Journal of Technology and Value Addition, Volume 5 (2), July, 2023: (1-21)

Research Article



The Impact of Natural Colourants on Quality Parameters of Cream Cheeses C.U. Withanage, V.A. Wijesekara, D.M.O.E. Ulapane,

W.V.V.R. Weerasingha*

*vviraj@agri.rjt.ac.lk

Department of Animal and Food Sciences Faculty of Agriculture, Rajarata University of Sri Lanka, Puliyankulama, Anuradhapura, Sri Lanka

Abstract

Physiochemical, microbiological and sensory properties were investigated for cream cheese which fortified with natural colourants, 4% Hibiscus, 3% beet root, 9% carrot, and 2% blue pea over 14 days at 4°C compared with colourless control. A decrease in lightness was observed over the storage, indicating pigment degradation. The highest ($61.42 \mu g \text{ GAEmL}^{-1}$) and lowest ($24.04 \mu g \text{ GAEmL}^{-1}$) Total Plate Count were observed in beet root and hibiscus incorporated cream cheese respectively, at the beginning and end of the storage. Since the coliform level was within the standard range the product safety was ensured. The highest sensory ranking for colour, aroma, texture and overall acceptability were observed in carrot incorporated cream cheese.

Keywords: Cream cheese, natural colourants, quality, storage

1. Introduction

Colour is one of the most important and appealing attributes that influences the preference, flavour perception, and ultimate purchasing decision of the consumer. Further, it indicates the food freshness and quality (Toldra and Nollet, 2021). The usage of natural colourants in food has become a highly penetrated trend that has been also moved to the dairy sector (Wijesekara *et al.*, 2022; Ghosh *et al.*, 2022). This user-friendly incorporation supports to overcome the detrimental health issues such as allergic reactions and attention deficit hyperactivity disorder (ADHD) in children caused due to the consumption of artificial colourants like E122, ponceau 4R E124, and Allura red AC E129 (Luzardo-Ocampo *et al.*, 2021).

Cream cheese is a fresh dairy product with growing economic significance. By 2026, it is expected that the worldwide cheese market would be worth \$8.3 billion US (Pombo, 2021). This acid-coagulated fresh cheese product has a mildly buttery flavor, a slight dairy sourness, complete lack of bitterness, a creamy texture, a firmness that ranges from brittle to spreadable, and a shining appearance (Pombo, 2021). Rennet is frequently employed as the coagulant in cream cheese, which is primarily acidified with mesophilic lactic acid starter cultures made up of Lactococcus spp. and Leuconostoc spp. (Phadungath, 2005). The minimum milk fat level of 33% and a maximum moisture content of 55% by weight should be in a quality cream cheese according to the US Food and Drug Administration (2019). High levels of polyunsaturated fatty acids in dairy products are quickly oxidized by molecular oxygen as storage time increases. It results in undesirable alterations such as flavor loss, colour loss, and compound buildup that is bad for the customers' health (Gad and Sayd, 2015). This research is an attempt to check the potential of beet root, carrot, *Hibiscus*, and blue pea as natural colourants (Figure 1) in cream cheese that would also improve the product bio functionalities.



Figure 1: A. Beet root, B. Carrot, C. Hibiscus, D. Blue pea.

Beet root (*Beta vulgaris L.* ssp. *vulgaris*) which is a biennial plant of the Chenopodiaceae family (Janiszewska, 2014) is abundant with red and yellow anthocyanins known as betalains that is made up of betaxanthins and betacyanin. The red pigment of the beet is mainly due to the betacyanin that proportionate 75 – 95%. Beet has being used in varieties of foods, including dairy products, chocolates, jelly beans, non-alcoholic beverages, and several emulsified meat products (Güneşer, 2016). Aforementioned pigments are sensitive to pH and temperature changes. Betanin is a potent antioxidant pigment that may offer protection and lower the risk of cardiovascular disease and cancer. Moreover, betalains sparked interest as it provides antioxidative, anti-inflammatory, and anticarcinogenic effects (Bet *et al.*, 2012).

Carrots (*Daucus carota* L.) belonging to Apiaceae (previously Umbelliferae) family are relatively affordable and highly nutritious, with significant amounts of vitamins B1, B2, B4, and B12. Carotene being the main pigments linked to a lower risk of various malignancies, including prostate cancer (Haq and Prasad, 2015). Furthermore, carrot contains vitamin A which act as an antioxidant that is essential for tissue growth and repair. Also, aids the body in fighting infections, maintaining eye health, and nourishing epithelial tissues in the lungs and skin (Singh, Panesar and Nanda, 2006). Reviewing the previous researches, probiotic soft cheese made out of buffalo milk, incorporated with carrot powder has shown that the amount of moisture and salt has changed at 0 days. Further, using carrot powder at a rate of 0.6% has caused the overall bacteria count in the cheese to drop from 7.5 to 7.3 log cfu/g. Adding carrot powder to the storage during the 28 days resulted in a decrease in the overall bacteria and *Bifidobacterium longum* at the same storage of 28 days (Kamel *et al.*, 2023).

Hibiscus rosa-sinensis L., (*Hibiscus*) extracts have been used as a natural food colourant and as an emulsifier for carbonated drinks, jams, and juices (Upadhyay and Upadhyay, 2011). Also, the use of water-soluble *Hibiscus* as a daily soft drink in Egypt, Sudan, and Mexico give clues that *Hibiscus* is a consumer-accepted natural product (Abou-Arab *et al.*, 2011). Red to violet hues in *Hibiscus* anthocyanin could be used in pharmaceutical and cosmetic industries (Cisse *et al.*, 2011). As well as the addition of 5% *Hibiscus* powder in milk candy production gives the best antioxidant activity, colour, and yield (Ekie and Evanuarini, 2020). The zobo drink produced by *Hibiscus* is consumed as a meat substitute due to its nutraceutical value and also, the use of *Hibiscus* extract in yogurt production has given positive impacts on biochemical and organoleptic properties (Iwalokun and Shittu, 2007).

Clitoria ternatea L. (Blue pea) treats many disorders such as liver problems, urine infections, anasarca, and enlargement of abdominal viscera and it is proved that there is a commercial potentiality of using blue pea extract in a beverage (Lakshan *et al.*, 2019). Delphinidin anthocyanins in blue pea are the main responsible pigment that gives the deep blue colour of *Clitoria* (Campbell *et al.*, 2019). Antineoplastic, antiatherogenic, and antioxidant effects and potential of using blue pea in producing healthy frozen sugar-free ice creams that have a lower glycemic response and good antioxidative properties are proven (Limsuwan *et al.*, 2014). Cupcakes incorporated with blue pea anthocyanins are said to be retained about 40% and increases the nutritional value and quality (Thanh *et al.*, 2020).

Hence, the current study on cream cheese with natural colourants will be a fastmoving product in the future market that is enriched with goodness overcoming adverse effects of artificial colourants.

2. Materials and Methods

2.1. Manufacturing process of cream cheese

2.1.1. Preparation of Anthocyanin aqueous extract form Hibiscus

The collected *Hibiscus* calyx were dried at 45°C for 24 hours until moisture content drop down below 10% (Inggrid, Jaka and Santoso, 2016). Extraction procedure was followed according to Wong *et al.* (2003) with some modifications. Boiled water was used for extraction and ratio of 1:40 (w/v) calyx to water was used in each extraction. The final extract was obtained after filtering with a cotton filter and stored it in the dark at -10°C until further analysis.

2.1.2. Preparation of Anthocyanin aqueous extract form blue pea

Collected unharmed disease - free blue pea flowers (*Clitoria termatea* L) were oven dried for 24 hours at 50°C (Lakshan *et al.*, 2019). In order to increase the surface area and mixing ability, the dried flower samples were ground into tiny particles. As per the maceration process, blue dye was extracted from the powder. (Baskaran *et al.*, 2019). A weight of 10 g of dried pea blossom powder with a moisture content of 3%–5% was mixed with 200 ml of water to begin the extraction process (from 15 to 60 minutes). After that, the samples were spun at 2000 rpm for 30 minutes (Thuy *et al.*, 2021). The final extract was produced after filtering with a cotton filter was used (Veggi, Santos and Meireles, 2011).

2.1.3. Preparation of Betaxanthins aqueous extract form Beet root

Raw beet roots were wash thoroughly using running tap water in order to remove dirt and debris. Initially Beet roots were cut into small dices of a thickness around 1mm following grinding was practiced using a sterile electrical blender (CML-7360065-Japan). Furthermore, peels were then blended in a mixer for 2 min to obtain a pulp (Zin, Majrxi and Banvolgyi, 2020).

2.1.4. Preparation of Anthocyanin aqueous extract form carrot

Aqueous extract of carrot was prepared according to Fuleki and Francis (1968), modified by Colin and Peter. Twenty grams of carrots were macerated in a warring blender at 2000 rpm speed for five minutes after being combined with 100 milliliters of food grade ethanol that had been acidified with 0.01% citric acid. Thereafter, the supernatant was filtered by using whatman No 1 filter paper via a buchner funnel.

2.1.5. Preparation of coloured cream cheese

Preparation of cream cheese was done according to Enwa *et al.* (2013) with certain modifications. Fresh cow milk was filtered and standardized to get an ultimate fat content of 3.5%. Milk base was pasteurized at 63-68°C for 30 min followed by cooling down to 30-32°C. Subsequently, 0.075% of mesophilic starter culture (CHN-22, Chr. Hansen) consisting *Lactococcus lactis* subsp. *cremoris, Leuconostoc, Lactococcus lactis* subsp. *lactis, and Lactococcus lactis* subsp. *lactis biovar diacetylactis* was added along with 0.05% w/v fermentation-produced bovine calf chymosin (chymosin 100%, 650 International Milk-Clotting Units per gram (IMCU g⁻¹); CHY-MAX). Thereafter, incubation was carried out for approximately 40-45 min until milk curd was formed. Curd was then cut with an incubated spatula for whey separation for 1 hr. Salt (2% w/w maximum) and an aqueous extract of natural colourants were also added. A food processor was used to thoroughly blend the cream cheese. For additional analysis, prepared samples were kept in a refrigerator at 40°C in sealed containers.

For preliminary studies, several concentrations of natural colourants were incorporated to cream cheese, among those the ideal level of each aqueous and powder extract to be integrated was determined using a trial-and-error procedure and a sensory evaluation. The most appropriate level of each treatment was chosen. 3%, 4%, 5% of beet root (T1), 4%, 5%, 6% of *Hibiscus* (T2), 7%, 8%, 9% of carrot (T3) and 2%, 1%, 0.3% of blue pea (T4) was incorporated respectively. After selecting the best level, the selected best levels of each colourant were replicated into three replicates for further studies.

2.2. Analysis of physiochemical properties

All the physicochemical properties were tested on 1, 7, and 14 days of storage at 4°C in triplicates. The sensory evaluation was done on 1, 7, and 14 days respectively.

2.2.1. pH and Titratable acidity

pH was measured using an electrode immersion with a pH meter (Thermo scientific pH 450, Singapore). Titratable acidity was measured according to the method described by Jayarathna *et al.* (2020), with few modifications. In brief, the filtrates of cream cheese were titrated against 0.1% NaOH in the presence of phenolphthalein indicator until a persistent pale pink colour was observed.

2.2.2. Moisture, crude fat, ash and crude protein content

Moisture content was determined according to Bradley Jr (2010) on triplicated cheeses. Crude fat content was determined by the Soxhlet extraction method which was described by Luque de Castro and Priego-Capote. (2010). By incineration of 2 g of each sample in a muffle furnace (HumanLab inc, DMF-05, Korea) for 5 hours at 550°C the ash content was determined. Crude protein content of each sample was determined by the kjeldhal method.

2.2.3. Syneresis

According to Arab *et al.*, (2020), the centrifugation method was used to conduct the analysis for syneresis (Labnet 6C). Cream cheese samples weighing 10 g from each treatment were centrifuged at 400 rpm for 30 minutes. The clear supernatant was weighed and the syneresis was quantified as a percentage of the original weight using the equation below of cream cheese.

Syneresis (%) = (Weight of whey / Weight of sample) x 100%.

2.2.4. Colour

A colourimeter (CR-10, KONICA MINOLTA, and Japan) was used for the analysis. Red/greenness, yellow/blueness, and lightness, the three colour parameters, were measured and were formulated using the L^* a* b* method.

2.2.5. Total phenolic content

Total phenolic contents were detected, using Folin-Ciocalteu reagent as stated by Moldovan, Iasko, and David I (2016). Expressed as mg gallic acid equivalents (GAE L^{-1}) of cream cheese.

2.2.6. Microbiological analysis

A serial dilution of cream cheese was prepared using 0.1% peptone solution, and diluted samples were plated using the pour plate technique. Potato dextrose agar (Biolab) was used for the enumeration of yeast and mold, at 37°C for 48 h. For the enumeration of coliforms, MacConkey agar was used while plates were incubated at 37°C for 24 h.

2.2.7. Sensory evaluation

The organoleptic quality attributes of coloured cream cheese were tested by 30 untrained panelists in terms of appearance, texture, flavor, mouth-coating, and overall acceptability based on a 5-point hedonic scale.

2.2.8. Statistical analysis

Physicochemical, and microbiological attributes were analyzed using two-way ANOVA of MINITAB software (Minitab® 17.1.0) with 95% confidence interval, Tukey test was used for mean separation while Kruskal wallis test along with 95% confidence interval was used to analyze data for Sensory evaluation tests by MINITAB software (Minitab® 17.1.0). Data for the antioxidant was analyzed by using completely randomized design (CRD) of ANOVA of SAS program (Version 9.0) and mean separation among the treatments were done by LSD method to determine the statistical difference among them at the significant level of p < 0.05.

3. Results and Discussion

3.1. Selecting the best level of incorporation

Trial and error method and preliminary sensory evaluation resulted 3% Blue pea, 4% *Hibiscus*, 9% Carrot and 2% Beet root as the best levels to add in the preparation of cream cheese (Figure 2).



Figure 2: Best level of natural colourants incorporated to cream cheese

3.2. Changes in pH, titratable acidity (TA), syneresis of natural coloured cream cheese during the storage period

Physicochemical properties were evaluated during the stipulated shelf life. Cream cheese treated with the aqueous pigment extraction of *hibiscus*, carrot, and blue pea did not significantly (p > 0.05) influence the pH on day 1. Thus, Beet root incorporated cheese was significantly differed (p > 0.05) from the rest of the treatments as well as the control. Surprisingly on day 14 beet root cream cheese showed no significance difference to the control and the carrot treated cream cheese. Comparing hibiscus and blue pea, they had no significant effect on pH at the end of the storage but they were significantly differed from rest. However, there was a gradual decrease (p < 0.05) in pH from 6.61±0.01 to 5.45±0.02 (Table 1) during the storage period. The reason would be that the fermentation of cream cheese, biomass may consume lactose as a food source and make lactic acid as a byproduct, which will gradually lower the pH (Abedi and Hashemi, 2020). The aforementioned reason get support by the stored room temperature that tends to accelerate lactose fermentation, which causes pH levels to fall (Perveen et al., 2011). Titratable acidity of beet root incorporated cream cheese and carrot treated cream cheese were insignificant on day 1 while the lowest pH was observed in blue pea cream cheese. Beet root and carrot incorporation had the same effect on pH recording the highest pH at the end of the 14 days as 4.56±0.02 (Table 1). The results obtained in the study are in agreement with Perveen et al. (2011), who observed gradual increases in titratable acidity with the storage of cream cheese at $4\pm1^{\circ}$ C. The higher TA in naturally coloured cream cheese samples in contrast to the control sample demonstrates that natural pigments accelerate the post storage fermentation process. All of the cream cheese samples displayed a growing syneresis patterns throughout the period of storage. There was a significant difference (p > 0.05) in the syneresis between the treatments (Figure 3). But the lowest syneresis value was for the control sample. One of the primary causes may be the higher fiber content of blue pea, carrot, Beet root, Hibiscus, and hibiscus. The fibers help to hold in the water molecules rather than release them. This information is consistent with the findings of recent research by Staffolo et al. (2017). They demonstrated that a semisolid dairy dessert made with plant-based fiber exhibits low syneresis and low impaired mechanical properties as compared to dessert without added plant fiber.

ST	$T_0 \pm SD$	$T_1 \pm SD$	$T_2 \pm SD$	$\begin{array}{c} T_{3} \\ \pm SD \end{array}$	$T_4 \pm SD$		
-	рН						
Day 01	6.59±0.03 ^{Ab}	6.39±0.05 ^{Ac}	6.61 ± 0.01^{Ba}	6.18±0.00 ^{Ba}	6.80 ± 0.04^{Aa}		
Day 07	6.02±0.01 ^{Bb}	5.99±0.06 ^{Bb}	$5.97{\pm}0.04^{\rm Bb}$	5.98 ± 0.08^{Bb}	6.57±0.03 ^{Ab}		
Day 14	5.83±0.03 ^{Cb}	5.77 ± 0.03^{Cb}	5.71±0.01 ^{Cc}	$5.45\pm0.02^{\text{Cb}}$	6.28 ± 0.02^{Ca}		
TTA							
Day 01	1.41±0.00 ^{Cc}	2.88 ± 0.05^{Ba}	2.49 ± 0.00^{Cb}	2.79±0.05 ^{Ca}	1.38±0.05 ^{Cc}		
Day 07	1.65±0.13 ^{Bc}	2.88±0.01 ^{Ba}	3.72±0.00 ^{Ba}	3.69±0.02 ^{Ba}	1.71±0.00 ^{Bc}		
Day 14	2.52±0.01 ^{Ad}	4.56±0.02 ^{Aa}	3.88 ± 0.02^{Ac}	4.56±0.25 ^{Aa}	3.33 ± 0.05^{Ab}		

Table 1: pH and Titratable acidity of coloured cream cheese at 4°C.

Mean values with average of triplicates are shown.

T₀: Control; T₁: Beet root; T₂: Hibiscus; T₃: Carrot; T₄: Blue pea; SD: Standard Deviation; ST: Storage Day; TTA: Titratable Acidity.

Means with different uppercase superscript are significantly different (p < 0.05) within the same parameter for three different storage days. Means with different lowercase superscript are significantly different (p < 0.05) within the same row for different treatments.



Figure 3: Changes of the syneresis period of storage in naturally coloured cream cheese

3.3. Colour stability

One of the important physicochemical characteristics examined in the current investigation is colour. By assessing the L^* , a^* , and b^* values, the study sought to assess the stability of colour throughout storage. The obtained data is in the Table 2. In comparison to the control sample, the lightness (L^*) was less due to the use of plant-based colourants. The lowest L* was found in cream cheese with hibiscus on day 14, while the lowest L* was found in blue pea on day 1. For all treatments, L* values generally increased by the conclusion of storage. This increase in L* suggests that the colour pigments may be deteriorating or oxidizing, which would increase the colourant's lightness. This is in line with a number of research, including those on fruity yogurt (Pires et al., 2018) and red rice coloured yogurt (Chen, Lv, Du, and Chen, 2012). Contrary to our findings, grape included kefir (Montibeller et al., 2018), and the L* value decreased over time. According to Pires *et al.* (2018), lactic bacteria may have a negative effect on the stability of anthocyanin by hastening their breakdown by enzymes such as glycosidase. Additionally, increased oxygen concentrations added during processing may result in significant anthocyanin losses. As expected, cream cheese that includes beet root has a greater a* (red/green) value than carrot, which has a higher b* (yellow/blue) value. On day 14, carrot-infused cream cheese had a stronger yellow hue than it had on day 1. Despite the fact that measured colour values changed after storage, no discernible colour change was found.

ST	T_0	T_1	T_2	T_3	T_4		
	$\pm SD$						
L* values							
Day 1	36.33±0.05 ^{Aa}	32.53±0.01 ^{Aa}	27.57 ± 0.04^{Aa}	40.00±0.01 ^{Aa}	24.93±0.04 ^{Aa}		
Day 07	36.47±0.00 ^{Aa}	32.53±0.00 ^{Aa}	29.57±0.00 ^{Aa}	41.37±0.04 ^{Aa}	25.23±0.00 ^{Aa}		
Day 14	81.60±0.05 ^{Aa}	72.43±0.07 ^{Ab}	70.33±0.04 ^{Ab}	79.13±0.04 ^{Aa}	71.87±0.02 ^{Ab}		
a* values							
Day 01	3.20 ± 0.01^{Ad}	14.73±1.3 ^{Aa}	12.5±0.2 ^{Ab}	6.40±0.04 ^{Ac}	-2.13±0.01 ^{Ae}		
Day 07	33.0±0.04 ^{Aa}	42.07±0.00 ^{Aa}	33.27±0.04 ^{Aa}	27.27±0.04 ^{Aa}	24.57±0.01 ^{Aa}		
Day 14	35.13±0.04 ^{Aa}	40.87±0.02 ^{Aa}	38.70±0.84 ^{Aa}	29.20±0.02 ^{Aa}	26.2±0.02 Aa		

Table 2: Changes of the L* a* b* value during period of storage in naturally coloured cream

 cheese

Journal of Technology and Value Addition, Volume 5 (2), July, 2023: (1-21)

b* values						
Day 01	22.67 ± 0.01^{Ab}	14.13±0.04 ^{Ac}	11.13±0.02 ^{Ac}	35.13±0.04 ^{Aa}	9.20±0.21 ^{Ad}	
Day 07	35.33±0.02 ^{Aa}	33.07±0.21 ^{Aa}	27.63±0.01 ^{Aa}	39.57±0.02 ^{Aa}	25.10±0.02 ^{Aa}	
Day 14	35.40±0.02 ^{Aa}	33.10 ± 0.02^{Aa}	29.50±0.2 ^{Aa}	39.70±0.40 ^{Aa}	25.83±0.02 ^{Aa}	

Mean values with average of triplicates are shown.

T₀: Control; T₁: Beet root; T₂: Hibiscus; T₃: Carrot; T₄: Blue pea; SD: Standard Deviation; ST: Storage Day

Means with different uppercase superscript are significantly different (p < 0.05) within the same parameter for three different storage days. Means with different lowercase superscript are significantly different (p < 0.05) within the same row for different treatments.

3.4. Changes in moisture, Crude Protein, Crude Fat, and ash content of naturally coloured cream cheese

The effect of adding aqueous forms of pigments on the physical and chemical properties of cream cheese is represented in Table 3 for the day 14. The highest protein content was reported in blue pea sample and the lowest protein in Beet root sample. Control has shown significant differences compared to the treatments. Crude fat was recorded highest as 43.38±0.65 in blue pea added cream cheese. It was significantly different (p > 0.05) from the rest of the treatments including the control. The fat content of the spreadable-type processed cheese samples varied from 11.8% to 29.3% as per the study by Dimitreli et al. (2017). In terms of content, reducedand low-fat cheeses are preferred but frequently fall short in terms of overall quality. One of the main issues with cheese that has had its fat content reduced is that it has developed a rigid texture that, unlike full-fat cheeses, does not crumble when chewed (Rogers *et al.*, 2010). Results indicate the largest proportion of ash in beet root which give the hint that it carries more minerals. Related to moisture, the control had lowest value compared to the treatments. This is supported by Senadeera et al. (2018). As mentioned before, it may be due to the high holding capacity of fiber enriched natural phenolics. Highest moisture content was observed in blue pea incorporated cream cheese.

	T0	T1	T2	T3	T4
	±SD	$\pm SD$	$\pm SD$	$\pm SD$	$\pm SD$
Crude Protein (%)	7.03±0.06 ^b	6.75±0.05°	6.80±0.04 ^c	7.16±0.03 ^{ab}	7.22±0.03 ^a
	27.04.0.57h	25.00 0.57h	26 57 0 00h	26.00 0 10h	10.00 0 65%
Crude Fat (%)	$3/.04\pm0.5/^{\circ}$	35.99±0.57°	$36.5 \pm 0.82^{\circ}$	$36.09\pm0.48^{\circ}$	43.38±0.65°
Ash (%)	$2.22+0.10^{\circ}$	3.49 ± 0.18^{a}	3.08+0.11 ^b	2.87 ± 0.07^{b}	2.98 ± 0.05^{b}
(,,,)	0.10	2	2.00_0.11	,,	
	20.00.005cd	41.04.1.250	20.27.0.77d	15 00 0 00h	40 71 . 1 103
Moisture content (%)	38.22±0.95 ^{cu}	$41.04\pm1.35^{\circ}$	$39.3/\pm0.77^{\rm u}$	$45.02\pm0.80^{\circ}$	$49./1\pm1.18^{a}$

Table 3: Physicochemical properties of naturally coloured cream cheese under 4 °C storage

3.5. Total Phenolic Content (TPC)

Highest (67.67 µg GAEmL⁻¹) and lowest (27.66 µg GAEmL⁻¹) TPC was observed on day 01, in Beet root incorporated cream cheese and the control sample, respectively (Figure 4). In general, cows' milk contains a variety of antioxidant molecules, including endogenous antioxidants like catalase and superoxide dismutase as well as exogenous antioxidants derived from vitamins A, E, and C (Khan et al., 2019). Proteins like lactoferrin and coenzyme Q1O may attract certain antioxidants. The transfer of phenolic compounds from plants results in their presence in milk and cheese products, which impacts the milk's total antioxidant activity. The TPC level observed in the control cream cheese may probably be derived from polyphenols in milk (Benzie et al., 2010). The presence of phenolic compounds in milk and cheese products is resulted from their transfer from plants which affects the overall antioxidant activity in milk (Vázquez et al., 2014). Additionally, El-Shafei et al., (2017) stated that higher TPC values of cream cheese was testified for increasing levels of pomegranate peel extract. Meanwhile plants are rich sources of polyphenols and have higher concentrations of bioactive compounds, the incorporation of plant materials into cream cheese resulted in higher TPC.



Figure 4: TPC of cream cheese incorporated with natural colourants under the storage of $4^{\circ}C$

3.6. Microbiological Analysis

Throughout the whole period of storage, all the cream cheese samples showed significant differences in viable counts of yeast and mold counts as the storage period extended (Table 4). Even though, there were not any significant difference among the samples. The highest viable yeast and mold count on the last day of storage was reported from the control which was 7.41 ± 0.00 CFU/ml whereas the lowest viable yeast and mold count was detected in T1, 7.37±0.01 CFU/ml, which included *Hibiscus* incorporated cream cheese. The reason for the aforementioned results could be the antimicrobial properties of Hibiscus. As per Farasayu et al. (2021), the hibiscus contains many phytochemicals, such as flavonoids and tannins that are inhibiting cytoplasmic membrane function, synthesis of nucleic acid, energy for metabolism, and biofilm formation. Dalie et al. (2010) stated that lactic acid bacteria (LAB) level has been an indicator for preventing the mold growth in foods. Result revealed that there was no change in the treatments with respect to viable coliform cell count over the storage period that was statistically significant (p < 0.05). However, there is a distinction between the hibiscus and blue-pea-infused cream cheese in terms of the total counts of viable coliforms. At the end of the storage

period, hibiscus-infused cream cheese had the highest viable coliform count (2-5 log CFU/ml), while blue pea-infused cream cheese had the lowest (2 log CFU/ml).

	T_0	T_1	T_2	T ₃	T_4
	$\pm SD$	±SD	±SD	±SD	±SD
Day 01	6.85±0.00 ^a	6.81±0.00 ^a	6.86±0.00 ^a	6.78±0.00 ^a	6.80±0.00 ^a
Day 07	7.02±0.01 ^b	7.01 ± 0.00^{b}	6.98 ± 0.00^{b}	7.01±0.01 ^b	7.02±0.01 ^b
Day 14	$7.41 \pm 0.00^{\circ}$	7.39±0.05°	7.37±0.01°	$7.40 \pm 0.00^{\circ}$	7.39±0.05°

Table 4: Impact of natural colours on the viable cell count in cream cheese

Mean values with average of triplicates are shown.

T₀: Control; T₁: Beet root; T₂: Hibiscus; T₃: Carrot; T₄: Blue pea; SD: Standard Deviation. Means with different uppercase superscript are significantly different (p < 0.05) within the same parameter for three different storage days. Means with different lowercase superscript are significantly different (p < 0.05) within the same row for different treatments.

3.7. Sensory Evaluation

The same 30 sensory panelists who participated in the preliminary screening scored each product on days 1, 7, and 14 of storage, along with the control, based on its colour, texture, flavor, aroma, and overall acceptability. The mean customer liking score separately from the product with regard to features instead of selecting the most favoured cream cheese was evaluated. As a result, each attribute had a different favourite product on each day. The additional plant pigments probably had a negative impact on the treated samples' fragrance. On the first day, the carrot aroma scored the highest, whereas on the fourteenth, the control aroma was the most popular. Control was the strongly suggested flavor for day 1. By day 14, control scored similarly to the carrot-cream cheese, in terms of flavour (Figure 5). According to Freitas-Sa et al. (2018), the flavor of non-coloured yogurt (Jabuticaba and Jamelao peel) received the highest mean consumer like score when compared to colourful yogurts. This conclusion is consistent with their findings. The flavor would have to live up to the expectations set by the hue in the minds of the consumers. On day 1, the colour of blue pea combined with cream cheese was the least liked. Throughout the storage, carrot had the greatest favorable colour rating, followed by beet root (Figure 5). In terms of general acceptability, carrot cream cheese had the highest value throughout storage, followed by beet root on day one, which was surpassed by the control later. Perhaps, the reason for carrot to be most preferred could be that it

is similar to the visible colour of authentic cream cheese with general colourant, annatto. Anyhow, the texture and general acceptance were not noticeably different on days 1 and 14.



Figure 5: Sensory analysis of natural coloured cream cheese (A. Day 1 and B. Day 14)

4. Conclusions

The current study simultaneously compares the physical and chemical characteristics of natural plant pigments in fermented milk. The outcomes showed that using natural pigments for colouring cream cheese did not have a negative impact on how the product was perceived by consumers. Did not simultaneously have a negative impact on the total lactic acid bacteria or the viable bacterial count, potentially maintaining bacterial counts above the necessary thresholds. Additionally, a higher level of sensory acceptability as well as an improvement in TPC values in coloured cream cheese were confirmed.

The conclusions and insights from this research will aid in the development of comprehensive technical and technological applications for fermented milk. The findings will have more industrial relevance if more research is done on the selection of natural pigments, health consequences, and technology for pigment extraction. Additionally, study will undoubtedly look into the most effective ways to extract the pigments from those plant sources in order to scale up the production of cream cheese. To confirm the ability of the examined plant materials to give health-promoting advantages, investigations that concentrate on nutrition and health benefits are also advised.

5. References

Abedi, E. and Hashemi, S.M.B. (2020). Lactic acid production–producing microorganisms and substrates sources-state of art. *Heliyon*, 6(10), p.e04974.

Abou-Arab, A.A., Abu-Salem, F.M. and Abou-Arab, E.A. (2011). Physico-chemical properties of natural pigments (anthocyanin) extracted from Roselle calyces (*Hibiscus subdariffa*). *Journal of American science*, 7(7), 445-456.

Arab, R., Freidja, M.L., Oomah, B.D., Benali, S., Madani, K. and Boulekbache-Makhlouf, L. (2020). Quality parameters, probiotic viability and sensory properties of probiotic stirred sesame yogurt. *The Annals of the University Dunarea de Jos of Galati. Fascicle VI-Food Technology*, 44(1), 9-25.

Benzie, I. F., and Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry*, 239(1), 70–76. https://doi.org/10.1006/abio.1996.0292

Bhavaniramya, S., Vishnupriya, S., Al-Aboody, M.S., Vijayakumar, R. and Baskaran, D. (2019). Role of essential oils in food safety: Antimicrobial and antioxidant applications. *Grain and oil science and technology*, 2(2), 49-55.

Bradley, R.L. (2010). Moisture and total solids analysis. Food analysis, 4.

Campbell, S.M., Pearson, B. and Marble, S.C. (2019). Butterfly Pea (Clitoria ternatea) Flower Extract (BPFE) and Its Use as a ph-Dependent Natural Colourant:(ENH-1309/EP573, 4/2019). *EDIS*, 2019(2).

Chen, S.H.A., Lv, B., Du, X. And Chen, F. (2012). Pigment from red fermented rice as colouring agent for stirred skimmed milk yoghurts. *International journal of dairy technology*, 65(2), 287-292.

Cissé, M., Vaillant, F., Pallet, D. and Dornier, M. (2011). Selecting ultrafiltration and nanofiltration membranes to concentrate anthocyanins from roselle extract (*Hibiscus sabdariffa* L.). *Food Research International*, 44(9), .2607-2614.

Dalié, D.K.D., Deschamps, A.M. and Richard-Forget, F. (2010). Lactic acid bacteria–Potential for control of mould growth and mycotoxins: A review. *Food control*, 21(4), .370-380.

De Castro, M.L. and Priego-Capote, F. (2010). Soxhlet extraction: Past and present panacea. *Journal of chromatography A*, *1217*(16), .2383-2389.

Dimitreli, G., Exarhopoulos, S., Antoniou, K.K., Zotos, A. and Bampidis, V.A. (2017). Physicochemical, textural and sensory properties of white soft cheese made from buffalo and cow milk mixtures. *International Journal of Dairy Technology*, *70*(4), .506-513.

Ekie, M.A.B. and Evanuarini, H. (2020), April. The quality of milk candy using rosella powder (*Hibiscus sabdariffa* L.) addition as natural food colouring. In *IOP Conference Series: Earth and Environmental Science*. 492(1), 012059. IOP Publishing.

El-Shafei, S., Abdeen, E.M.M. and Abozed, S.S. (2017). Using pomegranate peel extracts as natural antioxidant in cream cheese manufactured from goat's milk. *Zagazig Journal of Agricultural Research*, *44*(6), 2649-2664.

Enwa, F., Avbunudiogba, A., John, C. And Godstime, O. (2013). Physiochemical comparism of three different types of soft cheese. *Journal of Environmental Science, Toxicology and Food Technology*, *4*(5), 11-17.

Farasayu, R.D., Rachmawati, M.W., Ana, I.D., Syaify, A. and Listyarifah, D. (2021). The Effect of Hibiscus Flower Extract (*Hibiscus rosa-sinensis* L.) on the Growth of Streptococcus sanguinis Bacteria. In BIO Web of Conferences (Vol. 41, p. 07006). *EDP Sciences*.

Freitas-Sá, D.D.G.C., de Souza, R.C., de Araujo, M.C.P., Borguini, R.G., de Mattos, L.D.S., Pacheco, S. and de Oliveira Godoy, R.L. (2018). Effect of jabuticaba (*Myrciaria jaboticaba* (Vell) O. Berg) and jamelão (*Syzygium cumini* (L.) Skeels) peel powders as colourants on colour-flavor congruence and acceptability of yogurts. *Lwt*, *96*, 215-221.

Fuleki, T. and Francis, F.J. (1968). Quantitative methods for anthocyanins. 1. Extraction and determination of total anthocyanin in cranberries. *Journal of food science*, *33*(1), 72-77.

Gad, A.S. and Sayd, A.F. (2015). Antioxidant properties of rosemary and its potential uses as natural antioxidant in dairy products—A review. *Food and Nutrition Sciences*, 6(01), 179.

Ghosh, S., Sarkar, T., Das, A. and Chakraborty, R. (2022). Natural colourants from plant pigments and their encapsulation: An emerging window for the food industry. *Lwt*, *153*, 112527.

Güneşer, O. (2016). Pigment and colour stability of beetroot betalains in cow milk during thermal treatment. *Food Chemistry*, *196*, 220-227.

Haq, R.U., Kumar, P. and Prasad, K. (2015). Hot air convective dehydration characteristics of *Daucus carota* var. Nantes. *Cogent Food and Agriculture*, *1*(1), 1096184.

Inggrid, H.M., Jaka and Santoso, H. (2016). 'Natural red dyes extraction on roselle petals', *IOP Conference Series: Materials Science and Engineering*, 162(1).

Iwalokun, B.A. and Shittu, M.O. (2007). Effect of *Hibiscus sabdariffa* (calyce) extract on biochemical and organoleptic properties of yogurt. *Pakistan Journal of Nutrition*, 6(2), 172-182.

Janiszewska, E. (2014). Microencapsulated beetroot juice as a potential source of betalain. *Powder Technology*, 264, 190-196.

Jayarathna, S., Priyashantha, H., Johansson, M., Vidanarachchi, J.K., Jayawardana, B.C. and Liyanage, R. (2021). Probiotic enriched fermented soy-gel as a vegan substitute for dairy yoghurt. *Journal of Food Processing and Preservation*, 45(1), e1509.

Kamel, D.G., Hammam, A.R., El-Diin, M.A.N., Awasti, N. and Abdel-Rahman, A.M. (2023). Nutritional, antioxidant, and antimicrobial assessment of carrot powder and its application as a functional ingredient in probiotic soft cheese. *Journal of Dairy Science*, *106*(3), 1672-1686.

Khan, I.T., Bule, M., Ullah, R., Nadeem, M., Asif, S., and Niaz, K. (2019). The antioxidant components of milk and their role in processing, ripening, and storage: *Functional food. Vet World*, *12*(1), 12-33.

Lakshan, S.A.T., Jayanath, N.Y., Abeysekera, W.P.K.M. and Abeysekera, W.K.S.M. (2019). A commercial potential blue pea (*Clitoria ternatea* L.) Flower extract incorporated beverage having functional properties. *Evidence-Based Complementary and Alternative Medicine*, 2019.

Limsuwan, T., Paekul, N., Thongtan, J. and Tangkanakul, P. (2014). Total phenolic compounds, antioxidant activity and nutritional values of sugar-free and reduced-fat milk-based ice cream enriched with selected herb ingredients. *Asia-Pacific Journal of Science and Technology*, *19*(4), 515-525.

Luzardo-Ocampo, I., Ramírez-Jiménez, A.K., Yañez, J., Mojica, L. And Luna-Vital, D.A. (2021). Technological alications of natural colourants in food systems: A review. *Foods*, *10*(3), 634.

Manoharan, A., Ramasamy, D., Dhanalashmi, B., Gnanalashmi, K.S. and Thyagarajan, D. (2012). Organoleptic evaluation of beetroot juice as natural colour for strawberry flavor ice cream. *Indian Journal of Medicine and Healthcare*, *1*(1), 34-7.

Moldovan, B., Iasko, B. and David, L. (2016). Antioxidant activity and total phenolic content of some commercial fruit-flavoured yogurts. *Studia universitatis babes-bolyai, chemia*, 61.

Montibeller, M.J., de Lima Monteiro, P., Tupuna-Yerovi, D.S., Rios, A.D.O., and Manfroi, V. (2018). Stability assessment of anthocyanins obtained from skin grape alied in kefir and carbonated water as a natural colourant. *Journal of Food Processing and Preservation*, 42(8), p.e13698.

Perveen, K., Alabdulkarim, B., and Arzoo, S. (2011). Effect of temperature on shelf life, chemical and microbial properties of cream cheese. *African Journal of Biotechnology*, *10*(74), .16924-16928.

Phadungath, C. (2005). Cream cheese products: A review. *Songklanakarin Journal of Science and Technology*, 27(1), 191-199.

Pires, T.C., Dias, M.I., Barros, L., Calhelha, R.C., Alves, M.J., Oliveira, M.B.P., Santos-Buelga, C. and Ferreira, I.C. (2018). Edible flowers as sources of phenolic compounds with bioactive potential. *Food Research International*, *105*, 580-588.

Pombo, A.F.W. (2021). Cream cheese: Historical, manufacturing, and physicochemical aspects. *International Dairy Journal*, *117*, 104948.

Rogers, N.R., McMahon, D.J., Daubert, C.R., Berry, T.K. and Foegeding, E.A. (2010). Rheological properties and microstructure of Cheddar cheese made with different fat contents. *Journal of Dairy Science*, *93*(10), 4565-4576.

Senadeera, S.S., Prasanna, P.H.P., Jayawardana, N.W.I.A., Gunasekara, D.C.S., Senadeera, P. and Chandrasekara, A. (2018). Antioxidant, physicochemical, microbiological, and sensory properties of probiotic yoghurt incorporated with various Annona species pulp. *Heliyon*, *4*(11), e00955.

Singh, A., Auzanneau, F.I. and Rogers, M.A. (2017). Advances in edible oleogel technologies–A decade in review. *Food Research International*, 97, 307-317.

Singh, B., Panesar, P.S. and Nanda, V. (2006). Utilization of carrot pomace for the preparation of a value added product. *World Journal of Dairy and Food Sciences*, *1*(1), 22-27.

Staffolo, M. D., Sato, A. C., and Cunha, R. L. (2017). Utilization of plant dietary fibers to reinforce low-calorie dairy dessert structure. *Food and Bioprocess Technology*, *10*(5), 914–925. <u>https://doi.org/10.1007/</u> s1194 7-017- 1872-9

Thanh, V.T., Tran, N.Y., Linh, N.T.V., Vy, T.A. and Truc, T.T. (2020). Application of anthocyanin natural colours from Butterfly Pea (*Clitoria ternatea* L.) extracts to cupcake. In *IOP Conference Series: Materials Science and Engineering*. 736(6), 062014. IOP Publishing.

Thuy, N.M., Ben, T.C., Minh, V.Q. and Van Tai, N. (2021). Effect of extraction techniques on anthocyanin from butterfly pea flowers (*Clitoria ternatea* L.) Cultivated in Vietnam. *Journal of Applied Biology and Biotechnology*, 9(6), 173-180.

Toldrá, F. And Nollet, L.M. eds. (2021). *Handbook of dairy foods analysis*. CRC Press.

U.S Food and Drug Administration. (2019), *Part 133: cheeses and related cheese products*. https://www.acces sdata.fda.gov/scrip ts/cdrh/ cfdoc s/cfcfr/ cfrse arch.cfm.Cfrpa rt=133

Upadhyay, S.M., Upadhyay, P., Ghosh, A.K., Singh, V. and Dixit, V.K. (2011). Effect of ethanolic extract of *Hibiscus rosa sinensis* L., flowers on hair growth in female wistar rats. *Der Pharmacia Lettre*, *3*(4), 258-263.

Vázquez, C. V., Rojas, M. G. V., Ramírez, C. A., Chávez-Servín, J. L., García-Gasca, T., Martínez, R. A. F., García, O. P., Rosado, J. L., López-Sabater, C. M., Castellote, A. I., and Montemayor, H. M. A. (2015). Total phenolic compounds in milk from different species. Design of an extraction technique for quantification using the Folin-Ciocalteu method. *Food Chemistry*, *176*, 480–486.

Veggi, P.C., Santos, D.T. and Meireles, M.A.A. (2011). Anthocyanin extraction from Jabuticaba (*Myrciaria cauliflora*) skins by different techniques: economic evaluation. *Procedia Food Science*, *1*, 1725-1731.

Wijesekara, A., Weerasingha, V., Jayarathna, S. and Priyashantha, H. (2022). Quality parameters of natural phenolics and its impact on physicochemical, microbiological, and sensory quality attributes of probiotic stirred yogurt during the storage. *Food Chemistry: X, 14,* 100332.

Wong, P.K., Yusof, S., Ghazali, H.M. and Che Man, Y.B. (2003). Optimization of hot water extraction of roselle juice using response surface methodology: a comparative study with other extraction methods. *Journal of the Science of Food and Agriculture*, 83(12), 1273-1278.

Zin, M.M., Márki, E. And Bánvölgyi, S. (2020). Conventional extraction of betalain compounds from beetroot peels with aqueous ethanol solvent. *Acta Alimentaria*, 49(2), 163-169.