

REPLACEMENT OF ARTIFICIAL COLOURINGS OF A CARBONATED SOFT DRINK WITH NATURAL COLOUR PIGMENTS FROM RED-LAYERED HIBISCUS

SAS Jayawardana, SB Nawarathna*, KKDS Ranaweera

Department of Food Science and Technology, Faculty of Applied Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka

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ABSTRACT

A natural red colouring was developed from petals of Red-layered Hibiscus flowers, which are rich in anthocyanins, to replace artificial colourings that are being added to a carbonated soft drink. Boiling distilled water acidified with citric acid (0.8%) was found to be the most suitable solvent to extract the pigments from the petals. Immersing 1 g of petals for 10 minutes in 10 ml of the solvent was identified as the most suitable method to extract the pigments. Natural colouring was prepared in liquid form by concentrating 10 ml of pigment extract down to 1 ml. When storing in a clear glass bottle at 4°C, minimum shelf life of the colouring was 8 weeks. Carbonated soft drink test samples were prepared by adding the developed red colouring to match the colour of the standard beverage. Sensory evaluation, microbial analysis, Brix, pH and Carbonation Level measurements were done, using standard methods, for test samples stored under direct sunlight, indirect sunlight and in a refrigerator. No significant ($P > 0.05$) differences were detected between preferences for standard beverage and test sample, which also contained additional 2% citric acid amount and essence volume. The maximum shelf-life of test samples was limited to 5 days, when storing under direct sunlight. When storing under indirect sunlight or in a refrigerator, their minimum shelf-life was 8 weeks.

Key words: Red-layered Hibiscus, Natural red colour pigments, Carbonated soft drink

INTRODUCTION

Food colourings are added to carbonated soft drinks to make them attractive and pleasing. Usually artificial colourings are used to get the characteristic colour and the desired appearance of a carbonated soft drink. Artificial colourings are associated with several potential health risks as a result of possible presence of carcinogenic compounds in them. Therefore, the use of natural food colourings as substitutes for the artificial colourings is very important to eliminate or at least reduce these health risks. Any natural pigment that imparts colour when it is added to food or drink can be considered as a natural food colouring (Mortensen, 2006). Anthocyanins are a group of natural plant pigments, which have been used to colour food since historical times. Anthocyanins are sensitive to pH changes and give a red colour at acidic conditions (Mortensen, 2006).

Carbonated soft drinks are considered as acidic beverages and their pH values are normally less than pH 6.0. At low pH values (around pH

3), anthocyanins are most strongly coloured and exhibit their well-known purple-red colour. Anthocyanins are highly water-soluble and easily extractable (Mortensen, 2006). Although anthocyanins are commonly extracted from grape peels and black currants, there are many other good and abundant sources for anthocyanins. It is a known fact that the petals of Red-layered Hibiscus flowers are rich in anthocyanins. However, it remained to be shown that the petals of Red-layered Hibiscus flowers can be turned into a good, promising and commercially viable source of anthocyanins suitable to be utilized as a natural food colouring in food industry.

Therefore, this research was proposed and carried out with the objective of developing a natural red colouring from the petals of Red-layered Hibiscus flowers to replace the artificial colourings that are being added to a carbonated soft drink. During the first phase of this research, optimum conditions to extract anthocyanins from the petals of Red-layered

*Corresponding author: sbnaval234@yahoo.com

Hibiscus flowers were determined. Subsequently, a natural colouring was developed and the physico-chemical properties of the developed natural colouring were determined. The final phase of the research was devoted to produce carbonated soft drink test samples incorporating the developed natural colouring and to evaluate of the quality of the products by conducting sensory evaluations, microbial analysis and by determining Brix, pH and Carbonation Levels.

MATERIALS AND METHODS

Petals cut off from fully blossomed Red-layered Hibiscus flowers were used for experiments. Distilled water was selected as the solvent to extract the pigments from the petals. Citric acid was selected as the most preferable acid to acidify the extracting solvent.

Two samples of petals, each containing 1 g of petals, were used and one sample was immersed in 10 ml of the solvent maintained at room temperature. The other one was immersed in 10 ml of the solvent maintained at boiling temperature and the pigments were extracted. Then the mixtures were filtered through filter papers (Whatman No. 1) and 5 ml of the each filtration was diluted up to 100 ml using the extracting solvent. The colour intensities of the diluted solutions were measured at wave length of 520 nm using spectrophotometer (SHIMADZU UV mini - 1240). Total anthocyanins contents were calculated using the following equation and the results were analyzed to identify the most preferable temperature to extract the pigments.

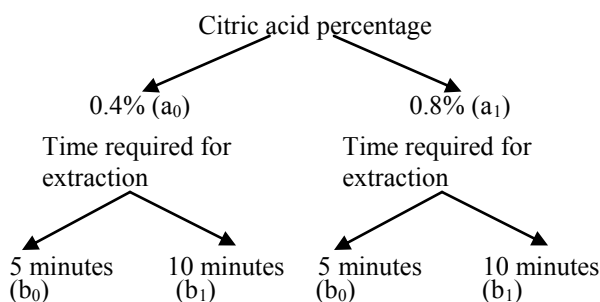
$$\text{Total anthocyanins (mg/100g)} = \frac{\text{Absorbance} \times \text{dilution factor} \times 100}{\text{Sample weight} \times 55.9}$$

(Source : Azza *et al.* (2011))

Pigments were extracted from 1 g of petals using 10 ml, 20 ml, 30 ml, 40 ml and 50 ml of the solvent. Total anthocyanins contents were calculated using the above mentioned equation and the results were analyzed to identify the

suitable volume of the solvent required for extraction.

Citric acid percentage and minimum time required to extract majority of the pigments were determined. The experiment was designed as a two factor factorial experiment and performed with four treatments as follows.



For the above treatments, four samples of 1 g of petals immersed in 10 ml (selected solvent volume) of the solvent maintained at boiling temperature (selected temperature) were used and the pigments were extracted. Total anthocyanins contents were calculated using the above mentioned equation and the results were analyzed to identify the best combination of citric acid and time to extract the pigments from the petals.

The variance of the colour of the pigment-extract with the pH value of the medium was studied. Pigments were extracted from the petals under the selected optimum conditions and the mixture was filtered through a Whatman No. 1 filter paper. Twelve solutions were prepared from pH 1 to pH 12, using the buffer solutions, N/10 HCl solution and N/10 NaOH solution. Then 1 ml of the pigments extract was added to 10 ml of the each solution and the colour changes were observed.

Natural colouring was prepared in liquid form. Petals were cut off from fully blossomed flowers. Then pigments were extracted from the petals by immersing 1 g of the petals for 10 minutes in 10 ml of 0.8% (w/v) citric acid solution prepared using boiling distilled water. Thereafter the pigment-extract was filtered through a Whatman No. 1 filter paper. When concentrating, 10 ml of the filtrate was concen-

trated down to 1 ml by evaporating the solvent.

The developed natural colouring was stored at a temperature of 4°C in a clear glass bottle. Brix value of the natural colouring was measured using digital Brix meter (PR201, Atago) and pH value was measured using pH meter (Orion 410 A+). Moisture content and ash content of the natural colouring were determined according to the methods described by AOAC (1996).

To determine the shelf life of the colouring, the variance of Brix value and pH value were monitored within a storage period of 8 weeks. Microbiological tests for Aerobic Plate counts, Yeasts and Moulds counts and *E.coli*/Coliform counts were carried out weekly within 8 weeks using petrifilms according to the methods described by AOAC (1996).

Carbonated soft drink test samples were prepared adding the natural red colouring to match the colour of standard commercial carbonated soft drink containing the artificial colourings. Since a citric acid solution was used in preparing the natural colouring, when adding the natural colouring to the beverage, certain amount of citric acid was also added. Therefore, the standard carbonated soft drink recipe was adjusted to balance the citric acid amount in the final product.

For the first sensory evaluation, two 400 ml carbonated soft drink test samples were prepared by adding 5.50 ml and 6.00 ml of developed natural red colouring per bottle. Prepared samples were compared with a standard commercial carbonated soft drink sample.

According to the results of the first sensory evaluation, it was decided to increase the citric acid content and the essence volume of the test samples prepared using the natural colouring in order to match odour, taste and after taste with the standard commercial carbonated soft drink. Therefore, two test samples were

prepared making the changes mentioned in Table 1.

Table 1: Differences between test samples prepared for the second sensory evaluation

Sample code	348	483
Natural colouring per 400 ml bottle / ml	5.50	5.50
Citric acid amount	Increased by 1%	Increased by 2%
Essence volume	Increased by 1%	Increased by 2%

Prepared samples were compared with a standard commercial carbonated soft drink sample during the second sensory evaluation. During the sensory evaluations the samples were assessed using a sensory panel containing 15 trained panelists with a five point hedonic scale and the results were analyzed using the Kruskal-Wallis non parametric ANOVA.

The quality of “Necto” samples which were produced by adding the natural colouring was monitored for a period of 8 weeks. Brix value and pH value of the samples were monitored weekly using standard methods. Carbonation Level was determined according to the method described by SLSI (1997).

Microbiological tests for Aerobic Plate counts, Yeasts and Moulds counts and *E.coli*/Coliform counts were carried out weekly using petrifilms according to the methods described by AOAC (1996). In order to analyze the colour changes with time, test samples which were produced by adding the natural colouring, were kept under direct sunlight, under indirect sunlight and in a refrigerator and the colours of the samples were compared with a freshly prepared standard carbonated soft drink sample. The changes were recorded within a storage period of 8 weeks.

RESULTS AND DISCUSSION

The extraction of anthocyanins from different plant materials using methanol, ethanol and water was reported and methanol extraction

was identified more effective than ethanol and water by Metivier *et al.* (Vankar and Shukla, 2011). The choice of an extracting solvent largely depends on the purpose of the extraction. Therefore, to avoid the toxicity of methanolic solutions, ethanol extraction was preferred for food use by Timberlake and Bridle (Azza *et al.* 2011). After extracting anthocyanins with ethanol, ethanol cannot be eliminated completely and as a result, there is a possibility of remaining some alcohol with the colouring. Therefore although the anthocyanins, extract with ethanol was higher than that of distilled water, distilled water was selected as the extracting solvent. Azza *et al.* (2011) stated that adding an acid to the extraction medium had a great effect in stabilizing anthocyanins, thus increased the extraction efficiency. They selected water acidified with citric acid as a more preferable solvent to extract anthocyanins. Vankar and Shukla (2011) extracted anthocyanin from *Hibiscus rosa-sinensis* flowers using both HCl and Citric acid and found that citric acid gave good yield and better colour too. According to above mentioned observations and recommendations of the researchers, citric acid was selected to acidify the extracting medium. Since citric acid is the acidulant which is used in the standard carbonated soft drink, the citric acid residues which remain in the anthocyanins concentrate does not affect the taste of the beverage. Therefore, distilled water acidified with citric acid was selected as the medium to extract anthocyanins from the petals of Red-layered Hibiscus flowers.

When using boiling distilled water, the extracted anthocyanins content was more than 200 times higher than the anthocyanins content extracted when using distilled water at room temperature. Therefore 100°C was selected as the preferable temperature among the two tested temperatures to extract the pigments from the petals. When increasing the solvent volume, extracted anthocyanins content also increases. Although using 10 ml of the solvent gives lower anthocyanins content

when compared to 50 ml of the solvent, it lowers the energy and time required for concentrating the solvent. The difference between the extracted anthocyanins contents (0.0085 mg) was not much significant when comparing with the difference between the solvent volumes (40 ml). Therefore, when considering a cost effective method, 10 ml of the solvent was selected as the most preferable solvent volume to extract anthocyanins from 1 g of petals. According to the results of the factorial experiment it was concluded that the effect of citric acid percentage is highly significant ($P < 0.05$) for extracting anthocyanins from the petals, while there is no significant ($P > 0.05$) effect from the factor time used for extraction. Petals immersed in a 0.8% citric acid solution for 10 minutes gave the highest yield. Therefore, that combination was selected as the most preferable combination.

The study on the dependency of the colour of the pigment-extract on the pH value of the medium indicated the fact, that anthocyanins are most strongly coloured at low pH values giving a bright red colour. The temperature used for concentrating the pigment-extract did not show any significant ($P > 0.05$) effect on the anthocyanins content. The Brix value and the pH value of the natural colouring were 12.03° and 2.63 respectively. The average moisture content was 95.08% and the ash content was 0.71%. During the eight-week storage period Brix values varied within the range of 12.2 to 11.7 and pH values varied within the range of 2.78 to 2.44. Yeasts and Moulds Count and Coliforms Count were zero during this period and the maximum value recorded for Aerobic Plate Count was two. The natural colouring was stored in a clear glass bottle at 4°C and the minimum shelf life of the colouring was found to be 8 weeks.

According to the results of the first sensory evaluation, there were no significant ($P > 0.05$) differences among appearances and colours of the three samples. But majority of the panel-

ists commented about the bitter taste of the sample, which was prepared by adding 6.00 ml of the natural red colouring. Therefore the sample, which was prepared by adding 5.50 ml of the natural red colouring, was selected for further improvements. According to the results and the comments of the sensory panelists it was concluded to increase the essence volume and citric acid content of the selected sample.

According to the results of the second sensory evaluation, there were no significant ($P>0.05$) differences in the preferences for appearance, colour, odour, taste, carbonation, after taste, and overall acceptability of the standard carbonated soft drink and the test sample containing the natural colouring and additional 2% of the citric acid amount and essence volume. Therefore that sample was selected as the most preferable sample among the two carbonated soft drink samples produced incorporating the natural red colouring. The Sensory Profiles of the standard carbonated soft drink and the selected natural colouring added Test sample are given in Figure 1.

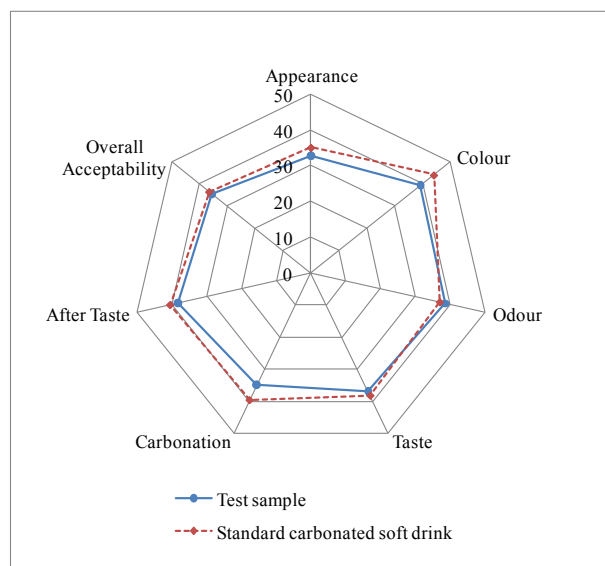


Figure 1 : Sensory profile of test sample and standard carbonated soft drink

The Brix values, pH values and Carbonation levels of the selected natural colouring added samples and standard carbonated soft drinks,

during the eight-week storage period, are given in Table 2.

Table 2 : Variance of Brix values, pH values and Carbonation levels of test samples (Test) and standard carbonated soft drinks (Std) during storage

Week	Brix value		pH value		Carbonation value	
	Test	Std	Test	Std	Test	Std
1	13.0°	13.0°	3.30	3.20	2.2	2.2
2	13.0°	12.9°	3.17	3.14	2.1	2.1
3	13.1°	13.0°	3.27	3.06	2.1	2.1
4	13.1°	12.9°	3.32	3.16	2.0	2.0
5	12.9°	13.0°	3.17	3.01	2.0	2.0
6	13.0°	12.9°	3.15	3.11	2.0	2.0
7	13.0°	12.9°	3.22	3.15	2.0	2.0
8	12.9°	13.0°	3.18	3.19	1.9	1.9

The changes of Brix value, pH value and Carbonation Level were found to be within the accepted ranges. Yeasts and Moulds Count and Coliforms Count were zero during this period. The Aerobic Plate Counts were within the accepted ranges too. The test samples gave acceptable results according to SLS standers (SLSI, 1997) in all tests performed.

The maximum shelf-life of the carbonated soft drink samples coloured using the natural colouring was limited to 5 days, when they were stored under direct sunlight. Therefore, storing under the direct sunlight cannot be recommended for natural colouring incorporated carbonated soft drink. It was also observed that the same samples have a minimum shelf-life of 8 weeks, if they were to store under indirect sunlight or in a refrigerator.

CONCLUSION

Immersing 1 g of petals for 10 minutes in 10 ml of 0.8% (w/v) citric acid solution prepared using boiling distilled water was identified as the most suitable method to extract pigments from the petals of Red-layered Hibiscus flowers. The natural colouring was prepared in liquid form by concentrating 10 ml of the pigment extract down to 1 ml by evaporating the solvent. The natural colouring was stored in a clear glass bottle at 4°C and the minimum

shelf life of the colouring was found to be 8 weeks. No significant ($P>0.05$) differences were noticed in the preferences for the standard carbonated soft drink and the natural colouring added samples prepared by increasing 2% of the citric acid amount and essence volume. All tested physicochemical and microbiological parameters of the test samples were found to be within the accepted ranges. When the test samples are stored under direct sunlight, their colour started to fade after 5 days. When these samples were stored under indirect sunlight or in a refrigerator, no significant ($P>0.05$) difference in colour was observed during the eight-week monitoring period. The results of this research, shows the possibility of replacing the artificial colourings of a carbonated soft drink with natural red colour pigments extracted from the petals of Red-layered Hibiscus flowers.

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