Journal homepage: https://journals.rifst.ac.ir

Research Institute of Food Science and Technology

Research and Innovation in Food Science and Technology

JRIFST

Original paper

https://doi.org/10.22101/jrifst.2024.418256.1520

eISSN: 2538-2357

The use of Transglutaminase and Gallic acid as Stabilizers and Thickeners in the Production of Ice cream

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Abstract

This study aimed to utilize transglutaminase and gallic acid as stabilizers and thickeners in ice cream production. Additionally, it explored the potential for enhancing the physicochemical properties of ice cream mixtures by partially replacing the stabilizer with transglutaminase (0.36g/100$g_{protein}$) (IC2), gallic acid (2.5g) (IC3), and a combination of transglutaminase (0.22g/100$g_{protein}$) and gallic (1.5g) acid (IC4). These mixtures were compared with a control mixture containing only the stabilizer (IC1). The properties of the prepared ice cream, including titratable acidity, pH, melting time, morphology, thermal stability, and sensory attributes, were studied. The results revealed that IC4 exhibited superior properties compared to the control mixture and other prepared mixtures across all evaluated aspects. Specifically, the study observed a decrease in pH alongside an increase in titratable acidity after aging, and IC4 demonstrated the highest melting time, stability, and overall acceptance. Furthermore, sensory evaluation results showed that the IC4 blend received the highest sensory rating at 91%. Overall, these findings highlight the potential for optimizing ice cream formulations through strategic use of additives, paving the way for advancements in ice cream production and product quality.

Keywords

Enzyme crosslinking
Gallic acid
Ice cream
Melting rate
Transglutaminase

Received: 27 September 2023
Revised: 12 February 2024
Accepted: 13 February 2024
Available online: 19 March 2024

Introduction

Ice cream is a significant food due to its high nutritional value (da Costa *et al.*, 2023). Typically sought by consumers for its refreshing, hydrating, and nutritious qualities during the summer (Andriani *et al.*, 2022; El-Maksoud, 2023; Elkot *et al.*, 2022), its production represents one of the most advanced technologies in the dairy industry (Mahdian *et al.*, 2023). The inclusion of new additives is expected to improve the sensory and rheological properties of ice cream, ensuring high quality (Asminaya *et al.*, 2022; Khosrow Shahi *et al.*, 2021; Shadordizadeh *et al.*, 2023). Ice cream is a complex colloidal system that, when frozen, includes ice crystals, air bubbles, fat globules, sugars, proteins, salts, and water (Liu *et al.*, 2023; Romulo & Meindrawan, 2021). The desired properties of ice cream depend on the physicochemical properties of the components in the mixture, including milk and its derivatives as well as other food ingredients. In addition, the properties are influenced by the air bubbles present in the liquid phase along with fine ice crystals and the liquid matrix containing emulsified fat, milk proteins, salts, and sugar crystals (Ghaderi *et al.*, 2021; Goktas *et al.*, 2022).

Functional foods refer to whole foods or food components ingested to boost health and wellness. Nutraceutical foods provide both health benefits, such as prevention, and medical benefits, such as medication for various diseases (Shamshad *et al.*, 2023). Exposing ice cream to temperature fluctuations can lead to deterioration in quality as the ice cream's ability to recrystallize is reduced, thus affecting its sensory properties. To alleviate this problem and make ice cream more resistant to changes while increasing viscosity, transglutaminase is added.

Transglutaminase is considered a safe enzyme and is widely used in various food industries (Rossa *et al.*, 2011). Transglutaminase (EC: 2.3.2.13) is an enzyme found in both the plant and animal kingdoms. It works by forming internal and external cross-links in proteins between lysine and glutamine residues (Al *et al.*, 2020; Motoki & Seguro, 1998). Transglutaminase is widely used to create crosslinks in most

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food proteins including casein, soy, etc. (this enzyme is known for its protein binding properties), (Xu *et al.*, 2022). Ice cream enriched with organic acids, including citric and phosphoric acid, also includes gallic acid (GA). GA is a phenolic acid derived from natural sources and possesses antioxidant, anti-inflammatory, antifungal, and antitumor properties (Cláudio *et al.*, 2012; Khalil *et al.*, 2021).

The aim of this study was to create bio-based polymers with antioxidant properties using monomers derived from gallic acid. The phenolic groups in GA are responsible for its antioxidant activity, which means they should be protected for maximum antioxidant activity in the final polymers (Khalil *et al.*, 2021). Gallic acid can be used to modify the functional and molecular characteristics of whey proteins and has the ability to form a gel with milk proteins, increasing water-holding capacity and product viscosity (Bayraktar *et al.*, 2019; Moreno *et al.*, 2020; van Lith & Ameer, 2016).

This results in ice cream that is more resistant to melting, has less shrinkage, increased viscosity, and higher yield.

In summary, this research showcases the potential for incorporating transglutaminase and organic gallic acid to improve the physicochemical properties of ice cream, resulting in a product with enhanced sensory and stability characteristics.

Materials and methods

Materials

The raw materials used in the manufacturing of this ice cream include Anchor milk powder of New Zealand origin, sugar, and Guar Gum (GG) (E412) from Sudan, as well as Gallic Acid (GA) procured from Sigma Aldrich (Wicklow, Ireland) and Transglutaminase (TGase) from Sigma (Darmstadt, Germany).

Ice cream preparation

Ice cream was prepared according to the method described by Akın *et al.* (2007) with some modifications. The first blend (IC1) was prepared by mixing 155 g of New Zealand-origin dried milk, brand "Anchor," with 665 g of water. The milk was then heated to a temperature of 85 °C for 15 min, followed by cooling to 30 °C. A mixture of 175 g of sugar and 5 g of stabilizer Guar gum was added gradually until complete dissolution of the sugar was achieved. The blend was then blended using a high-speed electric blender (Mix hand type MODEX model HM 560 series, China) for 5 min. Three other blends were prepared in the same manner, with the first one (IC2) substituting half of the stabilizer (2.5 g Guar Gum) with the addition of transglutaminase (TGase) at a ratio of 0.36 g/100 g protein. The blend IC3 replaced half of the stabilizer (2.5 g Guar Gum) with 2.5 g gallic acid, and in IC4 blend, equal proportions of stabilizer (1.5 g Guar Gum), enzyme (0.22 g), and gallic acid (1.5 g) were added. The four samples were stored in the refrigerator for 16 hours for aging purposes, after which they were transferred to the ice cream make, filled, and subjected to product-specific tests. The product was evaluated based on the assessment form provided by Soad *et al.* (2014), with some modifications.

Titratable acidity and pH value

Titratable acidity and pH value (pH-meter, Crisongl p22, Japan) with a glass electrode, was used for directly measuring the pH value of the ice cream mixtures. The acidity content, Titratable acidity was evaluated through titration with 0.1 N NaOH until the phenolphthalein endpoint was reached. This was then expressed as a percentage of lactic acid (%TA), following the method outlined in AOAC (2010). The percentage of total acidity was determined using the equation:

$$Titratable\ acidity\ \% = \frac{\text{NaOH (mL)} \times 0.1\text{N} \times 0.09}{\text{Weight of sample}} \times 100 \quad (1)$$

Melting rate

Melting rate was performed according to Olson *et al.* (2003) with some modifications. A 50-gram sample of ice cream was weighed, and the funnel was sealed using a clamp (strainer). The sample was then placed over the funnel, and subsequently transferred from the funnel to a balance to measure its initial weight. The weight was recorded at various time intervals (0, 15, 30, 45, 60, 75, and 90 min) until complete melting of the ice cream piece occurred. The samples were placed in a refrigerated incubator at a temperature of 25 °C, and the melting rate was determined according to the equation below:

$$Melting\ rate = \frac{\text{M1} - \text{M2}}{\text{M2}} \times 100 \quad (2)$$

M1 = weight of molten portion of sample at 15-minute intervals

M2 = weight of sample

Scanning electron microscopy (SEM)

Morphological characteristics were studied as described by Akin *et al.* (2019), using a scanning electron microscope, Model Balzer, type DEMG60A, Germany. Approximately 40 mg of freeze-dried ice cream was placed into the scanning device, and the sample was prepared by purging with argon and nitrogen gas. Subsequently, the samples were transferred to the scanning electron microscope and images were captured at an appropriate magnification of 5000× at 10 kV and 2000× at 15 kV for some samples.

Thermogravimetric analysis (TGA)

TGA was measured according to Er *et al.* (2019). The temperature of decomposition of freeze-dried ice cream was determined using a TGAQ40V6.3Build 19lk apparatus. Approximately 10-20 mg of the sample was placed in a platinum crucible specific to the device. The temperature range for the analysis was set between 20 to 600 °C at a heating rate of 10 °C/min, under nitrogen gas.

Sensory evaluation

Sensory evaluation of ice cream formulas was conducted by 10 assessors (comprising 5 women and 5 men, aged between 18 and 40 years), (Sanjeewa *et al.*, 2023) from the Department of Food Science at the College of Agriculture, University of Basra. This evaluation was carried out according to the assessment form designed by Soad *et al.* (2014), with some modifications. The evaluating scores for each parameter were as follows color and appearance (15 points), texture and consistency (35 points), flavor and taste (15 points), mouthfeel (20 points), overall acceptability (15 points), and total score (100 points).

Statistical analysis

A completely randomized design (One-way ANOVA) was used to analyze the experimental data using the SPSS program (version 2018), and the differences among means were assessed using the least significant difference (L.S.D.) test at a significance level of $p < 0.05$.

Results and discussion

Titratable acidity and pH value

Studying the physicochemical properties of ice creams is essential for understanding the impact of additives in comparison to the control formula, as well as the changes that occur during the manufacturing steps, including pasteurization, aging, cooling, freezing, filling, and storage.

It is important to note that the natural acidity of the blend varies with the composition of the ingredients used and is influenced by factors such as the fresh milk utilized in its preparation. Any decrease in pH or an increase in titratable acidity is undesirable as it can contribute to excessive viscosity, reduce the mix's whipping ability, and compromise the blend's stability. Such changes could be attributed to protein denaturation during the manufacturing processes.

Table (1) illustrates the relationship between pH and titratable acidity. The results showed variations before and after aging, depending on the nature of the stabilizer used and the occurring changes.

The results showed that the control blend (IC1) and the blend with added transglutaminase (TGase) enzyme (IC2) did not exhibit significant changes in titratable acidity and acidity function before and after aging. This is consistent with previous studies where adding TGase to ice cream blends with added Amla Fruit juice (containing ascorbic acid and malic acid) resulted in enzyme stability (Akin *et al.*, 2019; Rahmani *et al.*, 2014; Şanlidere Aloğlu *et al.*, 2018).

Furthermore, the results indicated that in the blends with added gallic acid (IC3) and the blend with both the enzyme and acid (IC4), the titratable acidity increased while the pH decreased after aging. This trend can be attributed to the addition of organic acids, which tend to decrease the pH and increase titratable acidity. The concentration, composition, molecular weight, and pKa dissociation constant of the acid are all factors influencing changes in pH and titratable acidity (6.2-0.25), (Hazrati & Madadlou, 2021; Minal *et al.*, 2017; Zagorska *et al.*, 2022). Several studies have revealed a relationship between product viscosity and its acidity, likely due to interactions between the hydroxyl groups of milk fatty acids with amino acids and phenolic compounds, which may decrease the acid function if the pH of the medium is decreased (El-Hadad *et al.*, 2020).

These results align with other studies assessing the influence of organic acids on ice cream blends For example, Singo and Beswa (2019) observed a reduction in pH after aging for ice cream blends with added plant extracts containing malic acid, and Zagorska *et al.* (2022) observed a reduction in acidity function from 6.36 to 5.93 due to the effects of lactic acid (lactobionic acid) on ice cream blends. Studies have demonstrated that the physicochemical properties, including natural acidity, are related to the composition of the mix, leading to increased acidity (0.23-0.27) and lower pH (6.7-5.6) when preparing ice cream blends with added Amla Fruit juice containing ascorbic acid and malic acid (Sanjeewa *et al.*, 2023).

Table 1. Changes in pH and titratable acidity of the triangle Formulas before and after aging process Control (add Guar Gum) IC, Transglutaminase and Guar Gum (IC2), Gallic acid and Guar Gum (IC3), Transglutaminase + Gallic acid and Guar Gum (IC4)

Formulas	pH value		Titratable acidity	
	Before aging	After aging	Before aging	After aging
IC1	6.65^{a}	6.72^{a}	0.15^{b}	0.14^{b}
IC2	6.59^{a}	6.50^{a}	0.16^{ab}	0.16^{b}
IC3	6.25^{b}	5.76^{b}	0.18^{a}	0.21^{a}
IC4	6.33^{b}	5.84^{b}	0.17^{ab}	0.20^{a}

Melting time

The stability of ice cream is characterized by desirable traits such as slow melting time, shape retention, and resistance to foam collapse.

Fig. (1) illustrates the effect of adding enzymes and acids to ice cream blends as stabilizers and thickeners on melting times compared to the standard sample at various time intervals. The melting times of the ice cream blends were assessed at a temperature of 22°C, over different time periods. It can be concluded that all types of stabilizers have an impact on melting resistance. Variations in the resistance to melting in blends may result from factors including overrun, emulsification, type of stabilizers, crystal size, percentage of solids in the blend, and the composition of the organic acid used (Gabbi *et al.*, 2018).

The results show that formula IC4, containing both the enzyme and acid, exhibited the highest resistance to melting at 38%, followed by formula IC3, containing the acid at 55%, and then formula IC2, containing the enzyme, at 40%, compared to the standard formula IC1 at 65%. The addition of the TGase enzyme to the formula resulted in the formation of cross-links between milk proteins, leading to protein polymerization through the formation of ç-glutamyl-lysine linkages. This increased the resistance to melting in the blend (Eissa & Khan, 2005; Rossa *et al.*, 2011). It was found by Kasprzyk *et al.* (2016) and Rossa *et al.* (2011) that the addition of the TGase enzyme to milk ice cream showed higher melt resistance compared to the control.

Additionally, the presence of organic acids containing multiple carboxylic groups binds to denatured proteins, forming a gel network that further increases the resistance to melting in ice cream blends (Bayraktar *et al.*, 2019). This aligns with the findings of Sanjeewa *et al.* (2023) when preparing ice cream blends with added Amla Fruit juice, resulting in higher melting resistance compared to the control sample. Similarly, Al Musa and Al Garory (2023) reported that ice cream blends, in which milk was replaced with fermented vegetable milk with added acid starters, exhibited higher melting resistance compared to the control sample.

The presence of both acid and enzyme in blend IC4 led to the formation of an ideal blend with high melting resistance. This is attributed to the binding of the acid with the enzyme and the formation of a stronger gel network. The enzyme's ability to bind the sulfur bridges released from protein denaturation (disulfide linkages), in addition to its primary role in gel formation through cross-linking, contributes to this effect (Eissa & Khan, 2005) .

Scanning electron microscopy (SEM)

Fig. (2) illustrates the microstructure of ice cream blends IC1, IC2, IC3, and IC4. The differences in the texture of the ice cream samples are evident, with sample IC4, containing the enzyme, acid, and stabilizer, exhibiting superior texture compared to sample IC1, which serves as the control. Sample IC2, containing the enzyme and stabilizer, and sample IC3, containing the acid and stabilizer, also show variations in texture. This indicates that the presence of the enzyme and acid alongside the stabilizer provided an ideal texture by enhancing the water-holding capacity of the binding entities present in the stabilizing agents, thereby improving the physical and chemical properties of the ice cream (Akin *et al.*, 2019).

Food products composed of biopolymers are linked through covalent bonds, hydrophobic interactions, and hydrogen bonds. The structure varies under the scanning electron microscope, causing variations in the appearance of the microstructure when viewed with a scanning electron microscope (Hernández-Rodríguez *et al.*, 2017). The cross-linking of glutamine and lysine by the enzyme leads to the formation of a stable and cohesive gel. Scientific studies have focused on the role and importance of the TGase enzyme in forming cross-linkages in milk proteins (caseins) due to their open chain structure, making them readily available for enzymatic action, resulting in their modification and easy alteration of their physical properties (Pereira *et al.*, 2020).

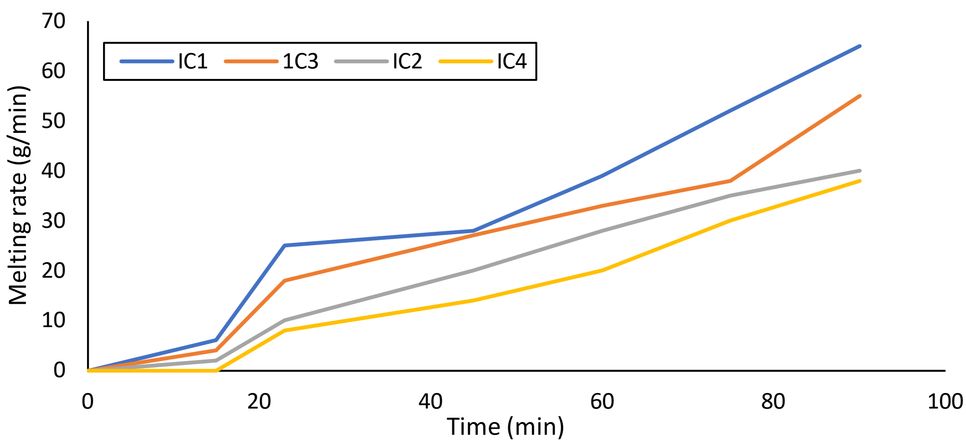

Fig. 1. Melting time measurement for 4 different ice cream Formulas Control (add Guar Gum) IC, Transglutaminase and Guar Gum (IC2), Gallic acid and Guar Gum (IC3), Transglutaminase + Gallic acid and Guar Gum IC4.

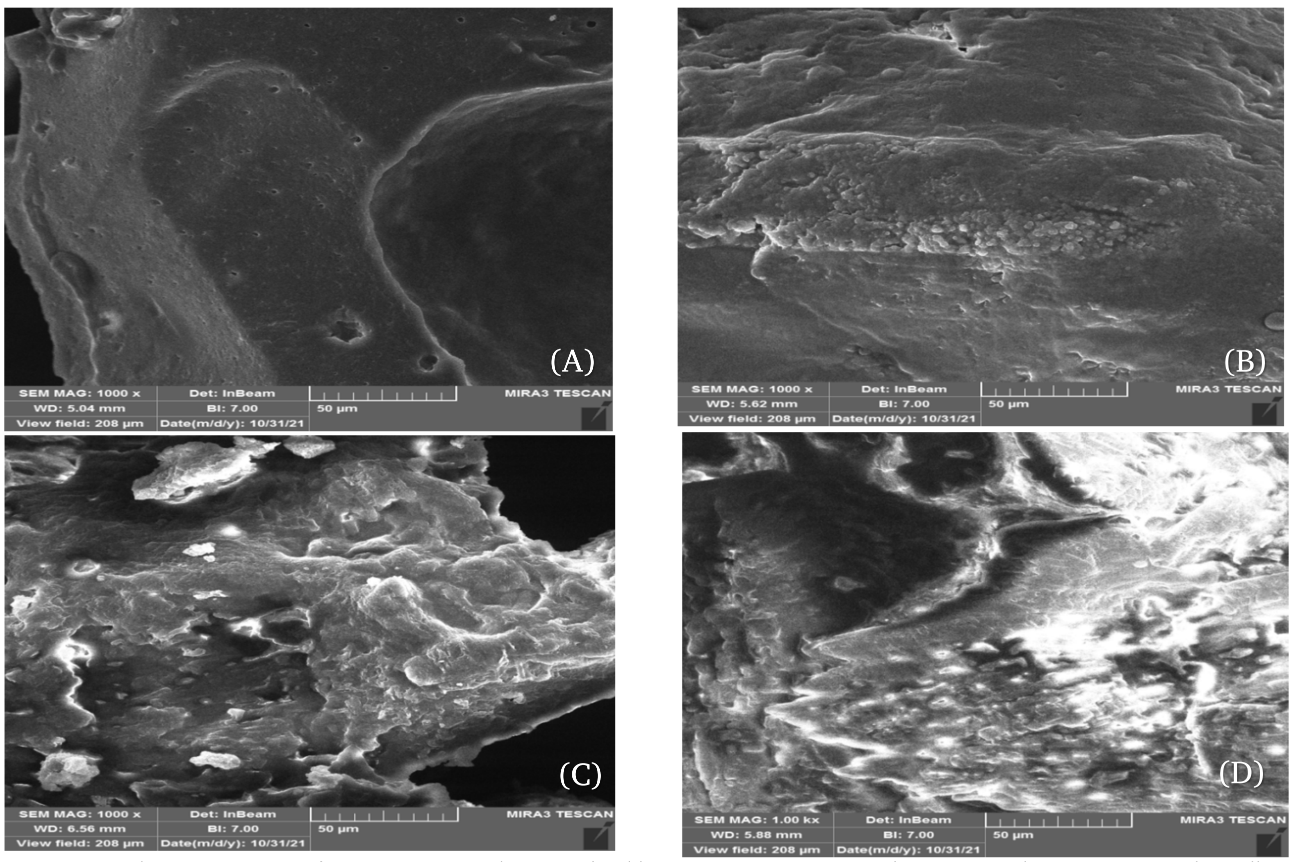

Fig. 2. Scanning electron microscopy for ice cream Formulas Control (add Guar Gum) IC1 (a), Transglutaminase and Guar Gum (IC2) (b), Gallic acid and Guar Gum (IC3) (c), Transglutaminase + Gallic acid and Guar Gum IC4 (d)

Thermogravimetric analysis (TGA)

Fig. (3A) and Table (2) show the thermal stability (resistance) of polymer networks for proteins using variable temperature thermal analysis. Thermogravimetric analysis (TGA) is a method for evaluating the thermal stability of stabilizing agents and monitoring changes in the physical and chemical properties of ice cream (Azevedo *et al.*, 2017). The thermal stability of the studied ice cream was measured and weight changes were monitored at different temperatures (50-550 ˚C) during the start and end of decomposition. The results showed that each ice cream goes through three stages of decomposition (Fig. 3). For instance, in the case of IC1, which contains only guar gum as the stabilizer, the first stage occurred at temperatures ranging from 50-160 ˚C. This stage represents the evaporation of free and bound water, resulting in a 5% weight loss. The second stage, from 350-220 ˚C, led to a weight loss of approximately 19.15%, primarily due to the decomposition of proteins and poly saccharides. The third stage, starting at around 350-550 ˚C, involved the decomposition of all organic materials, leaving behind only ash. In this stage, 80.85% of the weight was lost, with a half-weight loss temperature (T50%) at 280 ˚C and a charring rate of 19.15%. Protein decomposition occurred at a rate of 57%.

Fig. (3B) and Table (2) depict the thermal decomposition of the IC2 ice cream blend, which contains transglutaminase and guar gum as a stabilizer. This blend also undergoes three decomposition stages. The first stage, occurring between 50-150 ˚C, results in a 6% weight loss, attributed to the loss of free and bound water. The second stage, from 160-250 ˚C, leads to a weight loss of approximately 20%. The third stage, ranging from 290-550 ˚C, involves the decomposition of all organic materials, leaving behind ash. In this stage, half-weight loss (T50%) occurs at 310 ˚C, and the charring rate is 17.68%, with a protein decomposition rate of 80%. The increased thermal stability of the IC2 blend can be attributed to the role played by the transglutaminase in rearranging and linking proteins, resulting in the formation of a polymer that alters and increases the molecular weight of the protein. This makes it more thermally stable (Er *et al.*, 2019; Gaspar & de Góes-Favoni, 2015; Zhu & Gong, 2014).

Table 2. Thermogravimetric analysis of ice cream Formulas Control (add Guar Gum) IC, Transglutaminase and Guar Gum (IC2), Gallic acid and Guar Gum (IC3), Transglutaminase + Gallic acid and Guar Gum IC4

Formulas	Temperature of decomposition									T50%	Char content%	Rate of decomp. % min
	1st Decomposition ˚C			2nd Decomposition ˚C			3rd Decomposition					
	Ti	To	Tf	Ti	To	Tf	Ti	To	Tf			
IC1	50	100	160	107	220	350	350	420	500	280	19.15	2.28
IC2	50	90	150	160	210	250	290	550	550	310	17.68	2.28
IC3	50	100	200	204	220	260	350	450	550	336	26.37	2.14
IC4	50	90	150	160	208	300	-	-	-	338	29.3	2.24

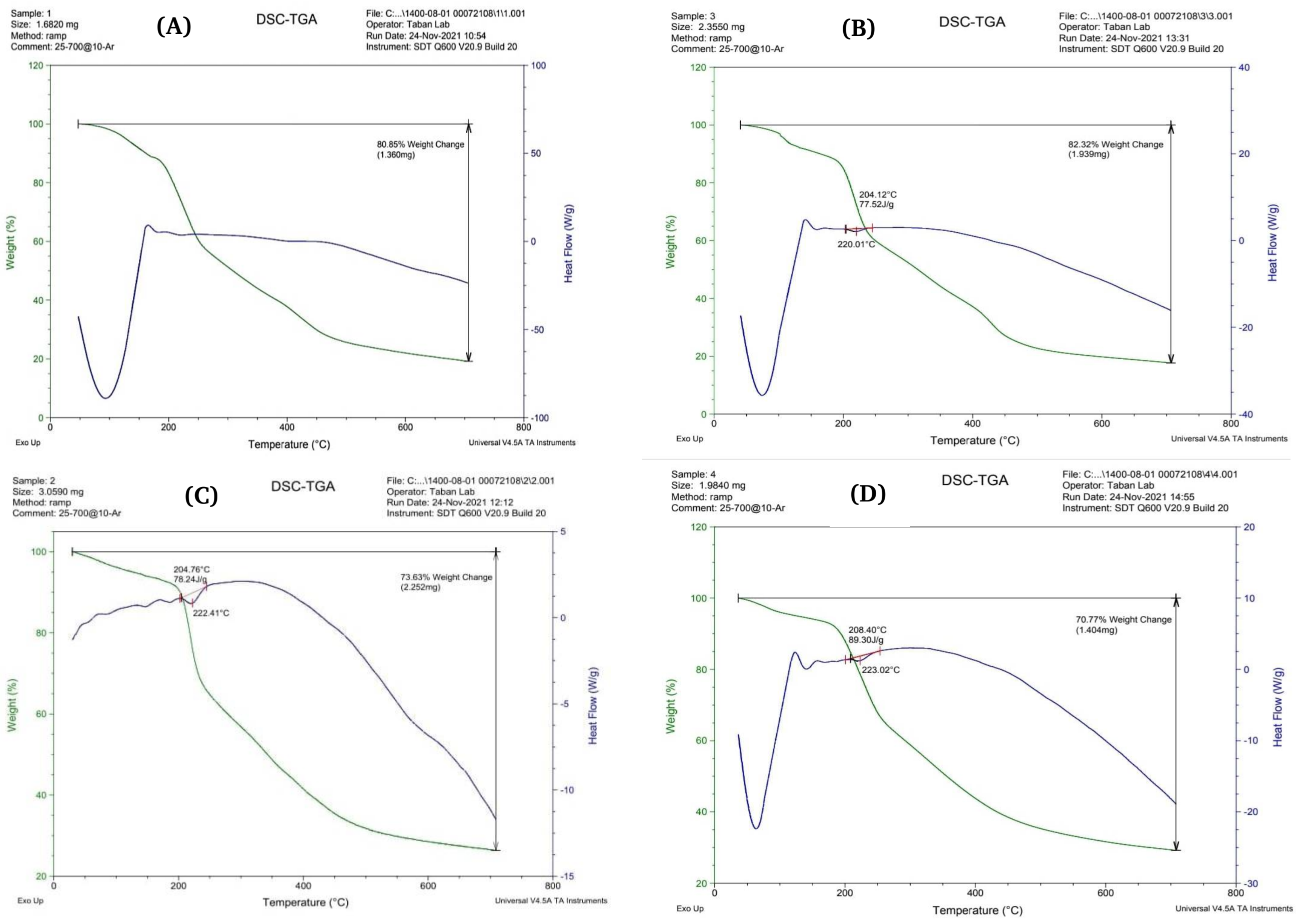

Fig. 3. Thermogravimetric analysis for ice cream Formulas Control (add Guar Gum) IC1 (A), Transglutaminase and Guar Gum (IC2) (B), Gallic acid and Guar Gum (IC3) (C), Transglutaminase and Gallic acid and Guar Gum IC4 (D)

Table 3. Sensory evaluation data of ice cream Formulas Control (add Guar Gum) IC1 (a), Transglutaminase and Guar Gum (IC2) (b), Gallic acid and Guar Gum (IC3) (c), Transglutaminase + Gallic acid and Guar Gum IC4 (d)

Formulas	Color and appearance (15 points)	Texture and consistency (35 points)	Flavor (15 points)	Mouthfeel (20 points)	Overall acceptability (15 points)	Total score (100 points)
IC1	11.50 ± 2.00^{a}	30.00 ± 2.00^{b}	11.00 ± 2.00^{a}	12.40 ± 0.90^{b}	10.43 ± 2.90^{a}	75.33 ± 4.46^{b}
IC2	12.40 ± 1.10^{a}	32.40 ± 0.60^{ab}	12.00 ± 2.00^{a}	15.00 ± 2.00^{ab}	12.00 ± 3.00^{a}	83.80 ± 4.70^{a}
IC3	12.50 ± 1.90^{a}	32.00 ± 2.00^{ab}	11.60 ± 1.70^{a}	15.50 ± 2.80^{ab}	12.80 ± 0.90^{a}	84.40 ± 2.10^{a}
IC4	13.50 ± 0.90^{a}	33.00 ± 1.00^{a}	13.00 ± 2.00^{a}	18.00 ± 1.00^{a}	13.50 ± 0.90^{a}	91.00 ± 3.80^{a}

All results were expressed as mean ± standard deviation values.
Means in the same column followed by different letters are significantly different (Tukey test, $P < 0.05$).

Fig. (3C) and Table (2) illustrate the thermal decomposition of the IC3 ice cream blend, which contains gallic acid and guar gum as a stabilizer. This blend also undergoes three decomposition stages. The first stage, occurring between 50-200 °C, results in a 6% weight loss, attributed to the loss of free and bound water. The second stage, from 204-260 °C, leads to a weight loss of approximately 20%. The third stage, ranging from 350-550 °C, involves the decomposition of all organic materials, leaving behind ash. In this stage, half-weight loss (T50%) occurs at 336°C, and the charring rate is 26.37%, with a protein decomposition rate of 80%. The increased thermal stability of the IC3 blend can be attributed to the presence of gallic acid, which promotes protein aggregation and the formation of disulfide bonds, responsible for increased cross-linking and, consequently, thermal stability (Patel & Velikov, 2011; Wang & Damodaran, 1991).

Fig. (3D) and Table (2) present the thermal decomposition of the IC4 ice cream blend, which contains transglutaminase, gallic acid, and guar gum as stabilizers. This blend also undergoes three decomposition stages, similar to the previous blends. The first stage, occurring between 50-150 °C, results in a 6% weight loss due to the loss of free and bound water. The second stage, from 210-250 °C, leads to a weight loss of approximately 20%. The third stage, ranging from 290-550 °C, involves the decomposition of all organic materials, leaving behind ash. In this stage, half-weight loss (T50%) occurs at 310 °C, and the charring rate is 17.68%, with a protein decomposition rate of 80%. The increased thermal stability of the IC4 blend can also be attributed to the action of the transglutaminase, along with the presence of gallic acid and guar gum, leading to enhanced cross-linking and thermal stability.

Sensory evaluation

The success of a product in the market is largely dependent on consumer acceptance, which is influenced by factors such as appearance, flavor, and mouthfeel sensation. Therefore, selecting the right qualities is crucial to enhance product acceptance and shelf life (Patel & Velikov, 2011).

Table (3) displays the results of sensory evaluations for the ice cream blends. It is apparent from the table that blend IC4 achieved the highest scores for sensory attributes, including color and appearance, texture and consistency, flavor and taste, mouthfeel, and overall acceptability, with a total score of 91. In contrast, the standard sample IC1 scored 75.4. Blends IC2 and IC3 achieved scores of 83.8 and 84.4, respectively. The table also indicates that there were no statistically significant differences between the four blends regarding color and appearance, flavor, and overall acceptability. This implies that the addition of the enzyme and acid did not significantly impact these attributes, which is consistent with previous findings (Aloğlu & Öner, 2013; Şanlidere Aloğlu *et al.*, 2018).

Statistically significant differences were observed in texture, consistency, and mouthfeel attributes. Specifically, blend IC4 provided the best results, scoring 33 and 18, followed by blends IC2 and IC3, which scored 32.4 and 32 for texture and consistency, and 15 and 15.5 for mouthfeel, respectively. These three blends outperformed the standard IC1 blend, which scored lower, with 30 for texture and consistency, and 12.4 for mouthfeel. This difference can be attributed to the presence of the enzyme, which improves the structure and consistency of the blend by binding water to milk proteins, enhancing texture stability (Şanlidere Aloğlu *et al.*, 2018). Additionally, the polymerization of milk proteins by the TGase enzyme leads to the formation of a cohesive gel network, further enhancing the characteristics of the dairy ice cream (Al *et al.*, 2020).

Conclusion

The research on the impact of additives in ice cream blends and the changes occurring during manufacturing steps, such as pasteurization, aging, cooling, freezing, filling, and storage, has provided valuable insights. The study emphasized the influence of titratable acidity and pH on the physicochemical properties of ice creams, with a focus on the impact of additives such as transglutaminase (TGase) enzyme and gallic acid. It was found that these additives have the potential to alter acidity and pH levels, affecting aspects such as viscosity, stability, and melting resistance of the ice cream blends. Furthermore, the study explored the relationship between the presence of additives and the melting time, texture, and thermal stability of the ice cream blends. The addition of both the enzyme and the acid was found to enhance melting resistance and thermal stability, leading to superior texture and consistency, as supported by scanning electron microscopy and thermogravimetric analysis. Notably, the sensory evaluation indicated that the blend containing both the enzyme and the acid achieved the highest scores for attributes such as color, appearance, texture, consistency, flavor, taste, mouthfeel, and overall acceptability. These findings underscore the potential of additives to improve the sensory attributes of ice cream blends, ultimately enhancing consumer acceptance and product quality. Overall, this research provides future insights into optimizing ice cream formulations by strategically utilizing additives to positively influence physicochemical properties, sensory attributes, and shelf life. The findings suggest that further exploration of the role of additives, such as enzymes and acids, could lead to advancements in ice cream production, resulting in products with improved quality and consumer appeal.

Acknowledgement

The authors wish to express their profound gratitude sincerely to the Research Deputy of the University of Basrah for financially supporting this project.

Author contributions

Zena Kadhim Al-Younis: Data collection, Data analysis and interpretation, Presenting the research idea and study design, Revising and editing the manuscript, Supervising the study; **Raqad R.Al-Hatim**: Data collection, Writing the draft of the manuscript, Data analysis and interpretation, Presenting the research idea and study design; **Sheren Fadhal Abbas**: Data collection, Writing the draft of the manuscript, Data analysis and interpretation, Presenting the research idea and study design; **Ammar B. Altemimi**: Data collection, Writing the draft of the manuscript, Data analysis and interpretation, Presenting the research idea and study design; **Mohammad Ali Hesarinejad**: Presenting the research idea and study design, Revising and editing the manuscript; **Mohamed Ibrahim Younis**: Presenting the research idea and study design, Revising and editing the manuscript; **Tarek Gamal Abedelmaksoud**: Presenting the research idea and study design, Revising and editing the manuscript. All authors have read and agreed to the published version of the manuscript. All authors read and approved the final manuscript.

Conflict of interest

There is no conflict of interest based on the writers.

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