cactus pear juice (data not shown).

Whereas for concentration at laboratory scale virtually no changes in both betaxanthin and betacyanin contents were noticed when using spectrophotometrical quantification, HPLC analyses revealed decreases for all betaxanthins under investigation except for isoproline-Bx. For the latter, a minor increase (JCL-33) followed by a small decrease during further concentration (JCL-65) was observed. The predominant proline-Bx amounting to 80% of the peak area monitored at 470 nm showed highest retentions confirming previous findings where betalain stability increased with pigment concentration (Merin, Gagel, Popel, & Bernstein, 1987; Moßhammer et al., 2005b). Betanin stability was in the range of the yellow proline-Bx, whereas isobetanin increased up to 120% of the initial content after concentration.

Following concentration at pilot plant-scale, the same trends were observed. However, betaxanthin degradation was higher compared to laboratory scale except for γ-aminobutyric acid-Bx showing similar retention. Interestingly, isoproline-Bx reached up to 200% of the initial values, while betacyanins

Table 7
Total betalain retentions of different cactus fruit juice products assessed by HPLC-DAD measurements [a]

	Product								
	JC	JCL-33	JCL-65	JCP-33	JCP-65	JFD	PFD	JSD	PSD
Total betalain retention [%]	100.0 (±0.0)	96.4 (±0.6)	94.9 (±0.9)	82.6 (±0.1)	70.8 (±2.6)	100.0 (±0.0)	93.0 (±1.2)	100.0 (±0.0)	96.2 (±3.4)

[a] Mean values of duplicate measurements±standard deviation.

Betaxanthins

Histidine-Bx

Glutamine-Bx

γ-Aminobutyric acid-Bx

Proline-Bx

Isoproline-Bx
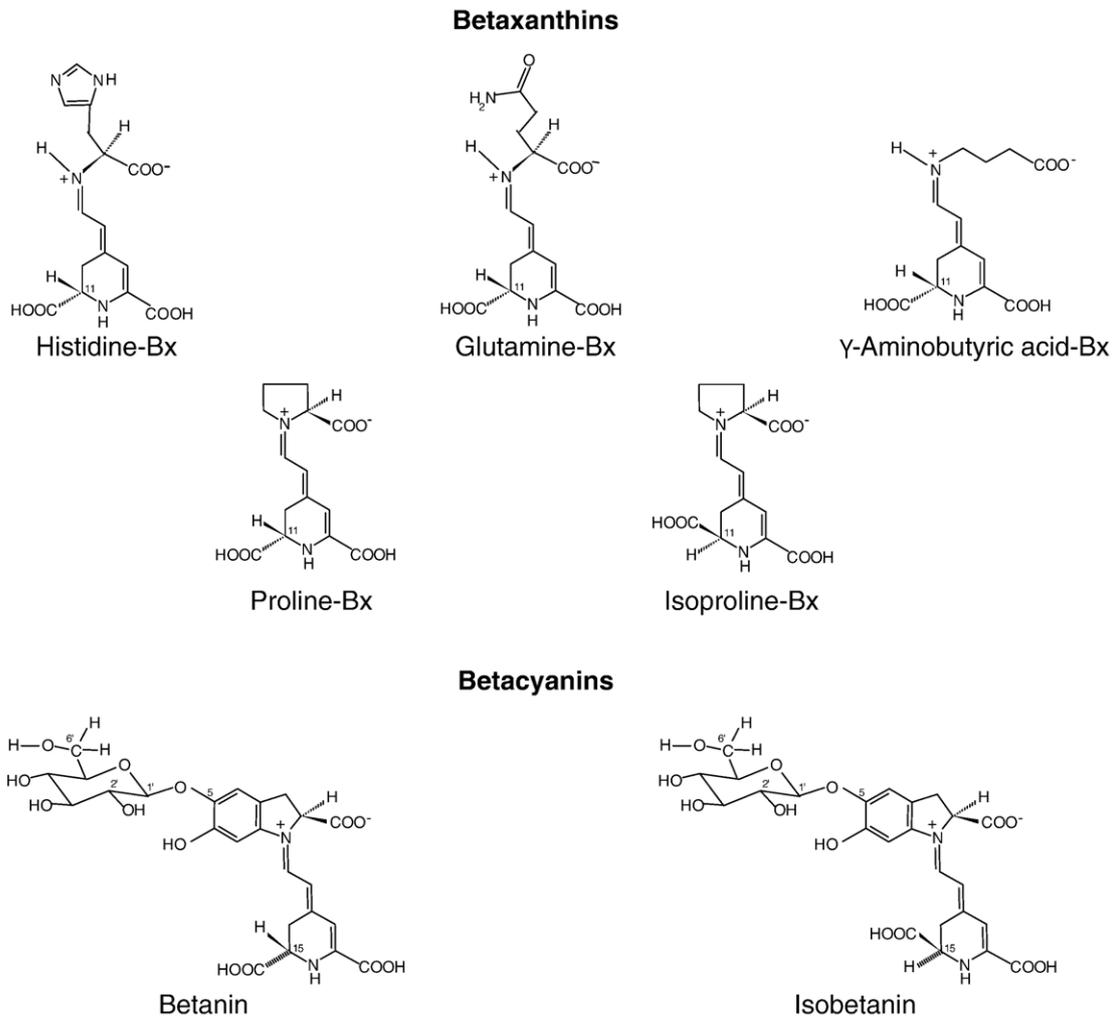
Betacyanins

Betanin

Isobetanin

Fig. 2. Chemical structures of the major betalains in cactus pear.

followed the same trend as observed during laboratory scale evaporation, with lower betanin and higher isobetanin retentions.

Whereas betacyanin stability was generally found to be superior to betaxanthin stability (Herbach et al., 2004b; Moßhammer et al., 2005b), proline-Bx showed a better pigment retention upon evaporation at pilot plant-scale compared to betanin (Table 6). Since cactus pear is rich in proline (Table 3, Stintzing et al., 1999, 2003), the apparently higher thermal stability of proline-Bx and the observed formation of its C_{11}-epimer can be ascribed to the association of proline and betalamic or isobetalamic acid generated after hydrolytic cleavage of betalains (Herbach et al., 2004b). Whereas thermal isomerisation of betanin has been described earlier (Herbach, Stintzing, & Carle, 2004a; Schwartz & von Elbe, 1983; Von Elbe, Schwartz, & Hildenbrand, 1981) this study marks the first report of proline-Bx isomerisation induced by heat.

To visualise the extent of isomerisation during processing, proline-Bx/isoproline-Bx and betanin/isobetanin ratios are shown in Fig. 3. Whereas changes of the proline-Bx/isoproline-Bx ratios were small upon evaporation at laboratory scale

resulting in slightly higher ratios after concentration, pronounced decreases were observed at pilot plant-scale. Decrementing betanin/isobetanin ratios were found in both concentrates, being stronger for pilot plant-scale products.

In freeze-dried products, minor pigment losses (5–20%) were registered for all betaxanthins as well as for betanin. Pigment retentions of about 100% for isobetanin indicates regeneration (Table 6). In contrast to both concentration variants, virtually no changes in both proline-Bx/isoproline-Bx and betanin/isobetanin ratios were determined after freeze drying (Fig. 3).

Upon closer inspection of pigment retentions during spray drying, only minor degradation was observed for all betaxanthins, except for glutamine-Bx (vulgaxanthin I), that unexpectedly showed an increase of 25.7% for PSD, respectively (Table 6). Czapski (1990) found a similar regeneration of glutamine-Bx after heating and regeneration of betanin solutions supplemented with glutamine from red beet. Analogously, betalamic acid released by pigment hydrolysis should have been condensed with free glutamic acid from cactus pear. The reason why this phenomenon was exclusively observed for spray-dried powder could not be disclosed further.
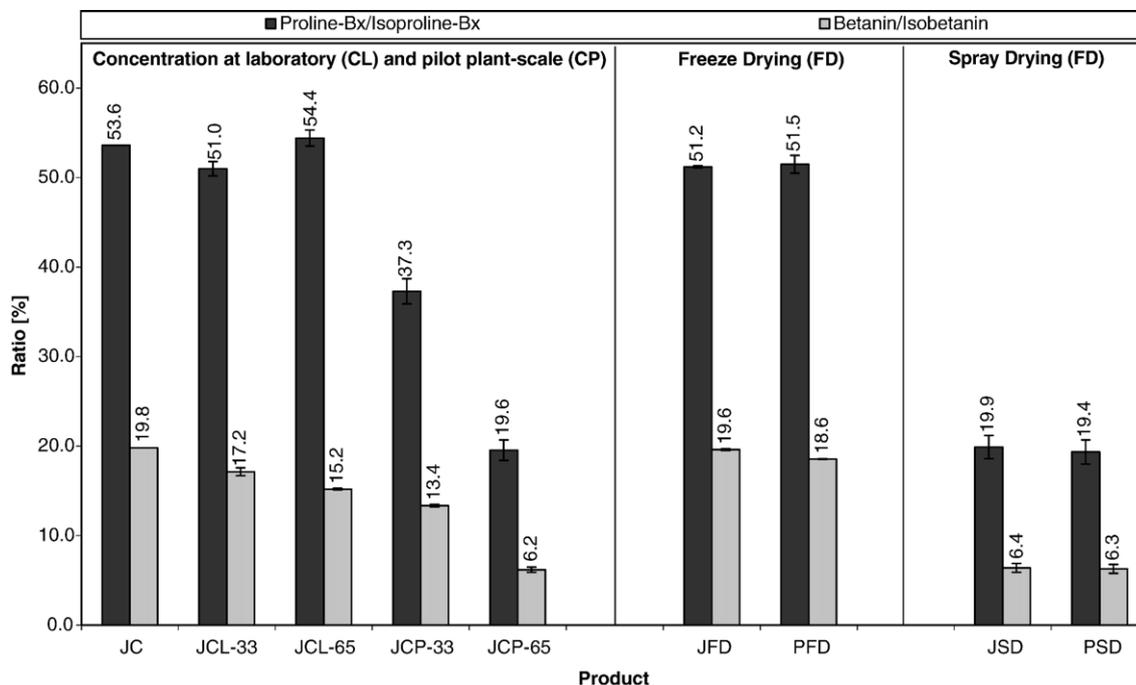
Fig. 3. Changes of proline-Bx/isoproline-Bx and betanin/isobetanin ratios depending on processing variants.

Betanin and isobetanin losses were minor after spray drying. In analogy to freeze drying, both proline-Bx/isoproline-Bx and betanin/isobetanin ratios remained virtually unchanged by spray drying (Fig. 3).

Although product appearance is not affected by isomerisation (Stintzing & Carle, 2004), the present findings demonstrate that such isomer ratios present a suitable indicator for the severity of the respective heat exposure.

3.6. Colour development during processing

During concentration at laboratory scale, virtually no changes in both tonality ($h°$) and Bx/Bc (HPLC) ratio together with minor increases of lightness ($L*$) and chroma ($C*$) were observed, respectively (Fig. 4). Interestingly, colour changes were mainly observed in the first concentration step (JCL-33), whereas the second concentration up to 65% TSS did not significantly affect colour. This may be ascribed to the low water activity which is expected to improve pigment stability (Cohen & Saguy, 1983).

Since a colour difference $\Delta E* = 1$ marks a significant threshold of perceptible changes in overall appearance (Gonnet, 1998), the $\Delta E*$ value of 3.5 for JCL-65 can be considered marginal translating into acceptable colour retention during evaporation at laboratory scale. Colour changes observed after evaporation at pilot plant-scale (JCP) were slightly higher than those caused by laboratory scale concentration resulting in a $\Delta E*$ value of 5.5 for JCP-65.

Despite high drying temperatures ranging from 165 °C (inlet) to 90 °C (outlet), spray drying did not cause significant colour changes reflected by minor total colour differences (Fig. 4). In contrast to spray drying, colour was more strongly affected by freeze drying (Fig. 4). The observed drop of

lightness by 6.5% for PFD may be ascribed to the formation of soluble pre-melanoidins as indicated in Table 4. The slight tonality ($h°$) shift towards red was accompanied by a minor decrease of the Bx/Bc ratio (HPLC), pointing to a superior stability of the red betacyanins.

4. Conclusions

In the present study, a previously published process (Moßhammer et al., 2005b) for cactus fruit processing into single strength juices was considerably improved and extended to obtain colour concentrates and fruit powders. First of all, the crushing process of the raw material was simplified through replacing carborundum peeling of frozen fruits by grinding fresh fruits. Thus, an easier handling of industrial batches together with an increase of the juice yield from 37% to 47% was achieved. By processing fresh instead of frozen cactus fruits, the process is considered more economic. However, milling of fresh instead of thawed cactus pears required a different enzyme preparation for complete degradation of pectin-like substances. Furthermore, the replacement of pasteurisation by cold-sterile microfiltration (0.2 μm) resulted in identical betalain retentions with the additional effect of virtually complete haze separation.

Despite high amounts of reducing sugars and amino acids, only minor Maillard browning effects were found both for concentrates produced by evaporation at pilot plant-scale and freeze-dried powder. In particular, basically early Maillard reaction products (MRPs, soluble pre-melanoidins) detectable between 340 and 360 nm were registered, whereas advanced MRPs (420 nm) were only observed in freeze-dried powders. Moreover, proline-Bx/isoproline-Bx ratios
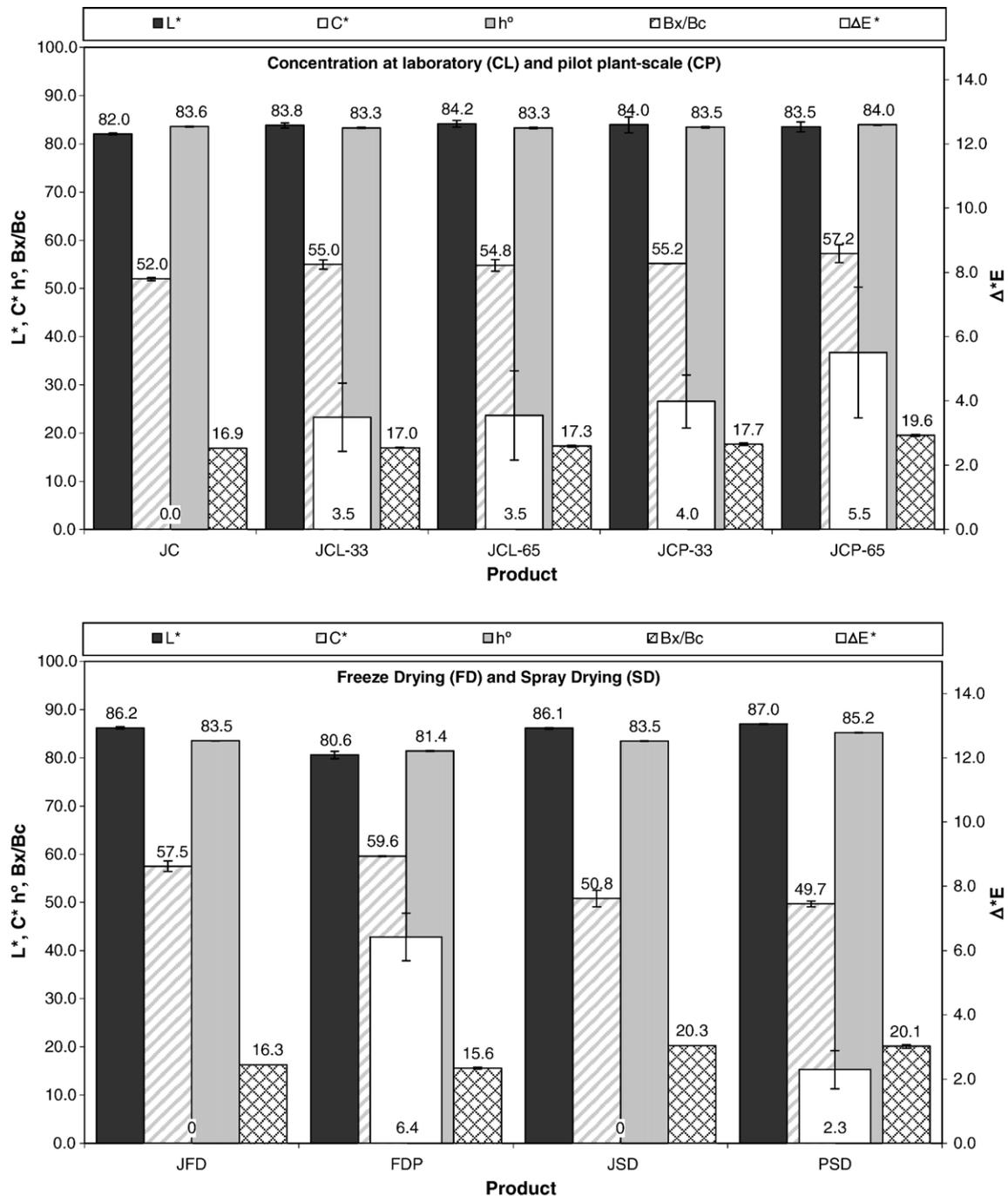
Fig. 4. Lightness (L^*), chroma (C^*), hue angle ($h°$) and total colour difference ΔE^* monitored after each processing step based on a tinctorial strength of 1.0 ± 0.1 at λ_{max}, and Bx/Bc ratio as determined by HPLC.

were shown to be a good indicator of heat exposure being even more sensitive than the respective betanin/isobetanin ratios.

Acceptable overall pigment retentions of 71–83% (Table 7) combined with the retention of the initial colour properties after reconstitution of semi-concentrated and concentrated preparations, proved the viability for industrial cactus pear juice processing. Finally, the use of fruit powders for colouring dessert preparations, fruit or cereals bars, instant dishes and chocolates opens new fields of application for cactus pear fruits.

Thus, cactus pear is considered a new valuable source of water-soluble yellow-orange colour preparations.

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