Selecting Best Extraction Method of Betanin from Beetroot (*Beta vulgaris*) and Evaluating Suitability as a Colorant for Flavored Yoghurt

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Introduction

Betanin are the pigments responsible for red colour of Beetroot (*Beta vulgaris*). It is derived from 3,4-dihydroxyphenylalanine through metabolic reactions such as respiration. Betanin (Beetroot red) is used as a food colouring and, as a natural dye for fabrics (Nottingham, 2004). Therefore, betanin may be useful to replace synthetic colours used in yoghurt manufacturing.

A variety of different colouring ingredients such as Tartrazine, Cochineal, Carminic acid, Erythrosine, Red 2G *etc.* are currently added to flavored yoghurt for making the products more attractive and to encourage wider popularity. The permitted colours and the concentrations used for flavored yoghurt differs from one country to another (FAO/WHO, 1990). According to recent studies, there are some evidence of carcinogenic effect in artificial food colourants. Similarly, many studies have shown that, heating and fermentation of the milk caused colour bleaching and increasing the fat content in the milk base leads to decrease the intensity of colour (Tamime and Robinson, 2007). Therefore, this study was carried out to select the best extraction method of betanin with low cost of production and to assess the suitability of extracted betanin as a colourant for strawberry flavoured yoghurt.

Methodology

Betanin was extracted from fresh beetroot Using three extraction methods; direct extraction, blanching and alcoholic extraction. For direct extraction, fresh beetroots were peeledand crushed in to small pieces. All the crushed beetroot were weighed using top loading balance. For blanching, a crushed beetroot sample was added in to 80 °C water and kept for 10 minutes and for alcoholic extraction, a crushed beetroot sample was added in to 70% ethanol and kept for 30 minutes. Then, these three samples were filtered using muslin cloth to get the extracts and extracts were pasteurized at 80-85 °C for 10 minutes. Finally, pasteurized extracts were cooled up to 30 °C and 1% (v/v) ascorbic acid was added. Prepared betanin extracts were stored in refrigerator at4 °C. Then, equal volume of three extracts were incorporated into yoghurt mixture and sensory attributes (appearance, colour and overall acceptability) were analyzed using 15 trained panelists. The selected extraction method was used for further experiments.

Set yoghurts were prepared using standerdized cow milk (fat-3.5% and SNF-8.25%). Then, it was heated up to 60 °C and gelatin, skimmed milk powder and sugar was added. Then the mixture was homogenized under 120 kg/cm^2 pressure and pasteurized at 105 °C for 20 minutes. Culture (Rich®, Chr. Hansen, Valinhos, Brazil) was inoculated once the

temperature reached45 °C. Then, betanin extracts and strawberry flavor (E124) were added in to the mixture and it was filled into plastic containers and incubated for nearly 3 hours (42 °C- 45 °C) until pH reached 4.46. Thereafter, plastic containers were transferred into the blast freezer (0 °C – 8 °C) for 8 hours and stored in cold room (0 °C - 5 °C). There were three treatments with respect to betanin % as 0.63% (v/v), 1.25% (v/v) and 1.88% (v/v). Existing strawberry flavored yoghurt with E124 artificial food colourant was used as the control. Each treatment was replicated three times. Optimum betanin% was selected by a sensory evaluation using 15 trained panelists. Sensory attributes were tested using a 5-point hedonic scale (5-extremely like, 1-extreamly dislike) for appearance, color, smell, taste, mouth feel, hardness, texture and overall acceptability. Shelf-life analysis was done by measuring titratable acidity, pH and microbial evaluation (coliform, *E. Coli* and yeast and mould) during refrigerated storage. Total solid, m-snf, protein and fat contents were measured in selected yoghurt sample. Data were analyzed using one way ANOVA (CRD) and Friedman non-parametric test in MINITAB 14 software package. Finally, cost of production was calculated to anlyze the profit margin.

Results and Discussion

According to the sensory evaluation results, direct extraction method of betanin had highest ($p<0.05$) overall acceptability among three samples and used for further experiments. Similarly, 1.2% (v/v) betanin contained yoghurt was most preferred by the panelist than the other treatments. Results of sensory evaluation is shown in Figure 1.

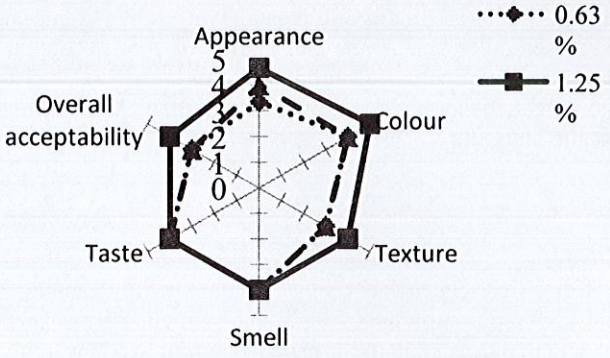

Figure 1: Sensory attributes of betanin incoporated strawberry yoghurt

The results indicate that, 1.25% (v/v) betanin incorporated yoghurt has highest ($p<0.05$) overall acceptability than other treatments. This may be the most favorable colour intensity to the panelists after fermentation. 0.63% betanin incorporated yoghurt showed lighter colour and 1.88% betanin incorporated yoghurt showed brighter colour than the selected level of betanin. The sensory attributes of selected yoghurt and control is shown in Figure 2.

According to the results, there was a higher ($p<0.05$) preference for betanin incoporated strawberry yoghurt with respect to appearance, colour, smell, taste and overall acceptability. Panenelists found no difference in mouthfeel, hardness and texture of two samples.

Figure 3 shows the variation in titratable acidity and pH of the selcted product over the storage period under refrigerated conditions.

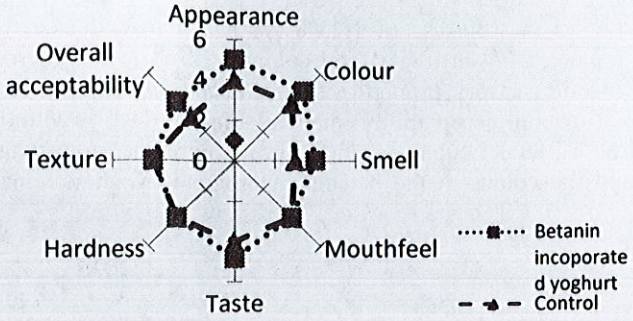

Figure 2: Sensory attributes of betanin incoporated strawberry yoghurt and control

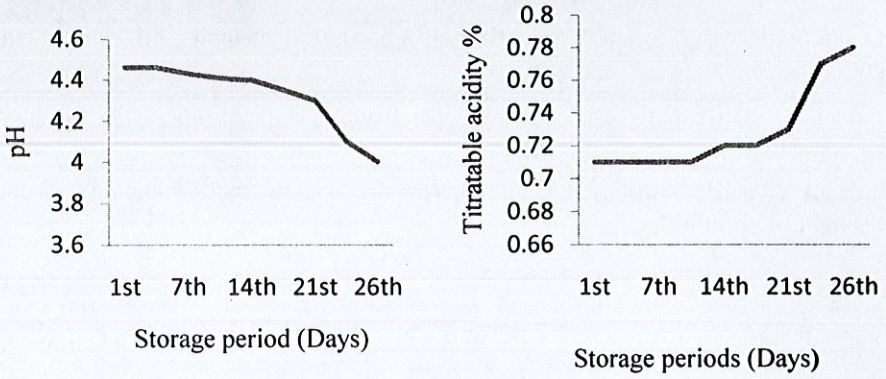

Figure 3: Fluctuation of titratable acidity and pH of selected sample over storage period

Betanin incoporated yoghurt had 0.71% titratable acidity and 4.46 pH value at 1st day of storage. During the storage period pH decreased ($p>0.05$) and titratable acidity increased ($p>0.05$). However, the post fermentation acidification did not affect the intensity of colour in both betanin incorporated yoghurt and control. According to the Sri Lanka Standards specifications, in set-yoghurts, the levels of titratable acidity should range between 0.8 and 1.25 lactic acid% (w/w) during the shelf-life. The selected yoghurt reached maximum levels of SLS specification at 21st day of refrigerated storage.

The results of proximate analysis showed that the selected sample is having 3.1% fat, 3.47% protein, 12.48% m-snf and 21.23% total solid content. The product can be stored for 21 days under 8 °C without any quality deterioration. There was no growth of coliform, *E.*

coli, yeast and moulds observed during this period. Extracted betanin solutions can be stored up to 1 week under refrigerated condition without any quality deterioration. Replacing E124 artificial food colourant by betanin reduce the cost of production of strawberry flavored yoghurt by Rs 1.80 per cup.

Conclusions

Results of this study support that the use of 1.25% (v/v) betanin extracted from direct extraction method can replace E124 artificial food colourant used in strawberry flavored yoghurts without reducing the sensory properties (appearance, color, smell, taste, mouth feel, hardness, texture and overall acceptability) than existing product. It will also reduce the cost of production by Rs 1.80 per cup of yoghurt. Furthermore, the product had 21 days shelf-life under 8 °C and the colour of the betanin incorparated yoghurt remains stable throughout the shelf life.

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