Research Article

Quality Assessment and Application of Red Natural Dye from Beetroot (Beta Vulgaris)

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Abstract | Beet root (*Beta vulgaris*) is an excellent source of natural red colorant. In the present work red beet root dye was prepared in the powder form and then candies were prepared using this red beet root dye as food color. The stability studies of both, the prepared red beet dye and candies were performed at different storage conditions 4°C, 25°C, 45°C for eight weeks. Betanin content (%) was measured to evaluate the stability of color which showed the maximum retention of betanin content in red beet dye and candies stored at 4°C while lowest %age of betanin content was observed at 45°C showing that at higher temperatures the color is deteriorated. Stability in pH of red beet dye was also monitored at three different temperatures (4°C, 25°C and 45°C). Increase in pH was observed with increasing storage temperatures. Maximum stability of the color was observed at 4°C at most stable acidic pH and color degradation was observed with gradual increase in pH at 45°C. Microbiological analysis of candies and beet powder at 4°C and 25°C ensure that they are safe for consumption at both storage temperatures.

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Introduction

Appearance of food is an important aspect as we assume about the taste of food from its color most of the times. To enhance the visual attraction towards food products many artificial colors are used in food industry (Clydesdale, 1993; Paakki *et al.*, 2019; Zeide, 2020). Food colors as additives are used in several processed foods for example; candies, soft drinks, sweets and in some prepared dairy products such as cheese and butter. Sometimes these artificial food colors cause allergic responses in children as short term effects while their long term effects result in carcinogenic diseases (Shanmugasundaram *et al.*, 2019). Due to which some of the colors are prohibited from use in foods especially red (Carocho *et al.*, 2014). The demand of safe and nutritious fresh products with high sensory quality and an appropriate shelf life has been increased in the last decade (Mejia *et al.*, 2020). The use of natural dyes in replacement of synthetic dyes is preferred with increasing awareness about side effects of synthetic dyes particularly in food industry. Natural colors obtained from plants and animals are considered to be safe to use because they are non-toxic, non-carcinogenic and biodegradable in nature (Leong *et al.*, 2018; Rodriguez-Amaya, 2016). The drawback in the use of natural dyes is that these are costly and less stable that's why their use is not that

easy as yet (Albuquerque *et al*., 2021; Ravichandran *et al*., 2014).

Beet root (*Beta vulgaris*) is a source of water soluble nitrogen-containing pigment, known as betalain or betanin, which is used as food colorant and food additive as well. Betalains have some properties which are beneficial to our health due to which they are also used to enrich food products (Azeredo, 2009). They are also used to enhance the color and flavor of tomato paste, desserts, jams, jellies, ice cream, sauces, sweets and breakfast cereals (Agrawal, 2013; Preethi *et al*., 2020). Betalains extracted from Beetroot are also known as "beetroot red". Right after the extraction it is exposed to degradation. There are several factors which influence pigment stability for example; temperature, enzymes, pH, light and oxygen (Pedreno and Escribano, 2001). According to Stintzing and Carle (2004). Betalain pigments showed more stability towards pH and temperature while they are suitable to those foods in which anthocyanin cannot be used as a coloring agent because of low-acid conditions. All these aspects regarding stability make the use of natural food colors less acceptable however there is still an increase in the consumer demand to add up safe and healthy ingredients to their food. This research work is aimed to improve the safety of food products regarding the use of food colors. Red natural color produced from Beetroot (*Beta vulgaris*) is a safe product to use in different foods. Red natural color is extracted from Beetroot, then this natural colorant is applied to make candies and the color stability at different storage conditions is studied.

Materials and Methods

Materials

All the chemicals and reagents used in the current study were of analytical grade, purchased from Merck chemicals (Germany), Scharlau (Spain), Sigma-Aldrich (UK) and Rapid (UK). Beetroot (*Beta vulgaris*) was procured from the local market of Lahore, Pakistan. The substrate material was then washed thoroughly to remove dust particles and then stored at 4°C.

Preparation of dye

Beet roots bought from local marketplace were washed and peeled off. Slices of beet root were placed in hot air oven overnight at 35-40°C for drying. Dried slices were grinded in common home grinder to get red beet dye in powder form. Red beet dye powder was stored in polythene bags at 4°C, 25°C and 45°C for further use.

Application of dye on a food product

Preparation of candy: 450g of sugar was dissolved in 500mL distilled water and solution was first slowly heated at 50-60°C until all the sugar dissolved and heated to boiling to remove moisture. Glucose syrup (230g) was added to the mixture to make it thick and sticky. The heating of the mixture was continued until temperature reaches 150°C and resulted in moisture less thick treacle which was kept to cool down till 35°C.

Application of Dye

Red beet dye powder was added to treacle kept at 35°C to avoid deterioration of color pigments at high temperature. The mixture was homogenized well by manual stirring to obtain uniform color in it. Before complete hardening of the treacle, different shapes of candies were made. Freshly made candies were stored in polythene bags at 4°C, 25°C and 45°C for further analysis (Figure 1).

Figure 1: *Red beet dye powder and candies stored at different temperatures.*

Spectrophotometric analysis of betanin (%) in red beet dye and candies

Red beet dye powder and candies were analyzed spectrophotometrically to observe the stability of color as betanin (%) at 4°C, 25°C and 45°C. 0.5 g of beet powder from each sample stored at 4°C, 25°C and 45°C was dissolved in McIlvain's phosphate buffer. The solution was centrifuged to take supernatant layer, diluted to 100mL with phosphate buffer and then absorbance was measured with water as reference. The maximum absorbance was in the range of 0.2 to 0.8. The color intensity of batanine in candies was also determined by the same procedure taking 10 g of each candy stored at 4°C, 25°C, and 45°C in McIlvain's phosphate buffer and diluted thereafter to 100mL. The color intensity was calculated on the basis of maximum absorbance (at about 530 nm), all red coloring matter being included under betanin with specific extinction (FAO, 1984).

$$E^{1\%}_{1cm} = 1120$$

$$\% \text{ Beet Red} = A \times V / 1120 \times L \times W$$

In which;

A= Maximum absorption; V= volume of test solution measured in ml; L= length of cell measured in cm; W= weight of sample in g.

Microbiological analysis of red beet dye and candies

Candies and dye powder were also analyzed microbiologically to ensure that they are safe for consumption in food. 10 g of sample was dissolved in 90 mL Butter Field Phosphate Buffer (BFB). For second dilution 1 mL of solution mixture was dissolved in 9 mL BFB. For third dilution 1 mL from second dilution was dissolved in 9 mL BFB again. These solutions were stored in test tubes for further use. Total plate count was carried out using Plate Count Agar (PCA) 20 – 25 mL/plate, Total coliforms and fecal coliform detection by Lauryl Tryptose Broth Single Strength (LTSS) media, Eosin Methylene Blue (EMB) Agar media for *E. coli*, XLD and HEA agar for the presence of salmonella., Baird Parker Agar (BPA) and Egg Yolk Agar (EYA) was used to detect staphylococcus aureus and Petro dextrose Agar (PDA) media was used for the detection of yeast and molds in samples. Incubation at 35°C for 24 - 48 hours and at 25°C for 5 days for yeast and molds was performed. The analyses were done in triplicates (FAO, 1992).

Stability of red beet dye in pH

pH of the samples of beet powder was measured by a pH meter to determine the effect of different temperatures on the stability of pH of the beet powder. Readings of pH were taken in triplicates after each week in the whole study of 8 weeks.

Statistical analysis

Spectrophotometric results were analyzed through Mixed Anova Test. These results were analyzed to regulate the significance or non-significance of percent beet in powdered beet dye and candies stored at increasing temperatures with increasing time (Steel *et al.*, 1997).

Results and Discussion

Storage stability of betanin (%) in red beet dye and candies

Temperature is the most important and influencing factor for the stability of betalain as far as food processing and storage is concerned (Herbach *et al.*, 2006). The betanin content (%) of red beet powder and candies stored at three different temperatures (4°C, 25°C and 45°C) are presented in Tables 1, 2 and Figures 2, 3. According to these results the highest percentage of betanin content was found retained in beet powder and candies stored at 4°C. While lowest retention of betanin content was observed at 45°C. This showed that at higher temperatures the color is deteriorated. However, at 25°C the retention was lower than the samples stored at 4°C and it was higher than the samples stored at 45°C. Statistical analysis shows that significant results ($P<0.05$) obtained from different storage temperatures of beet dye and candies and it is concluded that with increasing storage temperature color degradation is increased. According to reported studies, betalains are heat sensitive pigments so they lose their stability at higher temperatures (Reshmi *et al.*, 2012; Nisa *et al.*, 2015). Saguy *et al.* (1978) reported that degradation of betalains is increased with increase in temperature and time period of heating. A considerable decrease in betalain stability was observed at 50 and 60°C and between 70 and 80°C (Havlikova *et al.*, 1983). Thermal degradation of betalain is of great concern because various heat treatments are being used for food preservation to make sure the safety and quality of food products (Paciulli *et al.*, 2016). Thermal stability of betalains according to Herbach *et al.* (2006) depends on heating time and temperature as well as some other factors such as light concentration and

structure of the pigment. Another study also reported that degradation of betalain increases progressively with rise in temperature and extended storage time (Halwani *et al.*, 2018). Similarly, significant reduction in betalain content was observed at 40°C, room temperature and refrigeration temperatures (Mohammed *et al.*, 2021).

Table 1: *Stability in Betanin (%) and pH of red beet dye powder at different temperatures.*

Weeks	Red beet dye powder					
	4°C		25°C		45°C	
	Betanin (%)	pH	Betanin (%)	pH	Betanin (%)	pH
1st	0.211 ± 0.30	4.6	0.211 ± 0.25	4.7	0.211 ± 0.28	4.7
2nd	0.210 ± 0.20	4.7	0.201 ± 0.18	4.9	0.201 ± 0.32	5.0
3rd	0.200 ± 0.10	4.9	0.200 ± 0.10	5.2	0.190 ± 0.05	5.3
4th	0.190 ± 0.09	5.0	0.190 ± 0.09	5.4	0.180 ± 0.19	5.6
5th	0.180 ± 0.20	5.1	0.180 ± 0.20	5.6	0.170 ± 0.08	5.9
6th	0.160 ± 0.05	5.3	0.160 ± 0.17	5.7	0.150 ± 0.35	6.3
7th	0.140 ± 0.15	5.4	0.140 ± 0.20	5.8	0.120 ± 0.33	6.6
8th	0.100 ± 0.25	5.5	0.100 ± 0.09	6.0	0.092 ± 0.29	7.1

Table 2: *Stability in Betanin (%) of candies at different temperatures.*

Weeks	Candies		
	4°C	25°C	45°C
1st	0.00235 ± 0.02	0.00236 ± 0.06	0.00234 ± 0.08
2nd	0.00230 ± 0.05	0.00230 ± 0.12	0.00226 ± 0.06
3rd	0.00229 ± 0.09	0.00229 ± 0.09	0.00222 ± 0.17
4th	0.00222 ± 0.07	0.00223 ± 0.07	0.00204 ± 0.12
5th	0.00220 ± 0.10	0.00220 ±0.16	0.00198 ± 0.09
6th	0.00214 ± 0.09	0.00214 ± 0.19	0.00193 ± 0.15
7th	0.00204 ± 0.15	0.00207 ± 0.08	0.00185 ± 0.18
8th	0.00201 ± 0.08	0.00198 ± 0.15	0.00180 ± 0.16

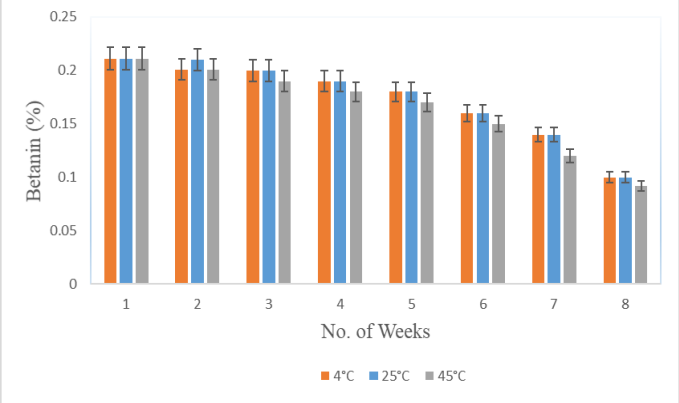

Figure 2: *Stability in Betanin (%) of red beet dye powder at different temperatures.*

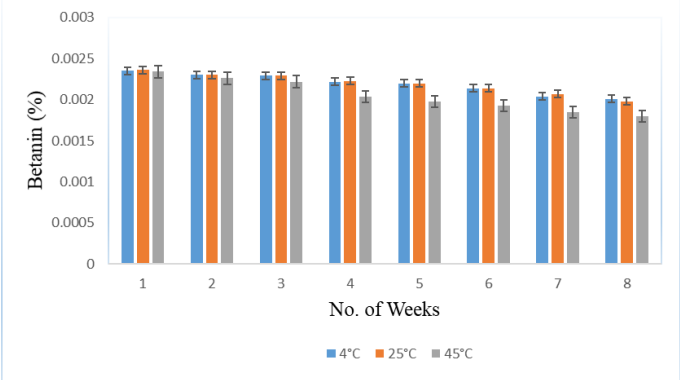

Figure 3: *Stability in Betanin (%) of candy at different temperatures.*

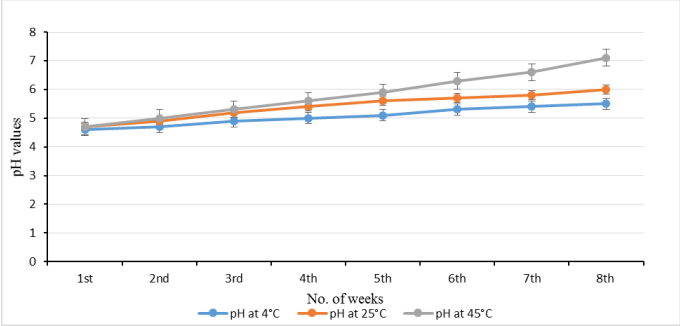

Figure 4: *Stability in pH of red beet dye powder at different temperatures.*

Table 3: *Microbiological analysis of red beet dye powder and candies at different temperatures.*

Microorganisms	Red beet dye powder		Candies	
	4°C	25°C	4°C	25°C
Total Plate Count (Cfu/g)	1.2 ×10^3	1.8×10^3	1×10^3	2×10^3
Total Coliforms (MPN/g)	ND	ND	ND	ND
Fecal Coliforms (MPN/G)	ND	ND	ND	ND
E.coli (MPN/G)	ND	ND	ND	ND
Staphylococcus aureus/g	ND	ND	ND	ND
Salmonella spp./25g	ND	ND	ND	ND
Yeast count/g	<10	<10	<10	<10
Mold count/g	ND	<10	ND	<10

**ND means not detected.*

Microbiological analysis of red beet dye and candies

Candies and beet powder were analyzed to ensure that they are safe for consumption. Total plate count, Total coliforms, Fecal coliforms, Staphylococcus aureus, *E. coli*, Salmonella, Yeast and Mold were determined. Results of Table 2 showed that candy stored at 4°C contains total plate count of 1×10^3 cfu/g while at 25°C it showed total plate count of 2×10^3 cfu/g. Beetroot powder stored at 4°C showed 1.2 ×10^3 cfu/g of total plate count and 1.8×10^3 cfu/g at 25°C. The observed values of total plate count at both temperatures is within standard limits (Codex

Alimentarius, 2003). Total coliforms, fecal coliforms, *E. coli*, Staphylococcus aureus and Salmonella were not detected in any sample of candy or beet powder. Yeast was detected in all samples (<10) but the number of yeast colonies were not more than the permitted limit. While mold was detected in candy and powder at 25˚C but it was within permissible limit. However, mold was not detected in samples of candy and powder stored at 4˚C. Antimicrobial activity of beetroot exhibits restriction of microbial growth (Nisa *et al*., 2015). Other studies also correlate the antibacterial and antimicrobial effect of beet root extract which is helpful to prevent the food from spoilage (Velicanski *et al*., 2011; Jasna *et al*., 2011).

Stability of red beet dye in pH

Figure 4 shows the pH of beet powder at three different temperatures (4˚C, 25˚C and 45˚C). According to these results a very slight change in pH was observed at 4˚C in the whole time period of 8 weeks. While at 25˚C the change in pH was slightly more than 4˚C. However, at 45˚C highest change in pH range was observed. The results are in agreement with other studies which showed that degradation of betanin pigment occurs with increase in pH. In a study by Mohammed *et al.* (2021), the beet root extract was found to be stable at pH 3-5 and the gradual decrease in the betanin stability was observed with increasing pH. Optimum pH for maximum stability of betanin is between pH 5.5 to 5.8 while oxygen is present. Betalains obtained from beetroot are normally stable at pH 5.5 (Huang and Elbe, 1987). Betalains extracted from *Amaranths* species exhibited maximum stability at pH 5 - 7 at 25˚C (Cai *et al*., 1999). Castellar *et al.* (2003) experimented that Betacyanins of *Opuntia* showed thermal stability at pH 5.0. Elbe *et al.* (1981) found that betanine solution in the presence of nitrogen is most stable at pH 4.0-5.0. Betanine degradation in solution is reversible (Huang and Elbe, 1987). Partial regeneration of betanine was experimented first after heating and results showed that amount of regeneration of the pigment depends on the pH of the sample (Elbe *et al*., 1981). Czapski (1985) noted the maximum regeneration of betanine affected by the storage temperature and the type of buffer solution. Betalain solution at pH 4.75 when kept at room temperature for 130 minutes after heating showed an increase in retention from 54% to 92% (Elbe *et al*., 1981). Czapski (1985) also obtained similar results in other studies of betanine regeneration. Hydrolysis of the aldimine bond may attributed as a possible cause of the degradation of the betanin under alkaline conditions pH>7 (Herbach *et al*., 2006).

Conclusions and Recommendations

The aim of the present research work is to promote the use of natural red dye obtained from Beetroot (*Beta vulgaris*) by replacing the synthetic red food dye with this natural one for making candies and then studying its stability in the prepared candies at different storage conditions 4°C, 25°C, 45°C for eight weeks. Stability in terms of betanin content (%) showed maximum retention at 4˚C while lowest %age of betanin content was observed at 45˚C showing that at higher temperatures the color is deteriorated. Increase in pH was observed with increasing storage temperatures. Maximum stability of the color was observed at 4˚C at most stable acidic pH and color degradation was observed with gradual increase in pH at 45˚C. The results are encouraging as this natural red beet dye is safe to use in candies because deterioration of color was observed only at high temperatures and pH. Moreover, microbiological analysis ensures the safety of natural red beet dye in candies. Therefore, it can safely be concluded that use of red beet natural colorant in product like candies which are more consumable items of children should be promoted. However, there is a need to explore different methods to increase the color stability of dye obtained from beetroot at high temperature.

Novelty Statement

To the best of our knowledge, application of natural red dye in candies and its stability studies is novel work.

Author's Contribution

Alim-un-Nisa: Conceived the idea and product development. Technical input at every step.
Sajila Hina: Did experimental work, overall management of the article.
Imran Kalim: Methodology spectrophotometric analysis.
Muhammad Khalid Saeed, Shahid Masood and Muhammad Ashraf: Methodology, stability studies.
Ijaz Ahmad: Technical input at every step.
Naseem Zahra: Experimental work, data compilation and analysis.

Sania Mazhar: Methodology, microbiological analysis.
Qurat-ul-Ain Syed: Technical input at every step.
Rabia Shad: Result and discussion, introduction, references.

Conflict of interest

The authors have declared no conflict of interest.

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