Research Article

Extraction and Utilization of Red Colour from Roselle (*Hibiscus subdariffa* L.) Calyces in Ice Cream

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Abstract

Colour of the food is one of the most important quality attributes that affects the consumer's acceptance since it gives the first impression of food quality. Due to proven safety and physiological advantage of the natural colourants over synthetic ones, interests are being increased into search of new natural colourants and the verification of the safety of existing ones. Roselle (Hibiscus sabdariffa L.) calyces have been known as a source that has characteristic intense red colouration due to presence of anthocyanins, which could be utilized as a natural colouring agent. This study was aimed to use of Roselle extract as a natural colourant in ice cream as a substitute to synthetic colourants. The physico-chemical properties were assessed using standard methods. The sensory evaluation was carried out to assess the colour, taste, texture, aroma and overall acceptability using a seven-point hedonic scale. The nutritional analysis of freshly made ice cream revealed that an increasing trend in titratable acidity from 0.24 to 0.37% (as citric acid), total soluble solid from 37.12 to 37.56 ºBrix, melting rate from 1.1 to 2%, ascorbic content from 2.75 to 3.45 mg/100 g, ash content from 0.73 to 0.79%, Colour parameter a value from 23.56 to 36.53 when the Roselle colour extract was increased from 0.10 to 0.25%. The results of this study revealed that the ice cream with 0.1% concentration Roselle colour is the best combination for maintain the physico-chemical and sensory qualities without any significant changes compared with the synthetic colourants.*

Keywords*: Colour, Encapsulation, Extraction, Ice cream, Physico-chemical quality, Roselle*

1. Introduction

In present world Food industry tries to provide visually appealing foods that have good taste and meet the consumers demand on quality and price (Downham and Collins, 2000). Many convenience foods would be colourless and would thus appear less attractable without the adding of colourants (Hirunpanish *et al*., 2006). Frequent usage of synthetic colourants started for decorative purposes and unfortunately it leads to reduction of nutritional quality of foods (Sulz, 1888). Not only that but also many of them have become toxic after prolonged use causing health problems such

as indigestion, anemia and allergic reactions as asthma and urticaria, pathological lesions in the brain, kidney, spleen and liver, tumors and cancer, paralysis, mental retardation, abnormalities in offspring, growth retardation and eye defects resulting blindness (Ashida *et al*., 2000). Due to this limitation and worldwide tendency towards the consumption of natural products, the interest in natural colourants has increased considerably (Huck and Wilkes, 1996).

Roselle calyces have been known as a source that has characteristic intense red colouration due to presence of anthocyanins, which could be utilized as a natural colouring agent (Shruthi *et al.,* 2016). Roselle has potential health benefits such as promoting cardio-vascular health and preventing hypertension, pyrexia and liver disorders, microorganism growth limitation, as well as a diuretic, digestive and sedative. Also red varieties of Roselle have antioxidant and cyclooxygenase inhibitory activity. The calyces of Roselle consist of brilliant red pigments of four anthocyanins including dephinidin 3-sambubioside or hybiscin and cyaniding 3-sambubioside as the major pigments and delphinidin 3-glucoside and cyanidin 3-glucoside as the minor ones (Du and Francis, 1973). Encapsulation facilitates light and heat-labile molecules to maintain their stability and improve their shelf life and activity. The coating film protects the core against deterioration, reduces the evaporation of volatile compounds, and releases the core under desired conditions (Kibry, 1991). Since ice cream products should have an attractive colour which accepted by consumers, addition of at least a small amount of colour should be required. This research was carried out to replace synthetic red colourants with extracted and encapsulated natural red pigment from Roselle calyces. This research was aimed to give pleasant, attractive, nutritional and healthy red colourants to Ice cream and to increase acceptability of Ice cream that produced using natural colourings.

2. Materials and Methods

2.1 Preparation of Roselle Colourant

2.1.1 Solar Drying of Roselle Calyces

Undamaged, healthy Roselle calyces were collected from Hatton city in Nuwara-Eliya district in June 2019 and it was authenticated as "*Hibiscus sabdariffa L*." from Hakgala botanical garden. Roselle calyces were washed and cleaned well. Then Seeds were removed and dried under sun for 2 days. Dried Roselle calyces were blended in to a fine powder using a blender.

2.1.2 Extraction of Colour

Ground dried calyces were thoroughly mixed with a 100 ml amount of 2% citric acid in 500 ml 1flask and incubated overnight in the refrigerator at 4°C. The soluble

extract was filtered using Whatman No. 1 filter paper to obtain Roselle pigment extract (Selim, *et al*., 2008).

2.1.3 Encapsulation of Roselle Extract Powder

Encapsulation procedure was done according to Selim, *et al,* 2008 with modifications. 20 g of gum arabic was dissolved in 80 ml of distilled water in 200 ml flask using a magnetic stirrer. Then, 5 ml Roselle extract was quantitatively transferred to the gum Arabic contain flask and the volume was adjusted to the 100 ml with distilled water (Selim, *et al*., 2008). The mixture was magnetically stirred for 15 minutes. After that the solution was vacuum dried (Model No. PZG Rotary vacuum drier) to get a fine powder under the condition of 44 cmHg at 70°C (Chumsri, 2007)

2.2 Preparation of Ice Cream

Preparation of ice cream was done according to Manuel *et al.,* 2013 with modification. 1 l Fresh cow milk was boiled and 200 g Sugar was added in to boiling milk. 200 g of milk powder and 25 g of Gelatin were added and manually stirred. 100 g of corn flour were dissolved in cooled milk separately and added to the boiling milk and stirred well. Boiling was stopped and allowed to cool 5 minutes. The extracted red colour powder was added according to relevant concentration and beat about 5 minutes. Mixtures were kept in freezer and it was beat 2 times in 1-hour interval. The contents were filled in to sterilized plastic cups and sealed with lids. Then sealed plastic cups were labeled according to the date of produced. Finally, ice cream cups were put in deep freezer and stored at -12°C.

2.3 Experimental Formulations

Five treatments that were used in this experiment are Ice cream with 0.015% synthetic colour (T_1), Ice cream with extracted Roselle colour concentration of 0.10% (T_2), 0.15% (T_3), 0.20% (T4) and 0.25%.

2.4 Physico-Chemical Analysis

Fat content was analyzed using the recommended AOAC (2002) methods. Titratable acidity, total soluble solids, colour parameters (L*, a*and b^* value) was measured using colour analyzer (Model PCE-RGB 2, Spain). Melting rate, Fat, ascorbic acid were analyzed after formulation and during the storage period. The titratable acidity was determined by titrating ice cream samples with standard NaOH and the results were expressed as prcentage of citric acid.

The Ascorbic acid content was titrimetrically estimated by using 2, 6-dichlorophenol indophenol dye method. The Total Soluble Solids (TSS) was measured by using the hand held refractrometer (Model ATAGO-S-28E). Colour measurement was carried out using colour analyzer (Model PCE-RGB 2). Fat

content was measured using Gerber centrifuge method (Astell Hearson Model GC 19-21, UK). Each parameter was triplicated during analysis. To estimate the melting rate of ice cream, hardenned ice cream was placed on a sieve (2 mm wide, square openings) and volume and weight was recorded every 10 minutes and melting rate was expressed as W/W.

2.5 Sensory Evaluation

The colour, taste, texture, aroma and overall acceptability were evaluated using a 7 – point hedonic scale. A questionnaire was used to assess the qualities of ice cream. Test was conducted between 9.00 – 11.00 a.m. in the morning and 2.00 - 4.00 p.m. in the evening. 40 panelists were asked to evaluate the samples from different treatment which were arranged randomly to assess the organoleptic qualities.

2.6 Statistical Analysis

Each formulation was replicated in experiments and they were in Complete Random Design (CRD). Data of the chemical analysis and storage study were analyzed by Analysis of Variance (ANOVA) ($\alpha = 0.05$) and mean separation was done with Duncan's Multiple Range Test (DMRT). Data related to sensory evaluation were analyzed using Turkey's test. Both chemical and organoleptic analysis was done through Statistical Analysis System (SAS) software statistical package.

3. Results and Discussion

3.1 Physico-chemical Parameters of Roselle Extract

Physico-chemical analyses were performed for the Roselle extract initially to develop the standard recipes of ice cream. Table 1 shows the physico-chemical parameters of Roselle (*Hibiscus subdariffa L.*) dried calyces and extract.

Table 1: Physico-chemical parameters of dried Roselle calyces and Roselle extract

Composition	Roselle Calyx	Roselle Extract
pH	2.80±0.04	2.89±0.06
Total Soluble Solids (°Brix)	15.40±0.01	44.00±1.20
Ash (%)	12.15±0.97	2.67±0.02
Titrable acidity (g/100 g) (% of citric acid)	16.95±1.2	17.87±1.10

The values are means of triplicates ± standard error

3.2 Quality Characteristics of Freshly Made Ice Cream

3.2.1 Titratable Acidity

Titrable Acidity of Ice cream Formulated with Roselle Red Colour is shown in the Table 2. According to that the titratable acidity of the ice cream samples varied significantly (<0.05) and increased from 0.17 to 0.37% with the increasing concentration of Roselle colour concentration from 0.1 to 0.25%. Normally ice cream formulations has the acidity ranging from 0.19 to 0.22% (Singo and Beswa, 2019) and the Control sample with 0.015% synthetic colour had 0.17% titrable acidy which present the lowest acidity level among all the treatments.

These results of the titratable acidity are agreement with the results reported by Choo *et al*. (2010) in ice cream formulated with virgin cococnut oil and Gabbi *et al*. (2018) in ice cream enriched with ginger juice. The control sample was observed as the lowest acidity because Roselle colour was not added to that treatment, but synthetic colour which has no effect to the acidity. The acidity of the Roselle-coloured ice cream may have been influenced by the presence of various compounds, which are dominated by citric acid, hibiscus acid, malic acid and tartaric acid are present in Roselle calyces in Roselle extracts (Mounigan and Badrie, 2007).

Table 2: Titrable acidity of Ice cream formulated with Roselle red colour

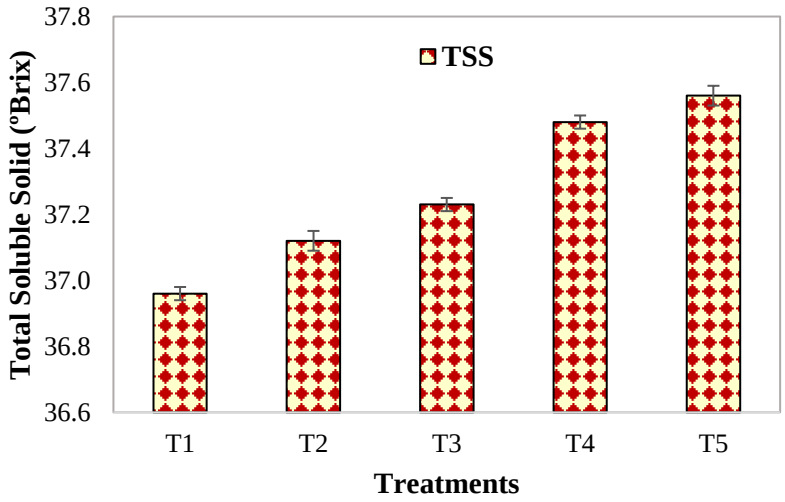
Treatments	Titrable Acidity (as % of citric acid)
T1	0.16±0.01 ^{e}
T2	0.24±0.00 ^{d}
T3	0.29±0.00 ^{c}
T4	0.33±0.01 ^{b}
T5	0.37±0.12 ^{a}

The values are means of triplicates ± standard error

3.2.2 Total Soluble Solids (TSS)

Total Soluble Solids is the sum of the sugar, acids and other minor components (amino acids, soluble pectin, ascorbic acid and minerals). There was a significant difference between all the treatments according to DMRT. Figure 1 shows Total Soluble Solids (TSS) Contents of Ice cream added with Roselle Red Colour. According to that TSS had increased while the formulated Roselle colour concentration increased. Roselle contains various types of organic substances and therefore TSS may be increased along with Roselle concentration level. Similar

results was reported by Roriz (2018) in ice cream formulated using beet root colour extract.



The values are means of triplicates± standard error

Figure 1: Total Soluble Solids (TSS) contents of ice cream added with Roselle red colour

3.2.3 Colour Parameters

The parameter a* represents the variation of the colour from red (+ values) to green (negative values), the parameter b* represents the variation of the colour from yellow (positive values) to blue (negative values), and the parameter L* represents luminosity, which varies from black to white (Granato and Masson 2010; Pathare *et al*., 2013).

Colour parameters of ice cream with different level of Roselle colour (0.1-0.25%) and synthetic colour (0.015%) concentration are shown in Table 3. According to that a^* value had increased and it means red colour intensity also had increased. Changes in colour according to the Roselle concentrations are shown in the Figure 2 according to L^* a^* and b^* values obtained from Table 3. It can be clearly seen the changes of colour according to Roselle concentration. Colour had become darker in ice cream formulated with concentration of 0.15%, 0.20%, 0.25%

Roselle extract subsequently while in ice cream formulated with concentration of 0.10% and synthetic colour had become slight similar. There was a significant increase in a^* (Red) value according to DMRT among treatments while concentration of Roselle colour increased. These results are in accordance with the results observed by Roriz *et al*. (2018) in ice cream formulated with beet root colour extract.

Table 3: Colour parameters of ice cream formulated with Roselle ice cream

Treatments	L^*	a^*	b^*
T_1	82.56±1.02 [a]	23.22±0.10 [b]	9.9±0.01 [c]
T_2	82.8±1.08 [a]	23.56±0.08 [b]	9.85±0.08 [c]
T_3	80.76±0.00 [a]	28.94±0.00 [b]	9.93±0.00 [c]
T_4	76.56±0.35 [a]	32.52±0.35 [b]	10.56±0.35 [c]
T_5	75.54±0.12 [a]	36.53±0.12 [b]	10.8±0.12 [c]

The values are means of four replicates ± standard error

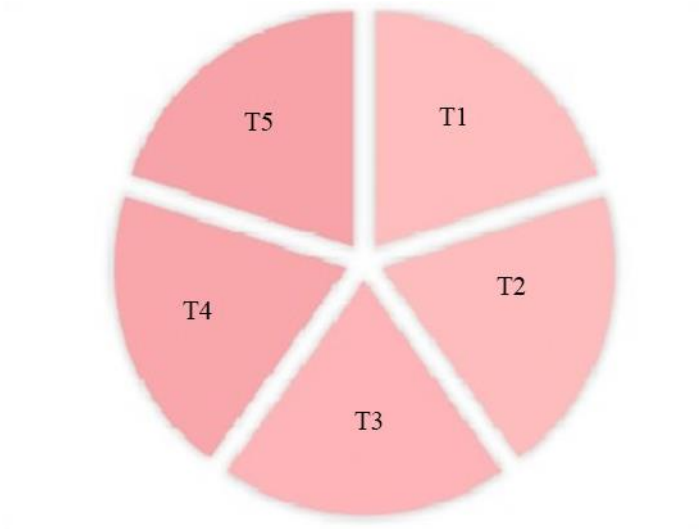


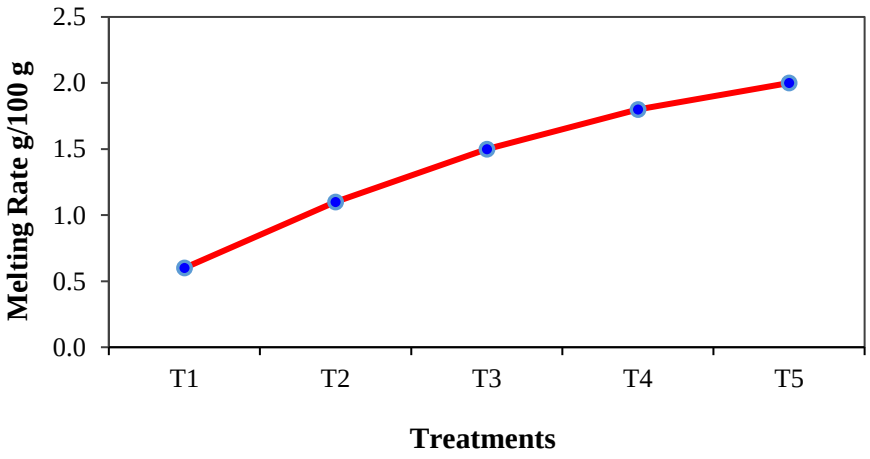
Figure 2: Colour of ice cream obtained according to L^* a^* and b^* values

3.2.4 Melting Rate

Melting Rate is a very important characteristic that deviation in the melting property from ideal condition can make the ice cream defective (Pelan *et al.*, 1997). Melting Rate of Ice cream formulated with Roselle Red Colour is shown in the Figure 3 and the lowest melting rate was in ice cream formulated with synthetic colour (0.6g/min) and the highest melting rate was 2.0 g/min in Sample with 0.25% of Roselle colour extract. These observations are agreed with findings of Manuel (2013). Air, fat

globules and ice are the main microstructural components of ice cream and have a significant effect on the meltdown behavior of ice cream (Warren, 2014). Roselle colour concentrations which was added to ice cream was in different level (0.1-0.25%) and that may be disturbed to the microstructure of ice cream samples and this may be the reason for significant difference of melting rate of ice cream. Ice cream samples incorporated with Roselle showed higher melting rate than control sample with synthetic colour.

According to Sun-waterhouse *et al*. (2013) a higher fat content could have facilitated a higher overrun, once more coalesced fat droplets would be available to trap a greater amount of air bubbles in the product. The reason for the increasing melting rate from ice cream formulated with 0.1% to 0.25% than control sample may be higher content of anthocyanin which is a fat molecule that present in Roselle extract. These results are in agreement with findings with Kumar *et al*. (2013) in ice cream formulation with Tulsi extract.

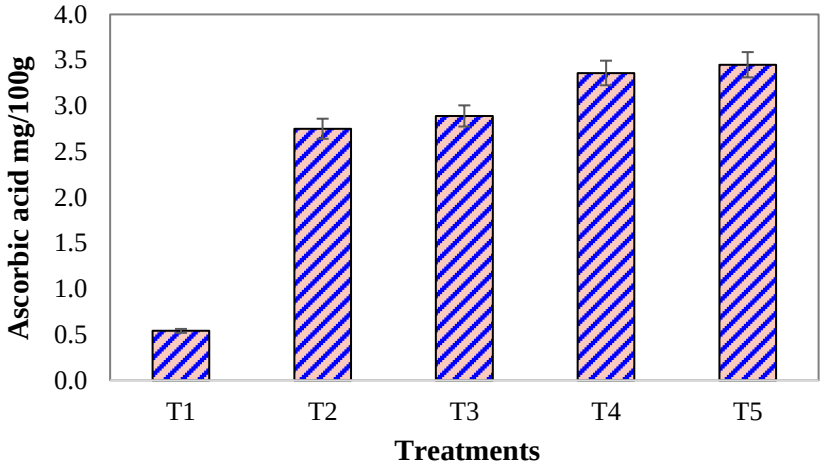


The values are means of triplicates± standard error

Figure 3: Melting rate of ice cream formulated with Roselle red colour

3.2.5 Ascorbic Acid

Ascorbic acid is a water-soluble vitamin and liable substance as it is easily degraded by enzymes and atmospheric oxygen. Ascorbic content had increased with the increasing of Roselle concentration. Normally Roselle calyces contain 11 mg/100g and rich in ascorbic acid (Mohammad *et al.*, 2012). Changes in Ascorbic acid in Ice cream formulated with Roselle Red Coloring is shown in the Figure 4. Ascorbic content of ice cream increased with the increasing of Roselle colour concentration. According to DMRT, there had a significant different (<0.05) between all treatments.



The values are means of triplicates± standard error

Figure 4: Changes in Ascorbic acid in ice cream formulated with Roselle red coloring

3.2.6 Fat

Fat affects all aspects of food perception including appearance, texture, flavor and mouth feel. The fat is also a concentrated source of calories and contributes heavily to the energy value of ice cream (Potter and Hotchkiss, 1995). Differences in fat content are shown in Figure 5. According to the DMRT, there had a significant difference in all treatments (<0.05).

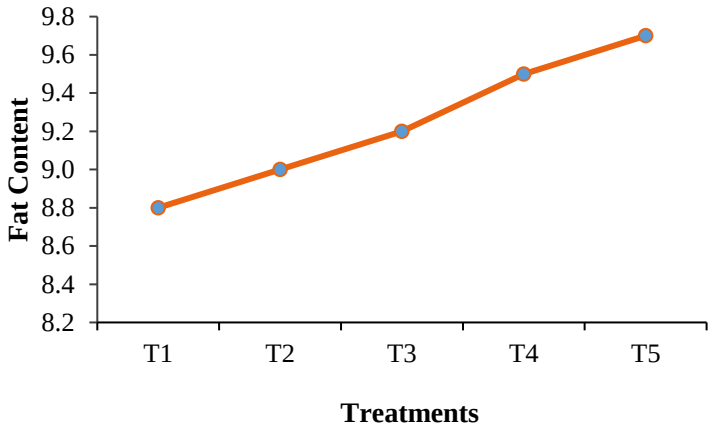


Figure 5: Changes in fat content in ice cream formulated with Roselle red coloring

Fat content had increased gradually along with the concentration of Roselle colour extract. These results are in accordance with the results obtained by Sun-waterhouse

et al. (2013) and they observed vary fat content levels in ice cream formulated with lycopene extracted from tomato and Erkaya *et al*. (2012) who studied the influence of cape goose berry in to ice cream. Fat content is important for conferring creamy and smooth texture, for the changes of viscosity and the melting rate of the ice cream. Anthocyanin which is a fat compound may have influenced on the increasing of fat content.

3.3 Sensory Qualities of Freshly Made Ice Cream

The sensory evaluation of the ice cream revealed that, there were significant differences between the treatments for colour, taste, texture and overall acceptability and nonsignificant difference was found in aroma at 5% level when the concentration of Roselle colour extract was increased from 0.1 to 0.25%. The mean values of sensory parameters according to Turkey's Studentized Range Test are shown in Figure 6. There was no significant difference between Ice cream formulation with 0.015% synthetic colour and Ice cream formulation with 0.1% Roselle colour whereas other three treatments had a significant difference in overall acceptability. According to the turkey's test ice cream formulated with 0.10% of Roselle had the highest score in colour, texture. There was no significant difference between ice cream formulated with 0.10%v and Ice cream formulated with synthetic colourant in taste and overall acceptability according to the Turkey's test. Based on physico-chemical and sensory analysis of freshly made ice cream formulated with Roselle red colour, the most preferred three ice cream formulations were selected for storage studies. They were stored at freezer at -12ºC.

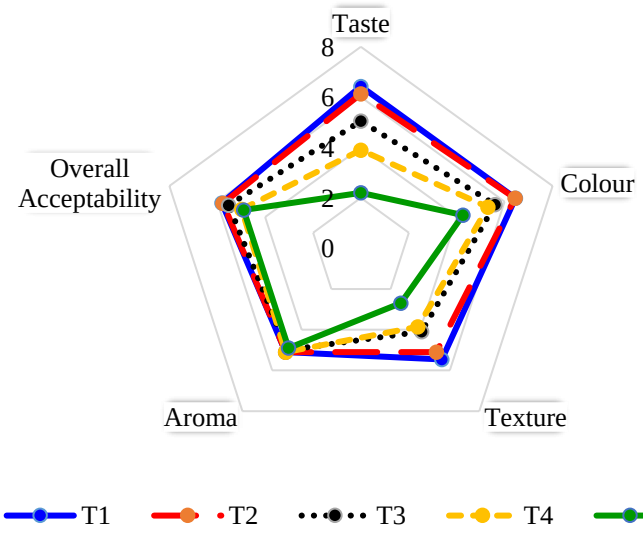


Figure 6: Sensory parameters of ice cream formulated with Roselle colour

4. Conclusions

The present study revealed that, the Roselle red colour extract could be used on the natural colouring material for ice cream. Physico-chemical analysis of ice cream revealed that there was decrease of pH while increase in a* value which is responsible for red colour. With the increase in the colourant concentration from 0.1 to 0.25%, titrable acidity, total soluble solids, melting properties, fat, ascorbic acid and ash increased. Sensory evaluation of freshly made ice cream formulations showed that the control ice cream sample with 0.015% synthetic colour concentration (T_1), ice cream sample with 0.1% Roselle concentration (T_2) and Ice cream with 0.15% (T_3) were most preferred formulation at the day on preparation based on the physico-chemical and organoleptic point of view. T_1 and T_2 samples were the best in chemical and organoleptic quality compared to other. This study shows that Roselle calyces can be effectively utilized as a natural colour in commercial purpose. Ice cream with 0.1% concentration Roselle colour is the best combination for maintain the physico-chemical and organoleptic qualities without any significant changes compare with the synthetic colourants and no any harmful effect for consumers.

5. References

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