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Red beetroot betalains as a novel source of colorant in ice-cream as compared with red dye 40 (E129)

Ruaa Tariq Mohamed Ali, Qaswaa Yousif Jameel *

Department of Food Science, Colleges of Agricultural and Forestry, University of Mosul, Mosul, Iraq

***Corresponding author**: Qaswaa Yousif Jameel, PhD, Department of Food Science, Colleges of Agricultural and Forestry, University of Mosul, Mosul, Iraq**.**

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ABSTRACT

Background: Although there is an increasing need for eco-friendly and non-toxic food colorants, plant-based colors have shown to be a promising alternative to synthetic food colors. The natural pigment (betalain) was extracted from red beetroot utilizing a magnetic stirrer shaking apparatus in the current study.

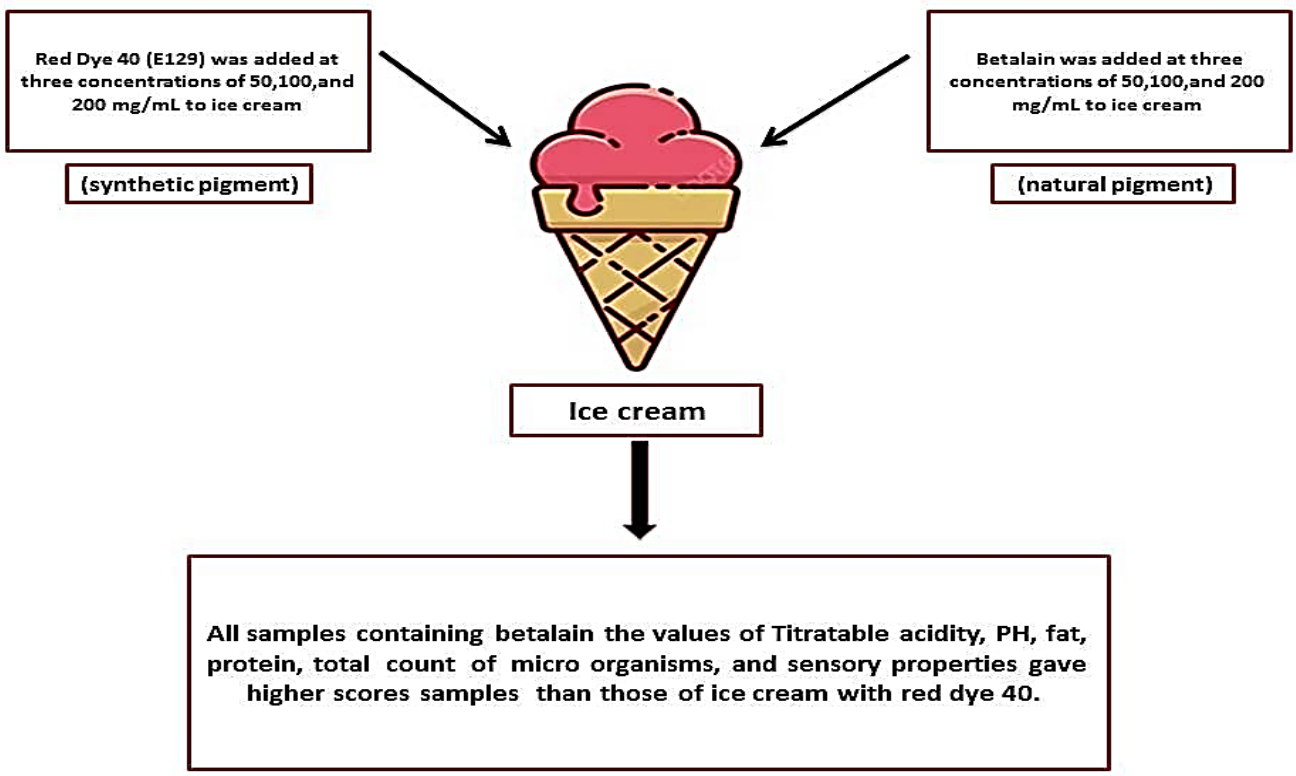
Objective: The purpose of this study was to evaluate the effect of betalain (natural pigment) and red dye 40 (synthetic pigment), on the chemical, microbiological, and sensory properties of ice cream.

Materials and Methods: Betalain and red dye 40 were added into ice cream at variable concentrations of 50, 100, and 200 mg/mL. Specimens were stored for 70 days and evaluated every ten days.

Results: The values of titratable acidity, PH, fat, protein, total count of microorganisms, and sensory characteristics scored higher for betalain ice cream in comparation to red dye 40 ice cream. Nevertheless, betalain doses of 50, 100, and 200 mg/mL have been reported to have a significant 70-day storage activity in ice cream, when compared to mixtures containing 50, 100, and 200 mg/mL of red dye 40 after 70 days.

Conclusion: According to the findings, betalain may be utilized as a natural pigment and food preservative to boost stability during storage.

Keywords: Antioxidants, Betalain, Functional foods, Red beetroot, Red Dye 40 (E129), synthetic colors, Storage period



INTRODUCTION

Ice cream is prepared from milk, sugar, emulsifiers, stabilizers, and flavoring ingredients. It is an aerated suspension of fat and water in a concentrated sugar solution with stabilizers, casein micelles, and proteins. Ice cream has a high protein and milk fat content. It is a valuable milk product due to its high nutritional and caloric density [1–3]. Ice cream is high in calories but low in natural antioxidants, dietary fiber, and minerals [4]. Consumers have recently shifted toward functional foods that have more natural antioxidants, natural colorants, and are devoid of synthetic ingredients. As a result, researchers aim to increase the nutrient quality of foods [5]. Functional foods are foods or dietary components that provide significant health advantages in addition to their nutritional value [6]. The functional properties of many plants, especially their potential use as novel nutraceuticals in functional foods are being investigated [7–9], and natural antioxidants have increased in ice cream manufacturing due to consumers' interest in healthier alternatives and functional foods. Vegetables and fruits have been shown to provide important health benefits because of their high antioxidants [5 –9].

Red beetroot (*Beta vulgaris L.*) is a traditional and popular vegetable in many parts of the world, and beetroot is regularly consumed as part of a normal diet. Red beetroot contains a high percentage of betalains, which can serve as naturally beneficial antioxidants and natural colors instead of industrial chemical colors. Natural colors have gotten a lot of attention lately because of their possible benefits in the prevention of chronic diseases [10 –13]. Betalains are natural pigments obtained from plants, which are growing in popularity as a natural dye in the food industry. While red beet

betalains are one of the most extensively used organic dyes, they have received less attention than other colorants such as anthocyanins, carotenoids, and chlorophylls [15]. Because of their innate colorant characteristics, high water solubility, and lack of toxicity, betalains are utilized as food additives [16 –18]. These pigments are also notable because of their bioactivity, which includes antioxidant capacity, immune system boosting, antibacterial and antiviral activities, and the ability to suppress the cell development of human cancers [9 –20].

The purposes of this research were to investigate the possibility of producing a new functional food, to increase the nutritive value of ice cream by using natural colors (betalains) rather than chemical colors [red dye 40 (E129)], to observe its effect on the chemical, microbiological, and sensory properties of ice cream.

METHODS

Chemicals and Reagents: In a local market in Mosul, Iraq, we bought raw cow's milk and red beetroot. All materials and chemicals were purchased from Scharlab S.L. (Spain), and they were of the highest analytical grades currently offered commercially.

Preparation of red beetroot extract: The extraction of red beetroot was conducted according to the method provided by [22]. Briefly, 100 g of dried red beetroot powder was macerated in 1000 mL of 50% ethanol for 24 h before being shaken for 24 h with a magnetic stirrer. After that, the solution was filtered using Whatman filter paper 0.15 cm. The resulting clear solution was concentrated by evaporation under vacuum to dryness at temperatures below 30°C using a rotary evaporator (PER FIT, Indian origin). The supernatant was then collected.

Quantification of betalains in red beetroot extract: Quantification of betalains in the red beetroot extract was performed according to the modified method of [7–23]. The sample was prepared by dissolving the beetroot extract in ethanol at a concentration of 1000 µg/mL, then 20 µL of the sample was injected into a high-performance liquid chromatograph (HPLC; model SYKAM, Germany) (through a C18 column (150 × 4.6 mm I.D., particle size 5 µm; Agilent Technologies, USA). The betalain was detected at 425 nm at 25 °C and the flow rate was 0.8 mL/min. The mobile phase consisted of 70:30 Methanol:water (% v:v) with 1% Acetic acid which was filtered using a 0.45 mm filter. Comparing unidentified extract samples to the standard betalain solution given by the Sigma Company (USA). The program made use of retention time and peak area to determine the concentration.

Preparation of ice cream with betalain and red dye 40 (E129): The six formulas listed in Table 1 were used to prepare the ice cream. The previously heated milk (45°C) was added to the dry ingredients, which included: Skim milk powder, whey powder, granular sugar, and emulsifier/stabilize and mixed well. The mixes underwent homogenization (at 70°C) and a one-minute Pasteurization at 80°C. They were then chilled to 15°C and stored for 16 hours at 4°C. Before freezing, vanilla extract was added to all ice cream mixtures at a rate of 0.4% (V/V). Betalain was added at three concentrations of 50,100, and 200 mg/ml in ICF1, ICF2, and ICF3, respectively, and red dye 40 (E129) was added at three concentrations of 50,100, and 200 mg/ml in ICF4, ICF5, and ICF6, respectively. The ice cream was made from the mixtures by ice cream maker (Moulinex, Italy 03051 410). Then, they were divided into weights of 50 g and packed in plastic containers and transferred to a -30°C hardening freezer, The samples were kept at -18°C and taken for analysis after 1, 10, 20, 30, 40, 50, 60, and 70 days of storage.

Table 1: The different ice cream mix ingredients and proportions.

Ice cream mix ingredients									
Ice-cream formulation (ICF)	Raw milk %	Vegetable Oil %	Granular Sugar %	Skim milk Powder %	Whey Powder%	Emulsifier Stabilizer %	Vanillin %	Betalain (mg/ml)	Red Dye 40 (mg/ml)
ICF0	70	8	18	2	1.4	0.5	0.1	-	-
ICF1	70	8	18	2	1.4	0.5	0.1	50	-
ICF2	70	8	18	2	1.4	0.5	0.1	100	-
ICF3	70	8	18	2	1.4	0.5	0.1	200	-
ICF4	70	8	18	2	1.4	0.5	0.1	-	50
ICF5	70	8	18	2	1.4	0.5	0.1	-	100
ICF6	70	8	18	2	1.4	0.5	0.1	-	200

Chemical and microbiological analysis: The titratable acidity and pH were assessed utilizing the modified method of [25]. The fat content in ice cream was measured using the Gerber method [26]. The technique of [27] was used to assess the protein content of ice cream, with a few modifications. The total count in ice cream during storage periods was assessed utilizing the modified method of [28].

Sensory evaluation: The sensory testing of the ice cream samples was conducted using, with minor modifications, the technique outlined by [29]. The committee consisted of 12 members, and the participants were academics in the Department of Food Sciences, ranging in age from 30 to 49 years (6 females and 6 males). They participated in the sensory evaluation of ice cream focusing on flavor & taste, body & texture, color & appearance, and melting resistance, while adopting the 9-point hedonic scale, where the score 1 indicated a very low preference, and 9 indicated a very high preference. Prior to taking part in the analysis, each participant got a letter of information and a consent document. Then, with their consent, the participants received four samples of ice cream, a pencil, and water to rinse their mouths.

Statistical process: The outcomes obtained in the present study were expressed as the mean ± Std. Deviation, and the probability of $p > 0.05$ was considered significant. The statistical significance of group differences was calculated using one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Quantification of betalains in red beetroot extract: HPLC analysis was performed to evaluate the betalain in the beetroot extract. The data revealed that red beetroot ethanol extracts contained 3.12 mg/g betalain the findings demonstrate that there is a convergence in the retention time for both the standard where it was 3.599, and sample where it was 3.568, Figure 1.

This is evidence that there is a large amount of betalain in red beetroot extract. The results show that red beetroot has a high concentration of betalain when compared to other plant sources. As the polarity of the extraction solution rises as a result of the addition of

ethanol and water, betalain separates and precipitates from the other chemicals into the extraction solution.

Effect of betalains and red dye 40 (E129) on titratable acidity and pH during the storage period: The statistical analysis demonstrates that betalain and red dye 40 (E129) had a significant impact on titratable acidity ($p < 0.05$) as shown in Table 2. From day 1 to day 40 of storage, the titratable acidity of the specimens considerably increased in control ICF0 (0.1720 to 0.1930).

From day 1 to day 40, the titratable acidity ratios in ICF1 increased from 0.1730 to 0.1770; ICF2, 0.1740 to 0.1763; ICF3, 0.1750 to 0.1760; ICF4, 0.1720 to 0.1840; ICF5, 0.1730 to 0.1850; and ICF6, 0.1730 to 0.1847. Following 70 days of cold storage, the specimens ICF0, ICF1, ICF2, ICF3, ICF4, ICF5, and ICF6 all showed an increase in titratable acidity of 0.3513, 0.1807, 0.2240, 0.2233, and 0.2260, respectively. This titratable acidity levels demonstrate that after 70 days of storage, in the formulas ICF0, ICF4, ICF5, and ICF6, ice cream quality significantly declined, in comparison to the 3 treatments formulas (ICF1, ICF2, and ICF3). The treatment (ICF1, ICF2, and ICF3) recorded a lower ratio of titratable acidity (0.1807, 0.1793, and 0.1793) respectively on day 70, compared with formulas ICF0 (0.3513), ICF4 (0.2240), ICF5 (0.2233), and ICF6 (0.2260). On the other hand, the pH of the samples on the first day of formulas ICF0, ICF1, ICF2, ICF3, ICF4, ICF5, and ICF6, were observed to be 6.6300, 6.6300, 6.6400, 6.6500, 6.6300, 6.6400, and 6.6400, respectively. Following day 40, the pH in each of the six formulations was 6.0633, 6.5600, 6.5700, 6.5800, 6.1700, 6.1667, and 6.1633, respectively. In contrast, after 60 days, pH in the formulas ICF0, ICF1, ICF2, ICF3, ICF4, T0C5, and ICF6 had reduced to 5.8600, 6.4400, 6.4700, 6.4800, 5.8500, 5.8600, and 5.8700, respectively. After 70 days, pH had decreased to 5.7600, 6.3933, 6.4233, 5.4600, 5.8400, 5.8300, and 5.8400, in those same formulations. Due to the antibacterial properties of betalain, the pH of ice cream with betalain decreased slightly when compared to ice cream without betalain.

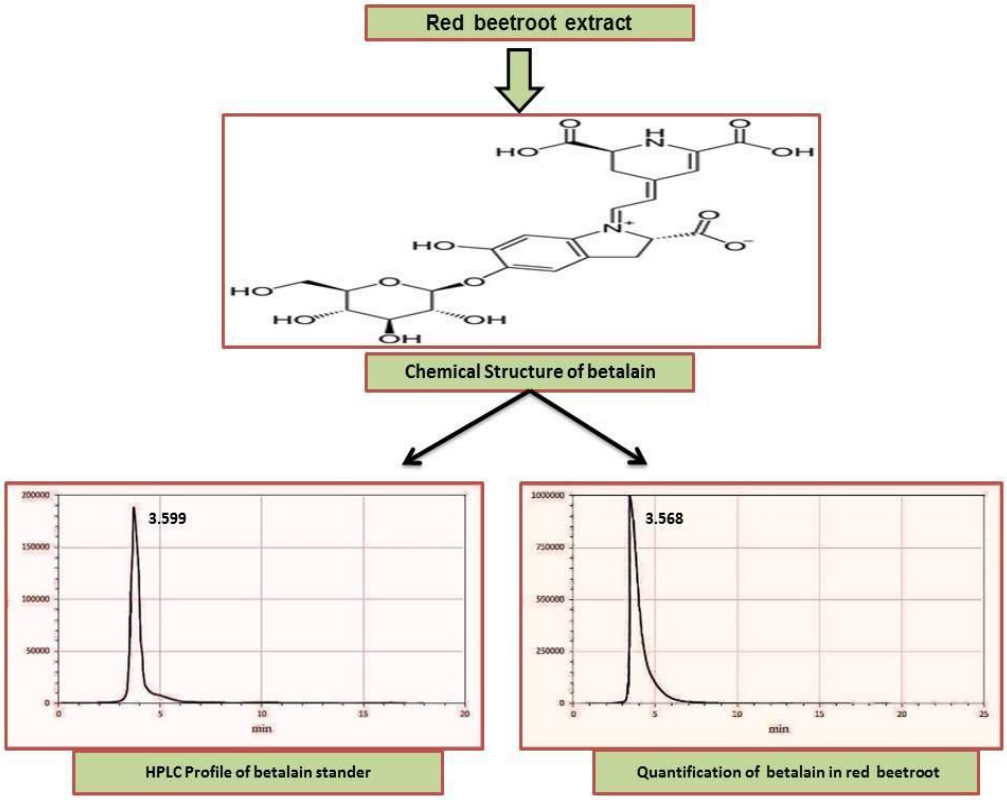


Figure 1: Concentration of betalain in red beetroot extract.

Effect of betalains and red dye 40(E129) on fat content during the storage period: The results of statistical analysis demonstrated that betalains and red dye 40 (E129) had a significant impact on fat ($p < 0.05$). As indicated in Table 2, all specimens' fat levels tended to decline during the storage period. The fat content in betalain-containing ice creams marginally decreased when compared to ice creams without betalain. With the ICF0, ICF1, ICF2, ICF3, ICF4, ICF5, and ICF6 formulas, the first day's fat content was 10.5200, 10.4800, 10.4867, 10.4933, 10.5000, 10.4867, and 10.4933, respectively. On day 20, the following fat levels were reached for each of the Seven formulas: 9.8300, 10.3600, 10.3900, 10.3867, 9.8600, 9.8433, and 9.8500. The fat value in the control ICF0 (9.8300 to 8.7667) decreased from day 30 to day 70, showing that the quality of the ice cream considerably decreased after 30 days of storage. The fat levels decreased in ICF1 (10.3600 to 10.2100), ICF2 (10.3900 to 10.2200), ICF3 (10.3867 to 10.2600), ICF4 (9.8600 to 8.7333), ICF5 (9.8433 to 8.7467), and ICF6 (9.8500 to 8.7600) from day 30 to day 70. The treatment ICF1, ICF2, and ICF3 recorded the highest fat content (10.2100, 10.2200, and 10.2600) at day 70, mainly due to less lipolysis of fat in the treatment specimens, compared with formulas ICF4 (8.7333), ICF5 (8.7467), and ICF6 (8.7600). This lowered lipolysis activity was primarily due to betalain's highly active and strong antibacterial properties. Lipase enzymes reduced the amount of fat by lipolyzing the fat and releasing free fatty acids as a result. It is possible for microorganisms to synthesize lipases, either in raw milk or in sterilized products. Many of them have the ability to produce lipases that are extremely heat-resistant [30], bacterial lipase are formed by these bacteria. Total lipase activity in raw milk is adequate to cause the rapid hydrolysis of a significant amount of the fat [31]. Due to betalain's antibacterial effect, which can prevent the growth of bacteria, yeast, and mold, fat levels in betalain-infused ice cream were slightly lower than in betalain-free ice cream [18]. Food spoilage is caused by betalain's antibacterial action, as established in [32]. Although [33] demonstrated that antioxidant activity is tightly connected with betalain content, demonstrating that betalain compounds are the dominating antioxidants, a high betalain content of the specimen causes higher antioxidant activity.

Effect of betalains and red dye 40 (E129) on protein content during the storage period: According to statistical analysis, the effects of betalain and red dye 40 (E129) on proteins had such a significant impact (p 0.05) as shown in Table 2.

ICF0, ICF1, ICF2, ICF3, ICF4, T0C5, and ICF6 all had protein concentrations on day one that were 3.9100, 3.9133, 3.9200, 3.9133, 3.9133, 3.9200, and 3.9000, respectively. After day 40, the protein concentrations in the 7 formulations had decreased to 3.4533, 3.8567, 3.8533, 3.8500, 3.4367, 3.4500, and 3.4533, respectively. Yet after 70 days, the concentrations of protein in the formulas ICF0, ICF1, ICF2, ICF3, ICF4, T0C5, and ICF6 were 2.8400, 3.6767, 3.6967, 3.7267, 2.8667, 2.8700, and 2.8767, respectively. The treatment with betalain recorded the highest content of protein ICF1=3.6767, ICF2=3.6967, and ICF3=3.7267 at day 70, compared with formulas which added red dye 40. The presence of betalains in the ice cream was the cause of the variations in the protein content; their best qualities were their high concentrations of 50, 100, and 200 mg/mL in the three formulations ICF1, ICF2, and ICF3. The proteolytic activity in ice cream can be functionally classified into peptide transport systems, which transport the oligopeptide and various intracellular peptidases, and cell surface associated proteinases, which hydrolyze caseins to oligopeptides [34]. The fact that there was less activity in the treatment specimens indicates that the betalains restricted the activity of bacteria that decompose proteins [21–35]. The betalains' ability to inhibit microbial activity by interfering with their enzymes through unspecialized interactions with proteins has been demonstrated [18 –37].

Table2: Effect of betalains and red dye 40 (E129), on titratable acidity, PH, fat, and protein during the storage period.

Ice-cream formulation (ICF)	Titratable acidity	PH	Fat%	Protein%
Storage period (Day 1)				
ICF0	0.1720±0.00100^{c}	6.6300±0.01000^{b}	10.5200±0.01000^{a}	3.9100±0.59758^{a}
ICF1	0.1730±0.00100^{bc}	6.6300±0.01000^{b}	10.4800±0.02646^{b}	3.9133±0.59475^{a}
ICF2	0.1740±0.00100^{a}	6.6400±0.01000^{ab}	10.4867±0.02082^{ab}	3.9200±0.58898^{a}
ICF3	0.1750±0.00100^{a}	6.6500±0.01000^{a}	10.4933±0.01528^{ab}	3.9133±0.59467^{a}
ICF4	0.1720±0.00100^{c}	6.6300±0.01000^{b}	10.5000±0.01000^{ab}	3.9133±0.594753^{a}
ICF5	0.1730±0.00100b^{c}	6.6400±0.01000^{ab}	10.4867±0.02082^{ab}	3.9200±0.58898^{a}
ICF6	0.1730±0.00100^{ab}	6.6400±0.01000^{ab}	10.4933±0.01528^{ab}	3.9000±0.60696^{a}
Storage period (Day 10)				
ICF0	0.1740±0.00100^{b}	6.6200±0.01000^{b}	10.4900±0.01000^{a}	3.9000±0.59758^{a}
ICF1	0.1740±0.00100^{b}	6.6300±0.01000^{ab}	10.4767±0.02082^{a}	3.9067±0.60053^{a}
ICF2	0.1747±0.00153^{b}	6.6400±0.01000^{ab}	10.4833±0.01528^{a}	3.9133±0.59475^{a}
ICF3	0.1753±0.00153^{ab}	6.6500±0.01000^{a}	10.4900±0.19616^{a}	3.9067±0.60044^{a}
ICF4	0.1740±0.00100^{b}	6.6200±0.02000^{b}	10.4900±0.01000^{b}	3.9033±0.59475^{a}
ICF5	0.1760±0.00100^{ab}	6.6300±0.01000^{ab}	10.4767±0.02082^{b}	3.9100±0.58898^{a}
ICF6	0.1770±0.00100^{a}	6.6300±0.01000^{ab}	10.4833±0.01528^{b}	3.8900±0.60696^{a}
Storage period (Day 20)				
ICF0	0.1763±0.00153^{bc}	6.4200±0.02646^{b}	10.4767±0.01528^{a}	3.8000±0.45044^{a}
ICF1	0.1750±0.00100^{c}	6.6200±0.01000^{a}	10.4700±0.02000^{a}	3.9033±0.60352^{a}
ICF2	0.1750±0.00200^{c}	6.6400±0.01000^{a}	10.4800±0.01000^{a}	3.9100±0.59775^{a}
ICF3	0.1757±0.00153^{bc}	6.6400±0.01000^{a}	10.4800±0.01000^{a}	3.9033±0.60335^{a}
ICF4	0.1770±0.00100^{bc}	6.4133±0.01528^{b}	10.3733±0.02082^{b}	3.7833±0.40427^{a}
ICF5	0.1780±0.00100^{b}	6.4200±0.01000^{b}	10.3533±0.03055^{b}	3.7933±0.40427^{a}
ICF6	0.1807±0.00153^{a}	6.4167±0.01528^{b}	10.3600±0.01000^{b}	3.7767±0.42829^{a}
Storage period (Day 30)				
ICF0	0.1803±0.00153^{a}	6.1600±0.01000^{c}	9.8300±0.02000^{b}	3.5533±0.74527^{a}
ICF1	0.1760±0.00100^{b}	6.6100±0.01000^{a}	10.3600±0.01000^{a}	3.8867±0.60929^{a}
ICF2	0.1760±0.00200^{b}	6.6300±0.01000^{a}	10.3900±0.01000^{a}	3.8867±0.60053^{a}
ICF3	0.1760±0.00100^{b}	6.6300±0.01000^{a}	10.3867±0.01528^{a}	3.8800±0.59775^{a}
ICF4	0.1810±0.00100^{a}	6.2800±0.06557^{b}	9.8600±0.03606^{b}	3.5667±0.66516^{a}
ICF5	0.1820±0.00100^{a}	6.2900±0.03606^{b}	9.8433±0.01528^{b}	3.5567±0.69501^{a}
ICF6	0.1820±0.00100^{a}	6.2400±0.03606^{b}	9.8500±0.02000^{b}	3.5533±0.70501^{a}
Storage period (Day 40)				
ICF0	0.1930±0.00100^{a}	6.0633±0.01528^{c}	9.4233±0.02517^{d}	3.4533±0.82646^{a}

Ice-cream formulation (ICF)	Titratable acidity	PH	Fat%	Protein%
ICF1	0.1770±0.00100^{b}	6.5600±0.01000^{a}	10.3100±0.01000^{b}	3.8567±0.63634^{a}
ICF2	0.1763±0.00153^{b}	6.5700±0.01000^{a}	10.3433±0.01528^{a}	3.8533±0.63058^{a}
ICF3	0.1760±0.00100^{b}	6.5800±0.01000^{a}	10.3700±0.01000^{a}	3.8500±0.62482^{a}
ICF4	0.1840±0.00100^{a}	6.1700±0.01000^{b}	9.4367±0.01528^{d}	3.4367±0.7968^{a}
ICF5	0.1850±0.00100^{a}	6.1667±0.01528^{b}	9.4433±0.01528^{cd}	3.4500±0.79171^{a}
ICF6	0.1847±0.00100^{a}	6.1633±0.01528^{b}	9.4700±0.02646^{c}	3.4533±0.81206^{a}
Storage period (Day 50)				
ICF0	0.2080±0.00100^{a}	5.9600±0.01000^{c}	9.2233±0.02517^{c}	3.0867±0.43822^{a}
ICF1	0.1780±0.00100^{b}	6.4933±0.01528^{b}	10.3033±0.01528^{a}	3.8233±0.61330^{a}
ICF2	0.1773±0.00153^{b}	6.5067±0.01528^{b}	10.3133±0.01528^{a}	3.8367±0.63634^{a}
ICF3	0.1770±0.00100^{b}	6.5333±0.01528^{a}	10.3200±0.01000^{a}	3.8400±0.62426^{a}
ICF4	0.1880±0.00100^{a}	5.9600±0.01000^{c}	9.2267±0.02082^{c}	3.1100±0.45211^{a}
ICF5	0.1890±0.00100^{a}	5.9700±0.01000^{c}	9.2600±0.01000^{b}	3.1167±0.44411^{a}
ICF6	0.1880±0.00100^{a}	5.9700±0.01000^{c}	9.2700±0.01000^{b}	3.1067±0.45720^{a}
Storage period (Day 60)				
ICF0	0.2260±0.00200^{a}	5.8600±0.01000^{cd}	8.8600±0.01000^{c}	2.9767±0.40427^{a}
ICF1	0.1790±0.00100^{e}	6.4400±0.01000^{b}	10.2600±0.01000^{b}	3.7100±0.71190^{a}
ICF2	0.1780±0.00100^{e}	6.4700±0.01000^{a}	10.2900±0.01000^{ab}	3.7233±0.71766^{a}
ICF3	0.1780±0.00100^{e}	6.4800±0.01000^{a}	10.3000±0.01000^{a}	3.7467±0.69788^{a}
ICF4	0.2007±0.00153^{d}	5.8500±0.01000^{d}	8.8833±0.03055^{c}	3.0667±0.50063^{a}
ICF5	0.2210±0.00100^{b}	5.8600±0.01000^{cd}	8.8667±0.01528^{c}	3.0767±0.49369^{a}
ICF6	0.2110±0.00100^{c}	5.8700±0.01000^{c}	8.8800±0.03000^{c}	3.0700±0.50764^{a}
Storage period (Day 70)				
ICF0	0.3513±0.00153^{a}	5.7600±0.01000^{c}	8.7667±0.01528^{c}	2.8400±0.53019^{a}
ICF1	0.1807±0.00153^{c}	6.3933±0.01528^{a}	10.2100±0.01000^{b}	3.6767±0.69659^{a}
ICF2	0.1793±0.00153^{c}	6.4233±0.03786^{a}	10.2200±0.01000^{b}	3.6967±0.72280^{a}
ICF3	0.1793±0.00153^{c}	5.4600±0.01000^{d}	10.2600±0.01000^{a}	3.7267±0.71389^{a}
ICF4	0.2240±0.00361^{b}	5.8400±0.01000^{b}	8.7333±0.01528^{c}	2.8667±0.53687^{a}
ICF5	0.2233±0.00321^{b}	5.8300±0.01000^{b}	8.7467±0.04933^{c}	2.8700±0.53019^{a}
ICF6	0.2260±0.00100^{b}	5.8400±0.01000^{b}	8.7600±0.01000^{c}	2.8767±0.53594^{a}

The values are indicated as the means±standard deviation. Values with varied superscripts in each column differ significantly ($p \leq 0.05$). Formula (ICF0) ice cream Without adding any color, formula (ICF1) ice cream containing 50 mg/mL of betalain, formula (ICF2) ice cream containing 100 mg/mL of betalain, formula (ICF3) ice cream containing 200 mg/mL of betalain, formula (ICF4) ice cream containing 50 mg/mL of red dye 40, formula (ICF5) ice cream containing 100 mg/mL of red dye 40, formula (ICF6) ice cream containing 200 mg/mL of red dye 40.

Effect of betalains and red dye 40 (E129) on total count (log cfu/mL) in ice cream during storage periods: According to statistical analysis, the effects of betalain and red dye 40 (E129) on total count in ice cream during storage periods. total count was significant ($p < 0.05$) (Table 3). The total count concentrations on the first day of formulas ICF0, ICF1, ICF2, ICF3, ICF4, T0C5, and ICF6 were observed to be 5.3767, 5.3100, 5.2800, 5.0667, 5.3800, 5.4100, and 5.4267 log cfu/mL, respectively. After day 40, total count had decreased to 4.7933, 5.1533, 5.1100, 4.7767, 5.3600, 5.3800, and 5.4067 log cfu/mL, in the seven formulas respectively. After 70 days of cold storage, total count increased to 7.2333, 2.5600, 2.2800, 2.0667, 8.2100, 8.5267, and 8.4200 in the samples ICF0, ICF1, ICF2, ICF3, ICF4, ICF5, and ICF6, respectively, indicating that the ice cream quality significantly decreased after day 70 of storage in formula ICF0, ICF4, ICF5, and ICF6, compared with the three formulas ICF1, ICF2, and ICF3. The treatment (ICF1, ICF2, and ICF3) recorded a lower ratio of total count 2.5600, 2.2800, and 2.0667 log cfu/mL, respectively on day 70, compared with formulas ICF0 (4.7933 log cfu/mL), ICF4 (5.3600 log cfu/mL), ICF5 (5.3800 log cfu/mL), and ICF6 (5.4067 log cfu/mL). The results showed mild increase in total count in all specimens treated with betalain, when compares to specimens containing synthetic pigment (red dye 40).

Table 3: Effect of betalains and red dye 40 (E129), on total count (log cfu/mL) of ice cream during storage periods.

	Ice-cream formulation (ICF)						
Day	ICF0	ICF1	ICF2	ICF3	ICF4	ICF5	ICF6
1	5.3767± 0.03215b	5.3100±0. 01000c	5.2800±0. 01000d	5.0667±0. 01528e	5.3800±0. 01000b	5.4100±0.0 1000a	5.4267±0. 01528a
10	5.2967± 0.00577e	5.2300±0. 01000c	5.1800±0. 01000d	4.8100±0. 01000f	5.2600±0. 01000b	5.3100±0.0 1000ab	5.3223±0. 01528a
20	5.0700± 0.01000e	5.2200±0. 01000c	5.1700±0. 01000d	4.8000±0. 01000f	5.3100±0. 01000b	5.3233±0.0 1528ab	5.3333±0. 01528a
30	4.9300± 0.01000e	5.1633±0. 01528c	5.1200±0. 01000d	4.7800±0. 01000f	5.3300±0. 01000b	5.3500±0.0 1000ab	a5.36670. 01528±
40	4.7933± 0.01528e	5.1533±0. 01528c	5.1100±0. 01000d	4.7767±0. 01528e	5.3600±0. 01000b	5.3800±0.0 1000b	5.4067±0. 01528a
50	6.7800± 0.01000a	4.8100±0. 01000c	4.9800±0. 01000c	3.9100±0. 01000d	6.0267±0. 56871b	6.3867±0.0 2082ab	6.4300±0. 01000a
60	7.4867± 1.16337a	3.8800±0. 01000b	3.9667±0. 01528b	3.7100±0. 01000b	7.3200±0. 01000a	7.3800±0.0 1000a	7.4200±0. 01000a
70	7.2333± 0.01528d	2.5600±0. 01000e	2.2800±0. 01000f	2.0667±0. 01528g	8.2100±0. 01000c	8.5267±0.0 2517a	8.4200±0. 01000b

The values are indicated as the means±standard deviation. Values with varied superscripts in each column differ significantly ($p \leq 0.05$). Formula (ICF0) ice cream Without adding any color, formula (ICF1) ice cream containing 50 mg/mL of betalain, formula (ICF2) ice cream containing 100 mg/mL of betalain, formula (ICF3) ice cream containing 200 mg/mL of betalain, formula (ICF4) ice cream containing 50 mg/mL of red dye 40, formula (ICF5) ice cream containing 100 mg/mL of red dye 40, formula (ICF6) ice cream containing 200 mg/mL of red dye 40.

Effect of betalains and red dye 40 (E129), on sensory characteristics during the storage period: Betalains and red dye 40 can alter the chemistry of a product, affecting its sensory scores. Fig 2; Table 4 illustrates the mean sensory characteristics of the ice cream specimens relying on the acceptability of each group; there was an observed difference in mean scores of Flavor & Taste, Body & Texture, Color & Appearance, and Melting Resistance in the six formulas: ICF0, ICF1, ICF2, ICF3, ICF4, ICF5, and ICF6. The sensory evaluation of the specimens revealed that betalain concentrations of 50, 100, and 200 mg/mL had significant ($p < 0.05$) effects on the ice cream flavor and taste, body and texture, color and appearance, and melting resistance. At the end of storage, the formula ICF0 recorded the lowest scores for flavor, body texture, appearance, and melting resistance, whereas the higher scores were for ice cream specimens containing 50, 100, and 200 mg/mL of betalains. The sensory panel did not notice any significant defects in any formulations. To summarize, the addition of betalains had no effect on the overall acceptance of the ice cream. In comparison to the formulation that included Red Dye 40 (E129).

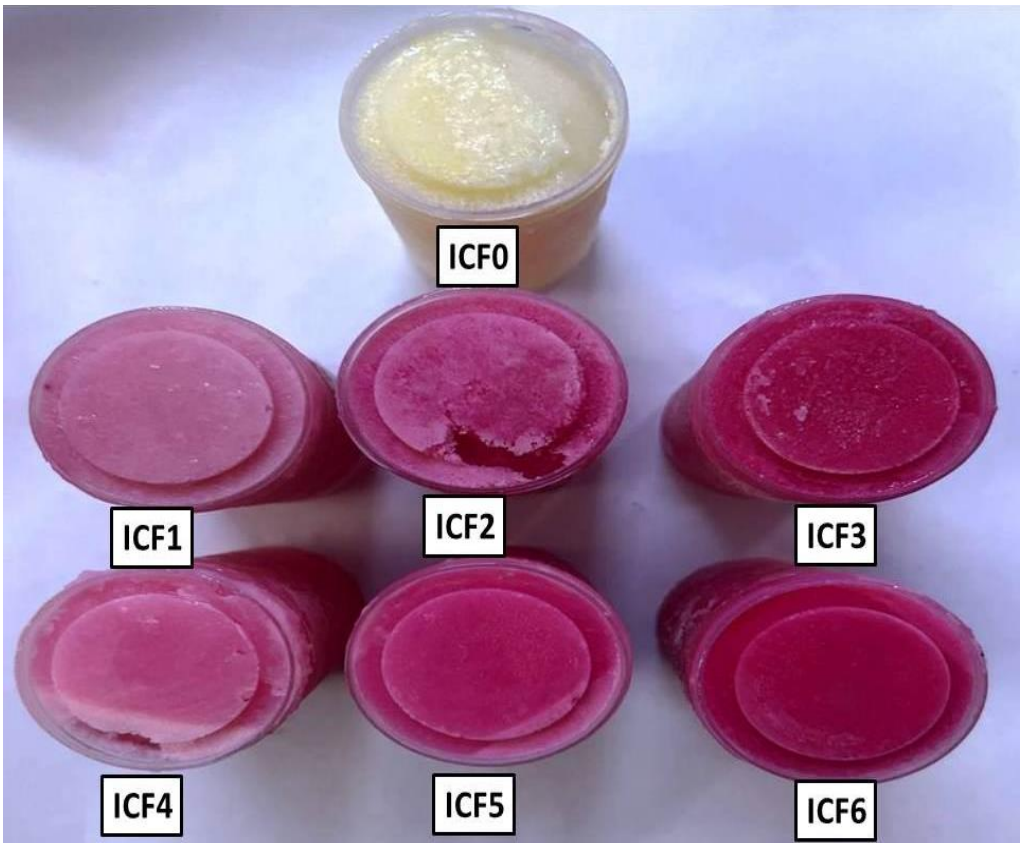


Figure 2: Ice cream prepared with betalain and red dye 40 pigment, (ICF0) ice cream Without adding any color, (ICF1) ice cream containing 50 mg/mL of betalain, (ICF2) ice cream containing 100 mg/mL of betalain, (ICF3) ice cream containing 200 mg/mL of betalain, (ICF4) ice cream containing 50 mg/mL of red dye 40, (ICF5) ice cream containing 100 mg/mL of red dye 40, (ICF6) ice cream containing 200 mg/mL of red dye 40.

Table 4: Effect of betalains and red dye 40 (E129), on sensory characteristics (Flavor and Taste, Body and Texture, Color and Appearance, and Melting Resistance) during the storage period.

Ice-cream formulation (ICF)	Flavor and Taste	Body and Texture	Color and Appearance	Melting Resistance
Storage period (Day 1)				
ICF0	7.5100±0.01000^{e}	8.1400±0.01000^{d}	8.0100±0.01000^{d}	8.0167±0.01528^{e}
ICF1	7.9100±0.01000^{d}	8.1600±0.02000^{cd}	7.6400±0.01000^{d}	7.7600±0.01000^{d}
ICF2	8.8700±0.01000^{c}	8.1700±0.02000^{c}	7.4300±0.01000^{d}	7.3800±0.01000^{d}
ICF3	8.9800±0.0100^{a}	8.1800±0.02000^{c}	7.3200±0.01000^{f}	7.3367±0.04041^{e}
ICF4	7.9200±0.01000^{d}	8.2100±0.01000^{b}	7.2900±0.01000^{c}	7.1900±0.16462^{d}
ICF5	8.9600±0.01000^{b}	8.2267±0.00577^{ab}	7.2800±0.01000^{c}	6.9400±0.01000^{f}
ICF6	8.9800±0.0100^{a}	8.2400±0.01000^{a}	6.9400±0.01000^{d}	6.5100±0.01000^{f}
Storage period (Day 10)				
ICF0	7.2600±0.01000^{d}	7.9500±0.01000^{d}	8.0700±0.01000^{ab}	8.2133±0.01528^{b}
ICF1	7.9000±0.01000^{c}	8.1533±0.01528^{c}	8.0600±0.01000^{ab}	8.2000±0.01000^{b}
ICF2	8.8733±0.02082^{b}	8.1600±0.01000^{c}	8.0567±0.00577^{ab}	8.1800±0.01000^{b}
ICF3	8.9767±0.01528^{a}	8.1733±0.01528^{c}	8.9200±0.01000^{c}	7.9600±0.01000^{d}
ICF4	7.9100±0.0100^{c}	8.2000±0.01000^{b}	8.8700±0.01000^{b}	7.8800±0.01000^{c}
ICF5	8.9533±0.01528^{a}	8.2100±0.01000^{ab}	8.8600±0.01000^{b}	7.6700±7.5233^{e}
ICF6	8.9367±0.04933^{a}	8.2300±0.01000^{a}	8.6100±0.01000^{c}	7.4800±0.01000^{c}
Storage period (Day 20)				
ICF0	7.1400±0.01000^{e}	7.6400±0.01000^{e}	8.0500±0.01000^{c}	8.3200±0.01000^{a}
ICF1	7.8867±0.01528^{d}	8.1300±0.01000^{d}	8.0400±0.01000^{c}	8.3067±0.01528^{a}
ICF2	8.8533±0.01528^{c}	8.1433±0.01528^{cd}	8.0300±0.01000^{c}	8.2800±0.01000^{a}
ICF3	8.9700±0.02000^{a}	8.1633±0.01528^{c}	8.9600±0.01000^{b}	8.1400±0.01000^{b}
ICF4	7.9000±0.01000^{d}	8.1900±0.01000^{b}	8.9400±0.01000^{a}	8.0800±0.01000^{b}
ICF5	8.9433±0.01528^{ab}	8.2000±0.01000^{ab}	8.9300±0.01000^{a}	7.8400±0.01000^{b}
ICF6	8.9267±0.04933^{b}	8.2200±0.01000^{a}	8.7100±0.01000^{b}	7.7600±0.01000^{b}
Storage period (Day 30)				
ICF0	6.9500±0.01000^{f}	7.5100±0.01000^{e}	8.0600±0.01000^{bc}	8.3300±0.01000^{a}
ICF1	7.8833±0.02082^{d}	8.1100±0.01000^{d}	8.0500±0.01000^{bc}	8.3067±0.01528^{a}
ICF2	8.8500±0.01000^{c}	8.1400±0.01000^{c}	8.0400±0.01000^{bc}	8.2900±0.01000^{a}
ICF3	8.9633±0.02517^{a}	8.1533±0.01528^{c}	8.9800±0.01000^{a}	8.2700±0.01000^{a}
ICF4	7.8133±0.01528^{e}	8.1800±0.01000^{b}	8.09400±0.01000^{a}	8.2500±0.01000^{a}
ICF5	8.8600±0.01000^{c}	8.1900±0.01000^{b}	8.9333±0.01528^{a}	8.1500±0.01000^{a}
ICF6	8.9167±0.04933^{b}	8.2100±0.01000^{a}	8.9067±0.01528^{a}	8.1100±0.01000^{a}
Storage period (Day 40)				

Ice-cream formulation (ICF)	Flavor and Taste	Body and Texture	Color and Appearance	Melting Resistance
ICF0	6.8200±0.01000^{d}	7.1400±0.01000^{f}	8.0400±0.01000^{a}	8.1633±0.01528^{d}
ICF1	8.2133±0.59475^{b}	8.0800±0.01000^{b}	8.0700±0.01000^{a}	8.1533±0.01528^{c}
ICF2	8.8400±0.01000^{a}	8.1100±0.01000^{a}	8.0600±0.01000^{a}	8.1400±0.01000^{c}
ICF3	8.9567±0.02082^{a}	8.1100±0.01000^{a}	7.8200±0.01000^{e}	8.0800±0.01000^{c}
ICF4	7.7800±0.01000^{c}	7.8400±0.01000^{e}	6.9600±0.01000^{e}	8.0600±0.01000^{b}
ICF5	8.8100±0.01000^{a}	7.8700±0.01000^{d}	5.8600±0.01000^{d}	7.7700±0.01000^{d}
ICF6	8.8600±0.01000^{a}	7.9100±0.01000^{c}	4.7600±0.01000^{g}	6.7400±0.01000^{e}
Storage period (Day 50)				
ICF0	5.8200±0.01000^{d}	6.8600±0.01000^{e}	8.0800±0.01000^{a}	8.1767±0.02082^{cd}
ICF1	7.8733±0.02082^{b}	8.0500±0.01000^{b}	8.0700±0.01000^{a}	8.1733±0.02082^{bc}
ICF2	8.8300±0.01000^{a}	8.0800±0.01000^{a}	8.0600±0.01000^{a}	8.1467±0.01528^{c}
ICF3	8.9500±0.02646^{a}	8.0800±0.01000^{a}	7.8100±0.01000^{e}	8.1400±0.01000^{b}
ICF4	5.9600±0.01000^{d}	7.5133±0.01528^{d}	6.8600±0.01000^{f}	8.1200±0.01000^{b}
ICF5	6.8133±0.00577^{c}	7.5300±0.01000^{cd}	5.8400±0.01000^{d}	7.7800±0.01000^{d}
ICF6	7.5267±1.16337^{bc}	7.5400±0.01000^{c}	4.8100±0.01000^{f}	6.7700±0.02000^{d}
Storage period (Day 60)				
ICF0	5.5100±0.01000^{e}	6.7600±0.01000^{f}	8.0600±0.01000^{bc}	8.1967±0.02517^{bc}
ICF1	7.8633±0.02082^{c}	7.8600±0.01000^{c}	8.0500±0.01000^{bc}	8.1733±0.02082^{bc}
ICF2	8.8200±0.01000^{b}	7.9600±0.01000^{b}	8.0400±0.01000^{bc}	8.1700±0.01000^{b}
ICF3	8.9333±0.01528^{a}	8.0600±0.01000^{a}	7.9800±0.01000^{d}	8.1600±0.01000^{b}
ICF4	4.9567±0.01528^{f}	7.4333±0.01528^{e}	6.9800±0.01000^{d}	8.1600±0.01000^{ab}
ICF5	5.9800±0.01000^{d}	7.4500±0.01000^{e}	5.8600±0.01000^{d}	7.8100±0.01000^{c}
ICF6	5.9700±0.02000^{d}	7.4800±0.01000^{d}	4.9200±0.01000^{e}	6.7600±0.01000^{de}
Storage period (Day 70)				
ICF0	5.2167±0.01528^{d}	6.5333±0.04933^{e}	6.8700±0.01000^{d}	6.4400±0.01000^{f}
ICF1	7.8533±0.02082^{c}	7.7600±0.01000^{b}	8.4200±0.02000^{c}	7.4800±0.01000^{c}
ICF2	8.8100±0.01000^{b}	7.7600±0.01528^{a}	8.6700±0.01000^{b}	7.6700±0.01000^{b}
ICF3	8.9233±0.01528^{a}	7.8800±0.01000^{a}	8.8400±0.01000^{a}	7.9800±0.01000^{a}
ICF4	4.8600±0.01000^{g}	7.1400±0.01000^{d}	4.5500±0.01000^{g}	6.5100±0.01000^{e}
ICF5	4.8900±0.01000^{f}	7.1800±0.01000^{c}	4.7800±0.01000^{f}	6.6967±0.03215^{d}
ICF6	4.9200±0.01000^{e}	7.1800±0.01000^{c}	4.8700±0.01000^{e}	6.5100±0.01000^{e}

The values are indicated as the means±standard deviation. Values with varied superscripts in each column differ significantly ($p \leq 0.05$). Formula (ICF0) ice cream without color added, formula (ICF1) ice cream containing 50 mg/mL of betalain, formula (ICF2) ice cream containing 100 mg/mL of betalain, formula (ICF3) ice cream containing 200 mg/mL of betalain, formula (ICF4) ice cream containing 50 mg/mL of red dye 40, formula (ICF5) ice cream containing 100 mg/mL of red dye 40, formula (ICF6) ice cream containing 200 mg/mL of red dye 40.

CONCLUSION

According to this study, adding betalain to ice cream had a big an impact on the ice cream's chemical and microbiological features, including titratable acidity, protein, pH, fat, overall number of microorganisms, and sensory characteristics. The results demonstrated that betalain concentrations of 50, 100, and 200 mg/mL maintained ice cream samples' consistency by inhibiting the oxidation of lipids and the hydrolysis of proteins, among other quality indicators. Due to its high acidity and other quality factors, the chemical, microbiological, and sensory assessment of ice cream using red dye 40 (a synthetic pigment) found that it cannot be stored for longer than 40 days. As a natural food preservative, betalain could be added to products to boost stability during storage. Further research is needed to evaluate whether the exceptional qualities of this natural pigment are appropriate for use in futuristic applications and can thus be added to the list of food additives.

Authors Contribution: Ruaa Tariq Mohamed: Formal analysis; Methodology; Validation; Writing-original draft. Qaswaa Yousif Jameel: Project administration; Data curation; Formal analysis; Writing-review and editing.

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