Bioencapsulation Research Group

XXII International Conference on Bioencapsulation

21st Bratislava International Conference on Macromolecules

Bratislava, Slovakia, September 17-19, 2014

Organizer

POLYMER INSTITUTE SAS

Polymer Institute
of Slovak Academy of Science

Sponsors

P&G

JDRF IMPROVING LIVES. CURING TYPE 1 DIABETES.

Stability and consumer acceptance of spray-dried betanin in model juices

Kaimainen M., Järvenpää E., Laaksonen O., Sandell M. and Huopalahti R.
University of Turku, Turku, Finland (mika.kaimainen@utu.fi)

INTRODUCTION AND OBJECTIVES

Industrial red beet (*Beta vulgaris*) peeling produces a large amount of red beet peel by-product or waste. This peel contains high amounts of betanin pigment, which potentially could be used for food coloring. As betanin is water soluble, it could be used for example in juices. Usually anthocyanins are used to color red juices, and the color of betanin is slightly different.

In this study, spray dried betanin from red beet is used to color model juices and the stability of betanin under different storage conditions is examined. Consumer acceptance of betanin color and comparison with beet extract and anthocyanins are studied with sensory evaluations of juices.

MATERIALS AND METHODS

Materials

Betanin pigment was extracted from industrial red beet (*Beta vulgaris*) peeling by-product or sliced red beets bought at local grocery store. Extraction was made with water at 40 °C for 40 min, and after extraction the extract was filtered through a gauze cloth. Salts and other solid reagents were analytical grade and organic solvents were HPLC grade.

Spray drying

Before spray-drying, the red beet water extract was concentrated with rotary evaporator (max 40 °C) to °Bx 9. Maltodextrin (from corn starch, DE 6) was added to raise °Bx to 17 and the mixture was filtered through cheesecloth and pasteurized 10 min at 65 °C. Betanin was spray dried with a Mini Spray Dryer B290 (Büchi, Flavil, Switzerland) using the following conditions: inlet air temperature 170 °C, outlet temperature 67 °C, air flow 450 l/h, feed flow 6 ml/min, aspirator 35 m^3/h and nozzle orifice 1,50 mm.

Storage test

Four different model juices were made for storage tests. Each model juice had a base mixture of 9 g/L citric acid and 0.3 g/L ascorbic acid and a sweetener of either 10 g/L saccharose (sugar) or steviol glycoside (stevia) of respective sweetness. Two concentrations of spray dried betanin pigment in two different pH values were tested. Compositions of juices are shown in table 1. Each storage test juice was pasteurized 10 min at 65 °C before packaging into either polypropylene cans with aluminum foil lid (ST1 and ST2) or polypropylene tubes with screw caps (ST3 and ST4).

Table 1: Compositions of storage test juices

	ST1	ST2	ST3	ST4
pH	3.4	3.4	5.0	5.0
betanin	0.07 %	0.07 %	0.14 %	0.14 %
sweetener	sugar	stevia	sugar	stevia

Juices were stored in three different conditions: in dark at 4 °C, in dark at 22 °C and in dark at 60 °C. Betanin content was analyzed after different storage times with HPLC analysis using a Shimadzu 10 HPLC instrument (Shimadzu, Kyoto, Japan) with a diode array detector, a Luna C-18 column (length 150 mm, i.d. 3.00 mm, particle size 5 µm, Phenomenex, Torrance, USA), and a gradient of acetonitrile in 0.2 % aqueous formic acid at a flow rate of 1 mL/min.

Sensory evaluation

Six model juices were made for sensory evaluation. Each had a base mixture of 9 g/L citric acid and 0.3 g/L ascorbic acid and 10 g/L saccharose. Spray dried betanin, red beet water extract and a commercial anthocyanin concentrate were used for coloring the juices. Amounts of pigments were adjusted to match the maximum UV absorption of juices colored with spray dried betanin to produce either 'weak' or 'strong' color. Compositions are shown in table 2. Storage test juices ST1 and ST3 are the same as sensory evaluation juices SE1 and SE3, respectively.

Table 2: Composition of sensory evaluation juices

juice	pH	pigment	color
SE1	3.4	spray dried betanin 0.07 %	weak
SE2	3.4	spray dried betanin 0.14 %	strong
SE3	5.0	spray dried betanin 0.14 %	strong
SE4	3.4	beet extract 1:50 dilution	strong
SE5	3.4	anthocyanin 1:1250 dilution	weak
SE6	3.4	anthocyanin 1:2500 dilution	strong

70 voluntary subjects (age 19–65; 43 females; 27 males) were recruited to the consumer acceptance test. They rated the pleasantness of appearance on 7-point balanced hedonic scales (from 1 = extremely unpleasant to 7 = extremely pleasant). Subjects rated also the question "How important it is to you whether added colors in the juice are natural?" on a scale 1 (Not important) to 5 (Extremely important). The samples were presented in randomized order and data was collected by using the Compusense five - software. Tests were carried out in controlled sensory laboratory conditions. ANOVA with Tukey's test (IBM SPSS Statistics version 21) was used to analyze the statistical differences between sample ratings.

RESULTS AND DISCUSSION

Storage test

The amounts of betanin were slightly higher in juices sweetened with stevia than in juices sweetened with sugar. The reason is probably an increase in juice volume due to added sugar, as the base mixture was made first without the sweeteners, then the batch was divided in two parts, and the sweeteners were added. Stability of betanin in all juices was poor at 60 °C, as no betanin was left after one week. Stability at 22 °C was only slightly better in pH 3.4 juices as practically all the betanin had degraded after two weeks. Stability was somewhat better in pH 5.0 juices stored at 22 °C, although some degradation could be seen after a few weeks. Retentions of betanin in different juices during storage at 22 °C are shown in table 3. The stability test is still going on, especially for storage at 4 °C, but based on preliminary results, the stability is better at low temperature. After two months of storage at 4 °C, there is still 5 % (sugar) or 40 % (stevia) of betanin left in juices with pH 3.4.

Table 3: % betanin left after 1-4 weeks of storage at 22 °C (nd = not detected, tr = trace)

Storage time	ST1	ST2	ST3	ST4
1 week	19 %	38 %	85 %	76 %
2 weeks	nd	tr	47 %	48 %
3 weeks	nd	nd	20 %	24 %
4 weeks	nd	nd	7 %	9 %

In juices with pH 3.4 the stability of betanin seemed to be better in juices with stevia than in juices with sugar. In juices with pH 5, the difference in stability of betanin due to sweetener was not that clear. HPLC detected also other peaks in the samples, which were different in juices with different sweeteners. These other compounds were also different in samples stored at different temperatures. We have not yet identified these other products, which are probably some kind of reaction products.

Temperature, pH, light and water activity (a_w) have been reported to be crucial factors for betanin stability (Herbach 2006). Temperature is the most important factor, and our results clearly support this. The pH optimum for betanin stability is 4-6, which is also well in line with our results, as stability at pH 5.0 was a lot better than at pH 3.4. The effect of a_w cannot be clearly seen in these samples, but we have also made preliminary studies on the stability of spray dried betanin stored as powder (very low a_w), and the stability is a lot better than in these juices.

Sensory evaluation

Beetroot extract (juice SE4) was the most liked sample (mean 5.6; between 5 = slightly pleasant and 6 = very pleasant) whereas spray dried betanin 'weak' (juice SE1) was the least liked (ANOVA, $p<0.05$). In general, the 'strong' colored juices were more liked or less disliked than the 'weak' colored samples. Internal preference map (principal component analysis, PCA) with the first two PCs showed a segment of 18 subjects (25%) with preferences in liking than the main group. Main group preferred the anthocyanin 'strong' (SE6) more than the segment (t-test, $p<0.05$). Simultaneously, the segment preferred the spray dried betanin juices (SE1, SE2 and SE3) more than the main group. In general, both groups liked equally the beetroot extract (juice SE4). Classification of subjects into two groups correlated with the importance of natural colorants (Pearson's correlation). The main group rated higher importance of natural colorants than the segment with group means 3.9 and 3.1, respectively (between classes 3 = moderately important and 4 = very important). The segment classification was not depending on age or gender.

CONCLUSIONS

The stability of spray dried betanin in model juices is not good enough for commercial use. However in a more complex real juice, the matrix effect may also affect the stability (Herbach 2006), and there could also be natural antioxidants from the berry or fruit material, which could enhance the stability. Majority of the sensory evaluation subjects preferred anthocyanin color over betanin color. However, in a real juice there would also be natural anthocyanin colors from the berry or fruit, and the overall color of the juice could be more favorable to consumers. Therefore, it could be interesting to repeat this study using a real fruit or berry juice instead of the simple model juice.

REFERENCES

- Herbach et al. (2006) *Betalain stability and degradation – structural and chromatic aspects.* J. Food Sci. 71(4) R41-R50

ACKNOWLEDGMENTS

Financial support from Raisio Plc Research Foundation (Raisio, Finland) is gratefully acknowledged.

Addresses of authors

Kaimainen M, Laaksonen O and Huopalahti R:
University of Turku, Food Chemistry and Food Development, Department of Biochemistry, 20014 Turku, Finland
Järvenpää E:
MTT Agrifood Research Finland, Biotechnology and Food Research, 31600 Jokioinen, Finland
Sandell M:
University of Turku, Functional Foods Forum, 20014 Turku, Finland