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Fermentation of *Opuntia stricta* (Haw.) Fruits for Betalains Concentration

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Fermentation of juice and homogenized fruits of *Opuntia stricta* fruits has been developed and optimized. The aim was to obtain the red food colorant betanin from prickly pear, at high concentration and low viscosity. Among three strains assayed, *Saccharomyces cerevisiae* var. *bayanus* AWRI 796 has been the optimum for this process. The optimum temperature value was found to be 35 °C for both sugar consumption and pigment preservation. After fermentation, biomass and residual vegetal tissue were discarded by centrifugation. Supernatant was concentrated under vacuum. Therefore, liquid concentrated betanin was obtained, with low viscosity and being sugar free. Besides, bioethanol was obtained as byproduct. Characteristics of the final product obtained were pH 3.41, 5.2 °Brix, 9.65 g/L betanin, color strength of 10.8, and viscosity of 52.5 cP. These values are better than obtained by other procedures.

KEYWORDS: ***Opuntia stricta*; betanin; betalains; betacyanins; color; food colorants; *Saccharomyces cerevisiae*; ethanol fermentation**

INTRODUCTION

Research on natural food colorants has been extensively conducted in recent years (*1–3*). Food colorants are usually employed to improve the appearance and consumer acceptability of processed foods. The red-purple color range is covered by some natural colorants such as cochineal (E-120), beetroot red (E-162), and anthocyanins (E-163).

The beetroot red pigments, usually called red-beet or betanin (E-162), are betalains. These are natural pigments typically associated with plants of the order Caryophyllales (*2, 3*). These water-soluble compounds comprise the red-violet betacyanins and the yellow betaxanthins. They are immonium conjugates of betalamic acid with cyclo-dopa and amino acids or amines, respectively (*3*). Besides their colorant properties, betacyanins exhibit significant antioxidant activity (*4–6*). Thus, the added value of these pigments is increased due to its double functionality, as food color and antioxidant.

The most important source of betalains as natural red colorant is the red beet roots (*Beta vulgaris* subsp. *vulgaris*) included in the Chenopodiaceae family. Other sources of betalains are some species from the genus *Amaranthus* (*7, 8*), from the Amarntaceae family, accepted as food colorant in China, and species that belong to the Cactaceae family, such as *Myrtillocactus geometrizans* (*9, 10*), *Hylocereus polyrhizus* (*11–14*), and some *Opuntia* species (*15–20*).

The use of cactus fruits as betalains source is an interesting possibility because these plants have minimal soil and water requirements and may be regarded as an alternative for the agricultural economy of the Mediterranean countries and other arid and semiarid regions. Recent research done with different *Opuntia* species has revealed that *Opuntia stricta* is a promising source of betacyanin pigments. It has high levels of betanin (800 mg kg^{-1}), even higher than that shown by some commercial red beets exploited for their purple color (*18*). In addition, *Opuntia* fruit is highly flavored and shows better nutritional properties than red beet root (*16*), which can help to add value to this ingredient. Extracts from *O. stricta* lack the yellow betaxanthins, betacyanins being the same chemical species as those present in beetroot red, a recognized and safe food color (*18*).

High concentrations of water-soluble compounds are extracted with the pigments of prickly pear, mainly carbohydrates. This fact results in a diluted pigment preparation, which has to be added at high concentrations to reach the desired technological effect. Thus, preparations of red colorants could contain low amounts of pigment and significant concentrations of sugar, with the consequent contamination risk.

There are different techniques to purify diluted extract with high carbohydrate content. Thus, membrane filtration can be used, but it is limited by operational problems and small differences in the molecular weights of the compounds to be separated. The retention of interesting colorants in a column filled with an adequate resin, such as Amberlite XAD 16, would also be difficult due to the high viscosity of the vegetal material. Use of pectolytic enzymes would decrease viscosity due to pectins but have no effect on the most abundant sugars in prickly pear, glucose and fructose (*20*). In this way, elimination of undesirable carbohydrates by fermentation is a simple and useful

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operation provided that the compounds of interest remain unchanged after the fermentation process.

Saccharomyces sp. is the safest and most effective microorganism for fermenting sugars to ethanol and is used in the industry for bioalcohol production. Besides, several alcoholic beverages such as wine or liqueurs are obtained from fruit juices fermented by *Saccharomyces cerevisiae* strains. Although not extensively, *S. cerevisiae* has also been employed to remove undesired compounds by fermentation. Thus, this yeast has been used to remove monosaccharide and disaccharide byproducts in carbohydrate preparations (*21*) and to remove benzoic acid in concentrated lingo berry juices (*22*). This technique is also employed in some industrial purification processes, where the yeast consumes the undesired byproduct, simplifying the purification and concentration procedures.

Fermentation of red beet juice (*23, 24*) and of some cactus fruits (*25, 26*) with *Saccharomyces* sp. has been previously reported and pigment recovery studied. Other researchers report fermentation of prickly pear juice for alcoholic beverage production (*27–29*).

In the present work fermentation is proposed as a technique to obtain concentrated betanin colorant from *O. stricta*. The carbohydrates are removed by fermentation, whereas bioalcohol is obtained as byproduct together with concentrated betanin. Ethanol produced can be recovered for bioalcohol uses. The aim was to obtain a sugar-free concentrated red-purple pigment preparation of low viscosity. In this way, fermentation of *O. stricta* fruits with *S. cerevisiae* strains was optimized, the fermented medium concentrated, and the obtained colorant solution characterized.

MATERIALS AND METHODS

Microorganisms. *S. cerevisiae* AWRI 350 and *S. cerevisiae* var. *bayanus* AWRI 796 were from the Australian Wine Research Institute, and *S. cerevisiae* var. *cerevisiae* was from Fermol Rouge (Pascal Biotech). The three lyophilized microorganisms were activated by suspension in water and incubation during 30 min at 30 °C, prior to inoculation.

Chemicals. 3,5-Dinitrosalicylic acid was from Sigma (Madrid, Spain). Ethanol, of HPLC grade, was purchased from Laboratory Scan (Dublin, Ireland). Water was purified in a Milli-Q water purification system from Millipore (Bedford, MA). All other chemicals employed were of analytical grade.

Plant Material. Ripened *O. stricta* (Haw.) fruits, of red-purple color, were selected for this study. This plant is grown in the Murcia municipal gardens (Spain) and in the experimental field of the Polytechnic University of Cartagena (Spain). The fruits were harvested in January 2007. After harvesting, they were carefully washed with water to remove the glochids and then homogenized with a T25 basic Ultraturrax (Ika-Werke, Staufen, Germany) without removal of the peel or seeds, as has been previously reported (*18*). Homogenized fruit fractions were frozen at −20 °C until use.

Fruit Juice and Fruit Extract. To obtain fruit juice, the homogenized fruits were clarified by centrifugation at 15000*g* and 10 °C during 15 min in a Z383K Hermle centrifuge (Wehingen, Germany) to remove the tissue residue. The fruit extracts were obtained as previously reported (*18*), by stirring of the homogenate fruits for 20 min in darkness using a 1:5 (w/v) ratio of fruit/water and later centrifugation as described above.

Fermentation. Assays were performed with fruit juice as fermentation medium, except in the substrate state study where four types of differently extracted/prepared fruit juices were used as growth media. These were the homogenized fruit, the fruit juice (clarified by centrifugation), a fruit water extract 1:5 (w/v), and homogenized fruit suspended in water 1:5 (w/v).

A different amount of fruit was used to obtain 50 mL of each fermentation medium. Thus, 50 g was necessary to obtain 50 mL of homogenized fruit and 73 g to obtain 50 mL of fruit juice; 10 g was employed for the homogenized fruit suspension 1:5 (w/v), and 14.6 g was used for the fruit water extract 1:5 (w/v).

To conduct the fermentation 250 mL Erlenmeyer flasks containing 50 mL of fermentation medium were incubated on an orbital shaker Rotabit (Selecta, Spain), at 200 rpm and temperature ranging from 25 to 35 °C. All assays were performed in triplicate. Samples were withdrawn during fermentation and analyzed. Sugar conversion (percent) was determined as the percentage of sugar consumed referred to the initial sugar concentration. Color recovery (percent) was expressed as the percentage of color preserved referred to the initial color amount. Color recovery productivity was determined by taking into account the color amount recovered and the fermentation time and was calculated as the ratio of betanin concentration (expressed as milligrams per kilogram of fresh fruit) to fermentation time.

Pigment Concentration. After fermentation, biomass and vegetal residue tissue were discarded by centrifugation at 15000*g* and 10 °C during 10 min in a Z383K Hermle centrifuge. Supernatants were concentrated using a vacuum concentrator Büchi Rotavapor R-200 (Flawil, Switzerland) at 30 °C and −30 mbar until the desired concentration was reached.

Biomass Determination. Biomass was determined as absorbance at 660 nm. To correlate absorbance with dry weight values, different cellular concentration samples were prepared at concentrations ranging from 0 to 0.2 g/L, and their absorbance at 660 nm was determined. A straight line of cellular dry weight versus absorbance at 660 nm was obtained.

Spectrophotometric Analyses. The visible spectra (380−780 nm) of the colorants were recorded using an Agilent 8453 UV−visible spectrophotometer (Waldbronn, Germany). Color strength of liquid samples was determined as absorbance units of a 1% (v/v) solution at 535 nm, the maximum betanin wavelength absorption. The prickly pear red pigment content was referred to betanin and determined by using the extinction coefficient of betanin at 535 nm ($E^{1\%}_{1cm} = 1120$) (*30*).

Other Analytical Tests. Sugar was measured according to the dinitrosalicylic method (*31*). Soluble solids content (°Brix) was measured using a Zeiss Opton refractometer at 20 °C. The pH was measured using a Crison micropH200 pH-meter. Viscosity (cP) was determined in a Canon-Fenske viscosimeter. Water content was determined using a Karl Fischer Titrator DL38 (Mettler-Toledo). All of the analytical measurements were performed in triplicate; values reported are the average of three analyses.

Statistical Analysis. The mean values, standard deviations, and analyses of variance (ANOVA) were calculated with Minitab statistical software, version 13.2 (Minitab Inc., State College, PA). Mean comparisons were performed using Tukey's test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Inoculum Size. For inoculum size optimization, fruit juice was used as fermentation medium. The three *Saccharomyces* strains assayed were activated by suspension at 100 g/L in water and incubation during 30 min at 30 °C. After activation, four different inoculum sizes, 0.2, 0.4, 0.8, and 1.2 g/L, were assayed. Therefore, 12 simultaneous fermentation runs were performed at 30 °C. **Table 1** reports biomass values obtained. Biomass was determined at exponential phase, 6 h fermentation after started, and at stationary phase, 30 h after fermentation started. As can be seen, biomass increased with inoculum size up to 0.8 g/L in all of the *Saccharomyces* strains studied. No significant differences in biomass were observed between 0.8 and 1.2 g/L. Thus, 0.8 g/L was selected as optimum inoculum size. A concentrated inoculum would also help to avoid juice contamination with microorganisms other than *S. cerevisiae*. These experiments also allow us to confirm that *O. stricta* fruit juice is easily fermented with *Saccharomyces* strains. Some problems with prickly pear juice fermentation have been previously reported by other authors. Thus, the addition of grape juice to facilitate the yeast growth has been reported (*27*). In

Table 1. Biomass Values Obtained in the *Opuntia stricta* Fruit Juice Fermentation Using Different Inoculum Sizes of Three *Saccharomyces cerevisiae* Strains[a]

inoculum (g/L)	biomass, 6 h (g/L)	biomass, 30 h (g/L)
S. cerevisiae AWRI 350		
0.2	1.08 ± 0.03 a	4.32 ± 0.04 a
0.4	1.47 ± 0.06 b	4.44 ± 0.07 a
0.8	2.16 ± 0.11 c	5.76 ± 0.08 b
1.2	2.07 ± 0.07 c	5.83 ± 0.10 b
S. cerevisiae Var. *bayanus* AWRI 796		
0.2	1.65 ± 0.02 a	4.44 ± 0.06 a
0.4	1.95 ± 0.12 b	4.74 ± 0.05 b
0.8	2.19 ± 0.06 c	4.92 ± 0.02 c
1.2	2.20 ± 0.08 c	4.87 ± 0.08 c
S. cerevisiae Var. *cerevisiae* FERMOL		
0.2	1.02 ± 0.09 a	4.26 ± 0.10 a
0.4	1.53 ± 0.05 b	4.50 ± 0.04 b
0.8	2.13 ± 0.07 c	5.04 ± 0.03 c
1.2	2.00 ± 0.02 c	4.95 ± 0.07 c

[a] Different letters indicate significant differences within each column at $p \leq 0.05$ (Tukey's test).

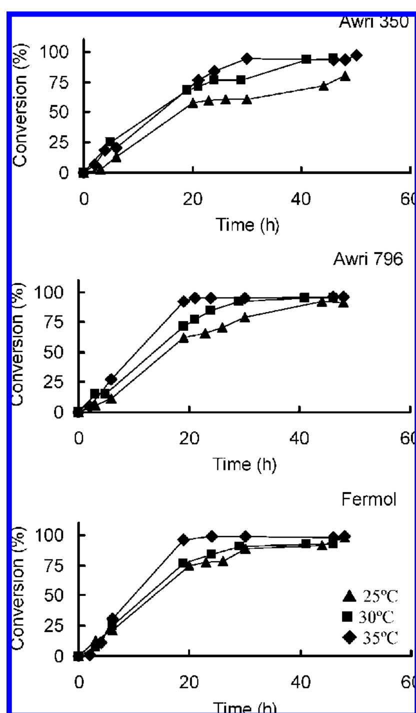

Figure 1. Sugar conversion in fruit juice with three *Saccharomyces* strains.

the present work no inhibition was observed, and prickly pear juice fermentation was performed in a short time with the three strains assayed.

Effect of Temperature. The fermentation temperature must be optimized to reduce the run time, obtaining simultaneously both the highest pigment concentration and the highest sugar conversion. In this way, nine fermentation runs were performed at 25, 30, and 35 °C with the three *Saccharomyces* strains studied, respectively. *Saccharomyces* grows slowly below 25 °C, and betalains degrade quickly at temperature above 35 °C (*19*). The temperature range was selected with attention to both the *Saccharomyces* growth rate and the betalains' stability. **Figure 1** shows the sugar conversion of the assays performed. With the three strains, sugar was more quickly consumed when temperature increased. Thus, the shortest runs were at 35 °C. *S. cerevisiae* AWRI 350 consumed sugar more slowly than the

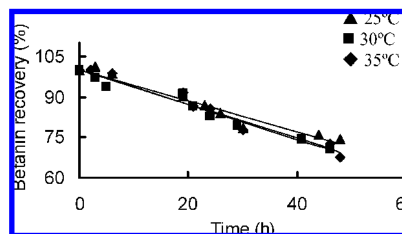

Figure 2. Betanin recovery from fruit juice fermented with *S. cerevisiae* var. *bayanus* AWRI 796.

Table 2. Effect of Temperature in *Opuntia stricta* Fruit Juice Fermentation[a]

temperature (°C)	sugar conversion (%)	color recovery (%)	color recovery productivity (mg/kg/h)
S. cerevisiae AWRI 350			
25	70.5 ± 0.4 a	72.3 ± 0.3 a	9.1 ± 0.6 a
30	77.0 ± 0.2 b	76.8 ± 0.4 c	15.6 ± 0.9 b
35	84.1 ± 0.5 c	81.5 ± 0.7 e	20.6 ± 0.9 c
S. cerevisiae Var. *bayanus* AWRI 796			
25	91.4 ± 0.3 d	74.2 ± 0.5 b	9.4 ± 0.7 a
30	94.0 ± 0.4 e	79.2 ± 0.7 d	16.0 ± 0.3 b
35	96.8 ± 0.6 f	85.6 ± 0.3 g	21.6 ± 0.4 d
S. cerevisiae Var. *cerevisiae* FERMOL			
25	98.1 ± 0.2 g	72.2 ± 0.7 a	9.1 ± 0.8 a
30	98.9 ± 0.3 g	78.2 ± 0.8 d	15.8 ± 0.5 b
35	98.9 ± 0.2 g	83.4 ± 0.1 f	21.1 ± 0.6 d

[a] Different letters indicate significant differences within each column group at $p \leq 0.05$ (Tukey's test). Values were determined 48, 30, and 24 h after inoculation, when temperature values were 25, 30, and 35 °C, respectively.

other two strains within the tested range. The behaviors of *S. cerevisiae* var. *bayanus* AWRI 796 and of *S. cerevisiae* var. *cerevisiae* FERMOL were very similar, and both achieved sugar conversion values up to 95% at 35 °C in 24 h. In these cases, a temperature increase from 25 to 35 °C led to a reduction of the fermentation time from 48 to 24 h, whereas *S. cerevisiae* AWRI 350 needed 30 h to ferment 95% sugar at 35 °C.

Betalalin stability was determined during fermentation as color recovery as a function of time. The pigment stability profiles were similar for the three strains. **Figure 2** shows results obtained with *S. cerevisiae* var. *bayanus* AWRI 796. As can be seen, betanin recovery was more affected by the incubation time than by the temperature within the studied range. Betanin recovery after 48 h at 25 °C was 72.6% and was 67.7% at 35 °C, only a 4.9% difference due to temperature and about 30% due to time. Therefore, reduction of fermentation time was a key to maximizing pigment recovery.

Sugar conversion, color recovery, and color recovery productivity are reported in **Table 2**. As can be seen *S. cerevisiae* var. *cerevisiae* showed the highest sugar conversion regardless of fermentation temperature. With the other two strains sugar conversion increased with temperature, achieving the best sugar conversion at 35 °C. *S. cerevisiae* AWRI 350 achieved the lowest sugar conversions in all assays. *S. cerevisiae* var. *bayanus* AWRI 796 showed sugar conversions higher than AWRI 350, and the value obtained at 35 °C (96.8%) was close to that obtained with *S. cerevisiae* var. *cerevisiae* (98.9%).

Color recovery showed a slight increase with temperature as a consequence of the incubation time reduction by the higher fermentation rate. With the three strains studied, the best results were obtained at 35 °C. *S. cerevisiae* var. *bayanus* AWRI 796 achieved the highest color recovery at the optimum temperature of 35 °C.

Color recovery productivity was determined as a function of fermentation time. At 25 and 30 °C no significant differences

Table 3. *Opuntia stricta* Fruit Juice Characteristics before and after Fermentation at 35 °C[a]

	pH	density (g/mL)	viscosity (cP)	viscosity reduction (-fold)
initial juice	3.31 ± 0.09 a	1.030 ± 0.060 a	107.31 ± 0.24 d	
fermented with				
S. cerevisiae AWRI 350	3.38 ± 0.08 a	0.996 ± 0.003 a	7.14 ± 0.23 c	15.03 ± 0.23 a
S. cerevisiae var. *bayanus* AWRI 796	3.38 ± 0.12 a	0.994 ± 0.008 a	4.78 ± 0.18 a	22.45 ± 0.20 c
S. cerevisiae var. *cerevisiae* FERMOL	3.30 ± 0.11 a	1.000 ± 0.006 a	5.72 ± 0.32 b	18.76 ± 0.25 b

[a] Different letters indicate significant differences within each column group at $p \leq 0.05$ (Tukey's test).

Table 4. Characteristics and Fermentation Parameters of the Different Substrates Assayed as Fermentation Media, at 35 °C, 200 rpm, with AWRI 796[a]

	homogenized fruit	juice fruit	suspension fruit 1:5 (w/v)	fruit extract 1:5 (w/v)
fruit amount[b] (g)	50	73	10	14.6
initial sugar (g/L)	72.3 ± 0.1	65.8 ± 0.4	14.2 ± 0.3	12.5 ± 0.5
initial betanin (mg/L)	1003.7 ± 0.6	885.6 ± 0.2	193.1 ± 0.3	166.1 ± 0.7
fermentation time (h)	26	24	14	12
color recovery (%)	89.1 ± 0.3 a	92.7 ± 0.4 b	94.5 ± 0.8 c	95.2 ± 0.7 c
betanin (mg/L)	894.2 ± 0.8 d	821.6 ± 0.6 c	183.3 ± 0.8b	157.4 ± 0.9a
betanin productivity (mg/L·h)	34.4 ± 0.4 b	34.2 ± 0.6 b	13.1 ± 0.2 a	13.2 ± 0.3 a

[a] Different letters indicate significant differences within each row group at $p \leq 0.05$ (Tukey's test). [b] To obtain 50 mL of fermentation medium.

were observed between the three strains assayed, whereas at 35 °C. *S. cerevisiae* AWRI 350 showed lower color recovery productivity than the other two strains. Results with *S. cerevisiae* var. *cerevisiae* and *S. cerevisiae* var. *bayanus* AWRI 796 were similar.

In all assays the best results for sugar conversion, color recovery, and color recovery productivity were obtained at 35 °C, which means the shortest fermentation time. Consequently, this temperature value was selected as the optimum for *O. stricta* fruit juice fermentation.

In previous works by other authors (*23, 24*), fermentation of red beet juices showed pigment recovery between 56 and 65%. These values have been improved in the present work, achieving up to 85.5% color recovery rate with *S. cerevisiae* var. *bayanus* AWRI 796.

On the other hand, the objective of the fermentation was to eliminate sugars and to reduce the viscosity of the final product. Thus, different juice characteristics were determined before and after fermentation in the three assays performed at 35 °C. Results are shown in **Table 3**. pH and density values did not suffer any significant variation, whereas viscosity was 15-fold reduced using *S. cerevisiae* AWRI 350, 18 times reduced using *S. cerevisiae* var. *cerevisiae*, and 22-fold reduced with *S. cerevisiae* var. *bayanus* AWRI 796. These results, together with those reported above, allowed us to select *S. cerevisiae* var. *bayanus* AWRI 796 as the most appropriate strain for *O. stricta* fruit juice fermentation. Therefore, further work was performed with this *Saccharomyces* strain at 35 °C.

Influence of the Substrate State. All assays reported above have been performed with fruit juice clarified by centrifugation. The fermentation of the homogenized fruit, directly without previous clarification, can also be done. Besides, in previous research we had worked with an aqueous extract 1:5 fruit/solvent ratio. Thus, to study the effect of substrate state on betanin concentration, fermentation was performed, with homogenized fruit, fruit juice (clarified by centrifugation), homogenized fruit suspended in water 1:5 (w/v), and water extract 1:5 (w/v) (clarified by centrifugation). All assays were run with 50 mL of fermentation medium, at 35 °C and 200 rpm.

Table 4 reports the characteristics of the four fermentation media used, obtained as reported under Materials and Methods. A different initial sugar concentration value was obtained for each assay, ranging from 72.3 to 12.5 g/L. Fermentation time was considered when 95% sugar conversion was obtained. Results of fermentation parameters are also shown in **Table 4**. Initial sugar concentration determined the fermentation time. Thus, fruit extract with 12.5 g/L sugar was quickly fermented, whereas homogenized fruit was fermented only after 26 h. Suspension fruit and fruit juice ended up at 12 and 24 h, respectively. Color recovery was also influenced by the fermentation time, as was expected. Betalains are degraded by prolonged incubation time, not by the yeast, as has been previously discussed. After 12.5 h, 4.8% of betanin is lost in the fruit extract fermentation, whereas after 26 h, 17.6% was degraded in the homogenized fruit assay. The other two assays achieved color recovery values between them.

Regarding betanin concentration and productivity, poor values were obtained for suspension fruit and fruit extract assays, due to the low betanin initial concentration of these fermentation media. Although they were fermented in shorter times, the low productivity achieved makes them inappropriate procedures for industrial scale-up. As the objective was to obtain concentrated betanin, it is most interesting to ferment the homogenized fruit or the fruit juice. Both of them were conducted at high betanin concentrations, 894 and 821 g/L, and also at high betanin productivity, around 34 mg/L·h. In comparison with suspension fruit or fruit extract, the fermentation time was duplicated, whereas betanin productivity increased 2.6 times and betanin concentration around 5 times.

Although the lowest color recovery value was for the homogenized fruit, this substrate state achieved both the highest betanin concentration and the highest betanin productivity after fermentation. There are no previous references in the literature about direct fermentation of homogenized fruit. Other works report fermentation of prickly pear juice for alcoholic beverages (*27–29*) or pigment (*26*) production. Results reported here demonstrate that homogenized fruit can be directly fermented, avoiding the clarification steps for juice preparation. This substrate could be considered as a semisolid culture, which offers some advantages, such as reduction of volume. In addition, the product is easily concentrated.

After fermentation, biomass and vegetal residue were discarded by centrifugation and alcohol and water were removed under vacuum to obtain a betanin-rich liquid. **Table 5** shows its characteristics in comparison with the results of a previous work without fermentation (*19*).

In the previous work a betanin-rich extract was prepared from prickly pear by extraction with ethanol/water at 60% w/v (*19*).

Table 5. Characteristics of Liquid Betanin after Fermentation and Concentration[a]

parameter	extracted fruit[b]	fermented fruit
concentration factor	40.2 ± 0.4 b	15.1 ± 0.1 a
pH	3.37 ± 0.01 a	3.4 ± 0.02 a
°Brix	56.0 ± 0.2 b	5.2 ± 0.3 a
betanin (g/L)	4.73 ± 0.07 a	9.65 ± 0.05 b
color strength (OD 535 nm, 1% sol)	3.9 ± 0.1 a	10.8 ± 0.2 b
moisture (%)	57.1 ± 0.2 b	50.3 ± 0.3 a
viscosity (cP)	59.0 ± 0.3 b	52.5 ± 0.2 a
density (g/mL)	1.23 ± 0.02 b	1.17 ± 0.03 a

[a] Different letters indicate significant differences within each row group at $p \leq 0.05$ (Tukey's test). [b] Castellar et al. (*19*).

This meant dilution of the juice extract and 40-fold concentration. With the new procedure reported in this work no solvent extraction has to be added and sugar transformation into ethanol allows for a preparation of more concentrated pigment by a simpler process. As can be seen from **Table 5**, fermentation allows for a higher color concentration (expressed either as betanin or as color strength) and much lower soluble solids (°Brix) due to alcohol elimination for a similar water content.

Furthermore, bioalcohol is produced as a byproduct in this process. Therefore, it could be possible to develop an integrated process including betalains concentration and bioethanol production.

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