Nisin based stabilization of novel fruit and vegetable functional juices containing bacterial cellulose at ambient temperature

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Abstract The current study reports the preparation and stabilization of novel functional drinks based on fruit and vegetable juices incorporating bacterial cellulose from *Acetobacter xylinum*. Pineapple, musk melon, carrot, tomato, beet root and a blend juice containing 20 % each of carrot and tomato juice with 60 % beet root juice has been studied. These juices have been stabilized over a storage period of 90 days at 28 °C, by the use of nisin and maintaining a low pH circumventing the need for any chemical preservatives or refrigeration. Instrumental color values have been correlated with the pigment concentrations present in the fresh as well as stored juices. There was 36, 72 and 60 % loss of total carotenoids in the case of carrot, pineapple and musk melon juices respectively while the lycopene content remained unchanged after 90 days of storage. The betanin content decreased 37 % in the case of beetroot juice and 25 % in the case of beetroot juice blended with carrot and tomato juices. Sensory analysis has revealed a clear preference for the beetroot blended mixed juice.

Keywords *Acetobacter xylinum* · Microbial cellulose · Nata

Introduction

Bacterial cellulose (*nata*) produced extracellularly by *Acetobacter xylinum* on the air-liquid interface, has many uses in the food industry such as to modify rheological properties, as a fat substitute, non-caloric bulking agent, stabilizer, thickener and texturizer. Most of these applications are related to its unique physico-chemical properties like high purity, water

holding capacity, unique fluid property, high surface area and being highly amorphous. Bacterial cellulose has been economically produced from a wide range of food process effluents like low-solids potato process effluents, cheese whey permeate, concentrated sugar beet raffinate (Thompson and Hamilton 2000), fruits and vegetable processing wastes like tomato peel waste, pineapple pomace waste, waste coconut water (Jagannath et al. 2011) and beet molasses (Keshk et al. 2006). Bacterial cellulose in the form of *nata* is virtually indigestible and therein lies its importance. Being fiber having high water holding capacity it could help in bowel movement and maintaining intestinal health.

Fruit and vegetables juices are important sources of vitamins, minerals, pigments etc., most of which have been established to have antioxidant and free-radical scavenging activities (Leonard et al. 2002). However these juices lack the fiber content originally present in their fresh forms. The supplementation of these juices with bacterial cellulose (*nata*), hence offers an alternative fiber source. Further, since *nata* is bland, it does not adversely affect the sensory properties. In fact the chewiness, springiness properties which *nata* offers in juices, provides a relishing experience when these juices are consumed.

The present work involves preparation of novel functional drinks based on fruit and vegetable juices incorporating bacterial cellulose from *Acetobacter xylinum*. The product has been stabilized over a storage period of 90 days by the use of nisin and maintaining a low pH circumventing the need for any chemical preservatives or refrigeration.

Materials and methods

Preparation of bacterial cellulose/*nata*

Bacterial cellulose or *nata* was prepared as described in our earlier papers (Jagannath et al. 2008, 2011). *Acetobacter*

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xylinum (NCIM 2,526) culture obtained from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune, India was grown under static conditions in modified medium (Hestrin and Schramm 1954). The layer of *nata* formed between 10 and 15 days time was harvested when it was about 1.0 cm thick, washed repeatedly with water and cut into small dices of equal dimensions (5 mm). The cut cubes of *nata* were immersed in water for 24 h with repeated changing of water to remove bacterial cell and medium residues, sterilized by autoclaving (stove top autoclave, 121 °C, 15 min, 15 psi pressure) and used in fruit and vegetable juices.

Preparation of fruit and vegetable juices

Carrot, tomato, beetroot, pineapple, musk melon were obtained from local market in Mysore, India, washed thoroughly, grated into thin slices and the juice extracted in a blender. The juice was filtered with many layers of muslin cloth. Carrot (CJ), tomato (TJ), beet root (BRJ), pineapple (PJ) musk melon (MMJ) individually and a mixed blend containing 20 % each of carrot and tomato juice with 60 % beet root juice (beetroot blend, BRB) were made. Wet bacterial cellulose cubes (100 g/200 ml) was added to the individual juices as well as to the beet root blend.

Nisin (Nisaplin, 1,000,000 units/g, 25,000 ppm, Aplin and Barrett Limited, Yeovil, Somerset, England) was added at 20 ppm level to the juices and pH adjusted to 4.0–4.2 with citric acid. Cane sugar was added to bring the TSS to 15 °Brix, the juice was packed in polyethylene standy pouches and subjected to heat treatment. Heat treatment was given in the form of tyndallization. The pouches were heated for 5 min on three successive days by immersing in a boiling water bath maintained at 98 °C. The juice was stored at 28 °C under dark and periodically analyzed for chemical, microbiological, sensorial attributes, pigment concentrations and color values as described below.

Chemical analysis

Total Soluble Solids (TSS), pH, content of relevant pigments like betanin, lycopene and total carotenoids in fresh juices, as well as after storage (28±5 °C) for a period of 90 days were measured at monthly intervals. Storage was done in an insulated wooden closed cabinet and temperature was monitored periodically using a thermometer. pH was measured using a pH meter (Cyber Scan, Eutech Instruments, India; Accuracy±0.01). Total soluble solids (TSS) content was determined using a digital refractometer (PAL-I, ATAGO, Tokyo, Japan; Accuracy±0.2 %).

Spectrophotometric determination of betanin, lycopene and total carotenoids

Beetroot juice (BRJ) or (BRB) was diluted with 0.05 M phosphate buffer (pH 6.5), such that the absorbance A_{538} of the sample was between 0.4 and 0.5 AU. The absorbance at 538 & 476 nm corresponding to the $\lambda_{\max}$ of betacyanins & betaxanthins respectively were measured spectrophotometrically (Nilsson 1970). In addition, the absorbance at 600 nm to correct for small amounts of impurities was also recorded. The light absorption measurement at A_{538} and A_{476} includes all minor betacyanins & betaxanthins respectively. Betanin (mg/100 g), the major betacyanin in beet root, was calculated based on the molar absorptivity value of 1,120 for a 1 % solution and applying the appropriate dilution factor.

Five to ten grams of the tomato juice was extracted with acetone until the residue was colorless. The acetone extract was mixed with petroleum ether in a separating funnel and the pigments were taken into petroleum ether by repeated extraction. A small quantity of sodium sulphate was added to the extract, volume made upto 50 and a 1 ml aliquot taken to measure the absorbance at 503 nm. The lycopene content was calculated applying appropriate dilution factor and based on the relationship that an optical density of 1.0=3.1206 µg of lycopene per ml.

Five to ten grams of pineapple, carrot or musk melon juice was extracted with 40 ml acetone, 60 ml hexane and 0.1 g magnesium carbonate in a blender for 5 min. The contents were transferred into a separating funnel and washed repeatedly with acetone hexane mixture. The extracts were combined and acetone removed with repeated washings with distilled water. The hexane layer was collected, made up to 100 ml with hexane, a pinch of sodium sulphate added to remove moisture and the absorbance read at 450 nm with hexane as blank. The total carotenoid concentration (mg/100 g) was calculated based on the molar absorptivity value of 2,500 for a 1 % solution and applying the appropriate dilution factor.

Sensory analysis

The sensory panel consisted of ten volunteers who were trained for the flavor attributes in a preparatory session using fresh juice reference. Sensory analysis of the functional juices were conducted using the following descriptors: typical flavor retention, typical color retention, pleasant aroma, sweetness, sourness, tanginess, bitterness in juice, extraneous taste, overall acceptability, chewiness of *nata*, ease of swallowing *nata*, fermented flavor and evaluated using 100 mm graphical non-structured abscissas with the description of extreme points (Jagannath et al. 2012).

CIE color values

Commission Internationale de l'Eclairage, CIE, parameters L^* , a^* , and b^* were used to study the pigment efficiency of juice samples using a Color Reader Mini Scan XE Plus, Model 45/O-S; (Hunter Associates, Laboratory Inc. Reston VA, USA). All the measurements were referenced to the CIE using the standard illuminant D65 and ten observers and the equipment was calibrated with a black and white standard ceramic tile. The parameters a^* indicates the chromaticity in the green (–) to red (+) axis, b^* indicates the chromaticity in the blue (–) to yellow (+) axis and L indicates variation from black (0) to white (100).

Statistical data analysis

The data were subjected to two way and one way analysis of variance (ANOVA) and significant differences between means ($P < 0.05$) were determined by Duncan's Multiple Range Test (DMRT). Stastitica 7.1 (Stat Soft Inc. OK, USA) was used for data analysis.

Microbiological analysis

Total bacterial counts, coliform, yeasts plus mold counts were determined by serially diluting the sample in 0.85 % physiological saline (w/v) and pour plating on plate count agar, Mac conkey agar and potato dextrose agar respectively (Himedia Laboratories, Mumbai, India). The plates were incubated at 37 °C for the bacteria and 30 °C for the yeasts plus mold counts and the number of colonies counted after 48 h. The microbial counts were expressed as mean colony forming units (cfu) per ml of the sample.

Results and discussion

The thermal process of tyndallization used in the current study was able to microbially stabilize the product and optimize the loss in pigment concentrations of various juices as compared to the fresh juices. Fresh juices of tomato and pineapple had a total plate count of 2.3 and 2.5 $\log_{10}$ cfu/ml while for carrot, beetroot, musk melon and beet root blend juices it was 3.2 ± 0.2 , 3.5 ± 0.2 , 3.8 ± 0.1 & 3.4 ± 0.2 $\log_{10}$ cfu/ml respectively. The total coliform and yeasts plus mold counts were less than 1.0 $\log_{10}$ cfu/ml for all the fresh juices. After tyndallization the total plate counts, coliform and yeasts plus molds count for all the juices were below 1.0 $\log_{10}$ cfu/ml. After the 90 day storage of juices, the levels of yeasts plus molds were less than 2.0 $\log_{10}$ cfu/ml, while the total plate counts were 2.0 ± 0.2 , 2.9 ± 0.2 , 2.7 ± 0.1 , 2.4 ± 0.1 , 2.5 ± 0.1 & 2.7 ± 0.2 $\log_{10}$ cfu/ml for tomato, pineapple, carrot, beetroot, musk melon and beet root blend juices respectively.

Table 1 shows the initial pH, TSS, pigment concentrations, color values of fresh juices and after 90 days of storage at ambient temperature (28 ± 5 °C). The pH and TSS adjusted to 4.2 and 15°Brix prior to storage remained unchanged after 90 days of storage in case of all the juices.

Fresh beetroot juice had the highest content of pigments (as betanin: 47–50 mg/100 ml) and tomatoes the lowest content (as lycopene: 0.9 ± 0.2 mg/100 ml). The average amounts of pigments reported in literature are 40–120 mg betalains/100 g fresh weight of beetroot (Marmion 1991) and 4–9 mg lycopene/100 g of tomatoes (Laleye et al. 2010). The pigment contents of the other juices were also within reported regional variations, which can be attributed to various factors.

In terms of CIE color values fresh carrot juice showed the maximum redness (18.3 ± 0.2) followed by tomato juice, musk melon juice, beetroot juice and beet root juice blend. It was interesting to note that although beetroot color can be visualized as red, the CIE color values gave a much lower value for redness (5.4 ± 0.4) and the lowest value for lightness (0.3 ± 0.2) among all the juices studied.

Fresh pineapple juice showed a greenness value of -1.3 ± 0.1 , which was not shown for any other juice and lightness L^* value of 15.6 ± 0.2 . The lightness L^* and yellowness b^* values were the maximum for carrot juice (28.4 ± 0.1 and 30.6 ± 0.2 respectively). Earlier efforts to use chromaticity values for correlating the lycopene and carotene content in vegetables have yielded mixed results. Good correlation has been noted for lycopene content but more than 55 % variability has been reported for carotene contents (Hyman et al. 2004). Color plays an important role in the acceptability of juices. High temperature processing for a longer time degrades the pigment contents and lycopene and carotene tend to precipitate while beetroot color changes from the fresh purple pinkish color to brown color. Although this change does not per se influence the sensory taste it affects the visual acceptability of the juices.

Irrespective of the nature of juice, the pigment concentrations decreased upon storage. There was 36, 72 and 60 % loss of total carotenoids in the case of carrot, pineapple and musk melon juices respectively while the lycopene content remained unchanged after 90 days of storage. Lavelli and Giovanelli (2003) in accelerated storage studies also found no change in the lycopene content for 3 months. Lycopene content is not stable when tomatoes are exposed to cooking temperatures near or above 100 °C (Mayeaux et al. 2006) and efforts like microencapsulation have been extensively tried to preserve the lycopene content (Choudhari et al. 2012). However in the current study the juices were exposed to very short duration (5 min) at 98 °C which ensured the retention of lycopene after the heating process and these levels were maintained during the 3 month storage period. The betanin content decreased 37 % in the case of beetroot juice and 25 % in the case of beetroot juice blended with carrot and tomato juices. Von Elbe et al. (1981) also reported 23 and 41 % losses

Table 1 The pH, TSS, pigment concentrations, color values for fresh juices and after 90 days of storage at ambient temperature (28 °C)

Juices	CJ	TJ	BRJ	PJ	MMJ	BRB		
Ph (Fresh) ³	6.2±0.2	4.8±0.1	6.5±0.3	4.0±0.2	6.3±0.2	5.9±0.3		
TSS (°Brix) (Fresh) ³	5.8±0.4	3.2±0.1	8.7±0.3	12.8±0.5	5.7±0.2	6.2±0.3		
Pigment concentration ¹ (mg/100 ml)						After 90 days		
Fresh juice	8.9±0.3b	0.9±0.2a	47.5±1.5b	5.4±0.2b	3.0±0.1b	Total carotenoids	13.8±0.3b	8.0±0.5a
						Lycopene	0.2±0.1a	0.4±0.1a
After 90 days storage	5.7±0.2a	1.3±0.1a	30.0±0.9a	1.5±0.2a	1.2±0.3a	Betanin	16.1±0.8b	12±0.5a
CIE Parameters ²								
L* Fresh juice	28.4±0.1b	15.5±0.1a	0.3±0.2a	15.6±0.2b	26.1±0.2b	6.6±0.2a		
After 90 days storage	22.9±0.2a	14.8±0.2a	9.3±0.4b	13.9±0.2a	14.3±0.2a	11.2±0.3b		
a* Fresh juice	18.3±0.2b	7.7±0.1a	5.4±0.4a	-1.3±0.1b	5.7±0.2b	5.0±0.2a		
After 90 days storage	12.1±0.2a	7.9±0.2a	4.2±0.2b	0.7±0.2a	2.5±0.3a	4.8±0.2b		
b* Fresh juice	30.6±0.2b	6.8±0.2a	0.3±0.2a	14.8±0.4b	14.7±0.2b	12.1±0.4a		
After 90 days storage	22.9±0.2a	6.3±0.8a	12.5±0.3b	13.5±0.2a	10.0±0.5a	14.7±0.2b		

¹ as: total carotenoids for pineapple juice (PJ), musk melon juice (MMJ), carrot juice (CJ), lycopene for Tomato juice (TJ), as betanin for beet root juice (BRJ) and all three pigments for mixed blend containing 20 % each of carrot and tomato juice with 60 % beet root juice (beet root blend BRB)

² Mean values ± sd (n=3). Values with different letters (a, b) for a particular CIE parameter or pigment concentration and for each type of juice are significantly different ($P<0.05$)

³ pH and total soluble solids (TSS) values remained unchanged at 4.2 and 15.0 °Brix after 90 days of storage

in pigments during processing and attributed it to leaching of pigments and to heat induced degradation. The total carotenoids decreased by 42 % in the case of blended beet root juice after 90 days of storage.

The CIE color parameters a^* , b^* and L^* decreased after 90 days of storage in the case of carrot and musk melon juices. The a^* value of redness did not change for tomato juice while for pineapple juice the value increased from greenness to redness after 90 days of storage. In the case of beetroot juice and beet root

juice blend the yellowness b^* and Lightness L^* values increased significantly ($P<0.05$) after 90 days of storage (Table 1).

Nisin as a biopreservative has been extensively used in fruit juices at levels of 1–25 ppm and has been reported to be particularly effective against *Alicyclobacillus* sp. an acid tolerant and a heat resistant organism which is the cause of major spoilage problem in pasteurized and heat treated juices. Nisin is most stable at pH 3 and reportedly maintains more than 70 % antibacterial activity at pH 4 when autoclaved at 115 °C

Fig. 1 Sensory profile of carrot juice CJ (Black square), tomato juice TJ (White square), beet root juice BRJ (White circle), pineapple juice PJ (White triangle), beet root blend BRB (Black circle) & MMJ (Black up-pointing triangle) after 90 days storage at 28 °C

(Davies et al. 1998). The presence of nisin during heating has been reported to decrease the D value of this bacterium by up to 40 % (Komitopoulou et al. 1999). In the current study, there was a reduction in total plate count of 0.5 to 0.8 log₁₀cfu/ml at the end of 90 days of storage as compared to the initial counts in fresh juices. The fact whether nisin is bactericidal or bacteriostatic depends on many factors like the nature of organisms originally present, the levels present, the food matrix, the duration of storage etc. These results indicate that use of low pH and nisin are potentially useful in controlling the microbial stability of juices and juice-containing products.

The sensory analysis of the functional juices is shown in Fig. 1. The panel gave the same scores for chewiness of *nata*, ease of swallowing *nata* (80) and for fermented flavor (10) in case of all the samples and hence these descriptors are not graphically depicted indicating that these parameters were not affected by the nature of juice. Beetroot juice blend scored highest in overall acceptability followed by pine apple, carrot and tomato juices. Musk melon juice was least preferred and scored the lowest overall acceptability scores of 40.

Mixed blend juice beverages are gaining popularity to satisfy consumer taste and preferences (Jan and Masih 2012). Making them with additional functional benefits will help in popularizing them further. Microbially produced cellulose is a good source of fiber which is indigestible and therein lies its therapeutic potential. Okiyama et al. (1993) has assessed the function of bacterial cellulose in food systems to determine its most advantageous end use and concluded that although bacterial cellulose is widely applicable to food hydrocolloids to improve their quality, further work is necessary to determine the optimum level of incorporation needed for maximal effectiveness and how to introduce bacterial cellulose to commercial product lines.

Conclusions

Sweetened fruit juices incorporating bacterial cellulose have been shown to be stable for 90 days at room temperature storage in this work. No chemical preservatives were added and the maintenance of low pH and 20 ppm nisin concentration ensured the microbiological stability of the product as well. There was no need for refrigeration and hence has logistical benefits especially for the defence forces and also serves as a source of fiber enhancing their intestinal health and well being. Beetroot juice blend incorporating 20 % each of carrot and tomato juice with 40 % beetroot juice scored highest in overall acceptability followed by pine apple, carrot and tomato juices.

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