Coffee ice cream as an innovative functional dairy food

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Abstract

Coffee ice cream is one of the best options when we're looking for a caffeinated dessert with a desirable taste and health benefits. In this study, the use of concentrated coffee extract (CCE) in ice cream production as an innovative functional dairy food was evaluated. The most abundant phenolic component in CCE was chlorogenic acid, with 7.39 mg/mL, followed by pyrocatecol, naringin, gallic acid, and catechin. Folic acid, with 4.67 mg/mL, was the most abundant B-complex vitamins in CCE, followed by thiamin and riboflavin. CCF also showed antibacterial activity against gram-positive and gram-negative bacteria as well as fungi. Ice cream formulated with 3% CCE showed higher overrun (41.76%), fat destabilization (15.14%), and melting rate (1.19 g/min) than that formulated with 5% CCE and control ice cream. Adding probiotic bacteria B. breve Bb-12 and L. plantaru enhanced the viscosity of the mixture, as well as the overrun and melting rate of the final ice cream. However, adding CCE to ice cream had no effect on survival of probiotic bacteria during storage at –20°C when compared to control ice cream. CCE-containing ice creams showed higher antioxidant activity against the DPPH and ABTS radicals, with the increase proportionate to the amount of CCE. Ice cream with 3% CCE had a favorable brown color, less bitterness, the desired coffee flavor, and a smoother texture than ice cream with 5% CCE.

1. Introduction

Coffee is one of the most popular beverages in the world due to its energizing caffeine effects, pleasant sensory qualities, and positive implications on human health. According to Statista (2021), around 9997.8 million kilograms of coffee were consumed globally in 2020/2021, representing a modest rise over the previous year (1.6%). Coffee contains more than 50% carbohydrates, 10% proteins, and 2% free amino acids and polyphenols. As a consequence of caffeine's content, those responsible for physiological activities result in powerful stimulation of the central nervous system, which affects many functions (Attokaran, 2017). Coffee possesses antioxidant, anticarcinogenic, antimutagenic, and anti-inflammatory properties, making it suitable for use in a variety of pharmacological formulations. In addition, it can help relieve headaches, so several over-the-counter and prescription pain relievers include it as an ingredient, usually with aspirin or another analgesic (Erskine et al., 2022). Coffee has a complex structure with varying types of chemicals such as alicyclic compounds, aromatic compounds, and heterocyclic compounds as well as furans, pyrazines, pyrroles, thiophenes, and thiazoles. Significant amounts of phenolic compounds are responsible for the above-mentioned health-promoting properties of coffee (Hofmann et al., 2007). It also contains a complex combination of chlorogenic acids (CGA), which are phenolic compounds with antioxidant properties that are mostly produced via the ester connection between ferulic or caffeic acids and quinic acid (Williamson et al., 2011).

Coffee extract can be used as a flavor in boiled sweets, candies, ice cream, malted beverages, liqueurs, and frozen dairy preparations. In recent years, cold coffee has also gained popularity and an important food item, only in some cases has it become a flavor in other food products (Attokaran, 2017). Ice cream production is an important and rapidly developing technology that has become a profitable industry because of recent advances. It uses many different additives and processing technologies, such as fruit

products, probiotics and other additives. Research shows that a mixture of ice cream with added additives affects nutritional value and sensory quality (Cakmakci et al., 2015; Sagdic et al., 2012). Several bioactive components, such as pomegranate peel phenolic (Cam et al., 2014), whey protein (Danesh et al., 2017), and vitamin D_3 were added to ice cream (Tipchuwong et al., 2017). Other studies recommended adding oils that are rich in phytochemicals to enhance the nutritional value of ice cream products, including chia (Ullah et al., 2017), hazelnut oil, and olive oil (Güven et al., 2018). Natural foods that are high in nutrients and may have biological activities are in high demand among modern consumers. Food manufacturers and researchers are responding to this need by developing new ice cream formulas that are supplemented with various components. Commercial ice cream, on the other hand, is a poor provider of essential vitamins, minerals antioxidant components (Sun-waterhouse et al., 2013). Therefore, the aim of the study was to use coffee extract as a natural ingredient in the production of a new functional ice cream characterized by healthy properties and a preferred taste for many consumers, as well as to evaluate the addition of coffee extract to some physicochemical properties and survival of probiotic bacteria during storage.

2. Materials And Methods

2.1. Materials

2.1.1. Ingredients: Fresh buffalo's milk (6% fat and 9.5% MSNF) and fresh cream (~60.0% fat) were obtained from the farm of Fac. Agric., Cairo Univ., Egypt. Skim milk powder made in the USA, and commercial grade granulated sugar cane produced by Sugar and Integrated Industries Co., Egypt and were purchased from a local market in Cairo, Egypt. Carboxymethylcellulose sodium salt was purchased from Sigma-Aldrich (CAS No. 9004-32-4, St. Louis, MO, USA).

2.1.2. Coffee extract: Concentrated coffee extract (CCE) with particles size ≤ 0.2 µm was obtained from Misr Cafe Co., Cairo, Egypt. Vietnamese coffee beans were roasted at 246°C, extracted at 97 °C, filtered through a 0.2 µm filter and then concentrated under vacuum at 70°C (43.51% TS, 10.07% protein and 6.12% ash).

2.1.3. Probiotic bacteria: *Bifidobacterium breve* Bb-12 and *Lactobacillus plantarum*, as probiotic bacteria, were obtained from stock cultures at the Dairy Microbiology Lab., National Research Centre, Cairo, Egypt. Both strains were individually activated by three consecutive transfers into modified MRS, followed by three subsequent transfers into sterile 11% reconstituted skim milk powder. The cultures were incubated at 37°C for 48 h under anaerobic conditions and prepared 24 h before use.

2.1.4. Chemicals: The 2,2-diphenyl-1-(2,4,6-trinitrophenyl)-hydrazinyl (DPPH), and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Methods

2.2.1. HPLC analysis phenolic compounds: The HPLC analysis of the phenolic compounds and B-complex vitamins in CCE were conducted using an Agilent 1260 series. The Eclipse C18 column (4.6 mm x 250 mm ID x 5 µm) was used to separate phenolic compounds. The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate of 0.9 mL/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A), 0–5 min (80% A), 5–8 min (80–60% A), 8–12 min (60% A), 12–15 min (60–85% A) and 15–16 min (82% A). For the separation of B-complex vitamins, the ZORBAX SB-C8 (4.6 mm x 150 mm ID x 5 µm) was used. Water (A) with 0.01% TFA (pH 2.9) and methanol (B) at a flow rate of 1.5 mL/min made up the mobile phase. The mobile phase was designed in this order: 0 min (90% A), 0–1 min (90–70% A), 1–4 min (70–50% A), 4–8 min (50–90% A), and 8–10 min (90% A). The injection volumes for phenolic compound and B-complex vitamin solutions were 10 and 5 µL, respectively. The multi-wavelength detector was monitored at 280 nm.

2.2.2. Antimicrobial activity: Six bacterial strains, *Escherichia coli* O157: H7 ATCC 6933, *Staphylococcus aureus* ATCC 20231, *Pseudomonas aeruginosa* ATCC 9027, *Listeria monocytogenes* ATCC 7644, *Yersinia enterocolitica* ATCC 9610, and *Salmonella typhimurium* ATCC 14028, as well as three fungal strains, *Aspergillus terreus* ATCC 10020, *Aspergillus niger* ATCC 16404, and *Candida albicans* ATCC 14053, were obtained from stock cultures of the Dairy Microbiological Lab, National Research Centre, Cairo, Egypt. The agar-well diffusion method was used to test the antibacterial activity of the CCE against reference strains, as described by Ghazya et al. (2021). Briefly, 1 mL of activated indicator strain culture (10^5 cfu/mL) was inoculated into 20 mL Mueller-Hinton agar (Becton Dickinson, USA), poured into sterile Petri dishes, and allowed to solidify. Then, wells of 5 mm in diameter were cut from the agar with a sterile borer and 50 µL of CCE was poured into each well. The plates were kept at room temperature for 2 h to allow the CCE to diffuse into the agar before being incubated at 37°C for 24 h. The antifungal spectrum activity was also evaluated using the agar-well diffusion method as described by Murray et al. (1995). Inoculated aseptically with 100 µL spore suspension (10^6 spores/mL) through the spread plate technique, the plates were kept in the laminar flow for 30 min. Wells of 5 mm in diameter were cut in the center of petri dishes using a sterile borer. 50 µL l of CCE was dropped into each well, kept at 4 °C for 3 h, and incubated at 25±1°C for 5 days. The inhibitory zone around each well was measured in mm in both tests.

2.2.3. Coffee ice cream making: Buffalo's milk and fresh cream, skim milk powder, sugar, and CMC as a stabilizer were used to make ice cream mixtures with final fat, milk solids, non-fat, sugar, and stabilizer content of 10.0, 11.0, 15.0, and 0.1%, respectively. The ice cream mixtures were preheated to 65°C, homogenized using a laboratory homogenizer (POLYTRON PT10-35-GT-D, KINEMATICA, Swaziland) at 21,000 rpm for 5 min and then pasteurized at 81 °C. After cooling to 40°C, CCE was added at rates of 0, 3, and 5% (based on preliminary studies), and each mixture was divided into two equal parts: one was inoculated with 2% probiotic bacteria (*B. breve* and *L. plantarum*, 1:1), while the other was not. After aging overnight at 5±1°C, all mixtures were frozen in a batch freezer (Staff Ice System, BTM 10, Rimini, Italy), and overrun was calculated for all treatments using the weight-volume method (Adapa et al., 2000). The resulting ice cream was filled into 100 mL plastic cups, covered, and hardened in a deep freezer at –20°C for 24 h before analysis.

2.2.4. Properties of coffee ice cream mixture: The pH value of ice cream mixtures was measured using a laboratory pH meter with a glass electrode (HANNA, instrument, Portugal). The surface tension (dyne) of the ice cream mixtures was measured according to the method of Arbuckle (1986). Briefly, a tube with a uniform bore was used, and the number of drops of the sample falling per measurement of time was compared with that of water. The surface tension of water is 72–73 dynes. A Brookfield digital viscometer (Model LVT; Brookfield Engineering Inc. Stoughton, MA) was used to determine the structure viscosity (mPa.s) of all ice cream mixtures. Readings were taken at speeds of 3 – 60 rpm using a spindle-00 at 5±1°C for an upward curve according to Naeem et al. (2019).

2.2.5. Properties of coffee ice cream

2.2.5.1. Fat destabilization: The fat destabilization of ice cream was determined according to Adapa et al. (2000) by dilution of the mixture or frozen ice cream with distilled water (1:500) and measurement of turbidity (absorbance) in a spectrophotometer (Shimadzu spectrophotometer, UV–Vis. 1201, Japan) at 540 nm. The following formula was used to calculate the fat destabilization percentage:

$$Fat\ destabilization\ (\%) = \frac{OD\ of\ mixture - OD\ of\ ice\ cream}{OD\ of\ mixture} \times 100$$

Where: OD, optical density (absorbance).

2.2.5.2. Melting rate: The melting rate of frozen ice cream was determined according to Muse and Hartel (2004). A plastic cup of ice cream (100 mL) was weighed and carefully cut into a block and placed on a wire mesh (6 holes/cm^2) above a glass funnel in a controlled temperature chamber at 27°C. Every 10 min, for up to 60 min, the dripped volume was recorded. The melting rate was calculated by graphing the dripping weight (g) against time and finding the slope of the linear portion of the curve.

2.2.5.3. Antiradical activities: The antiradical activities of coffee ice cream in ice cream filtrate were determined using the stable DPPH radicals (DPPH•) and stable ABTS radicals (ABTS•) assays developed by Brand-Williams et al. (1995) and Re et al. (1999), respectively. Briefly, 25 g of ice cream sample was left at room temperature (25±2°C) until completely melted, then diluted with distilled water at a ratio of 1: 3 for the DPPH assay or 1: 9 for the ABTS assay, and centrifuged at 15,000 xg for 10 min .The supernatant was filtered using Whatman filter paper No. 1 after the top layer was removed. To 3.9 mL of DPPH working solution (25 mg DPPH/L methanol) or ABTS working solution (7 mM ABTS solution with 2.45 mM $K_2S_2O_8$), 100 mL of diluted filtrate was added. After incubation for 30 min in the dark at room temperature (25±2°C), the degree of decolorization was measured in a spectrophotometer (Shimadzu spectrophotometer, UV–Vis. 1201, Japan) at 517 nm for the DPPH• and at 734 nm for the ABTS• radical-scavenging activity assays. Control solutions, DPPH• and ABTS• solutions without ice cream diluted filtrate, were prepared in the same manner as the assay mixture. The following formula was used to determine both ABTS• and DPPH• scavenging activities:

$$Antiradical\ activity\ (\%) = \frac{A_0 - A_1}{A_0} \times 100$$

A_0 is the absorbance of the control (DPPH• or ABTS• solution), and A_1 is the absorbance of the sample.

2.2.5.4. Viable counts of probiotic bacteria: Probiotic bacteria, *B. breve* Bb-12 and *L. plantarum*, were enumerated selectively in ice cream mixtures after aging and in the resulting ice cream stored at -18°C for 90 days using the methods indicated in standard methods for analysis of dairy products at 1, 15, 30, 60, and 90 days (Wehr and Frank, 2004). Before being analyzed, samples were melted for no more than 15 min in a thermostatically controlled water bath at 40°C and thoroughly mixed. MRS-sorbitol agar was used for *L. plantarum,* and MRS agar (Oxoid) enriched with L-cysteine and lithium chloride was used for *B. breve Bb-12*, with anaerobic incubation at 37°C for 72 h. The results were represented as log colony forming units (cfu/g) of sample, and the survival percentage at the freezing storage period was calculated as flow:

$$Survival\ (\%) = \frac{Log_{10}\ cfu\ /\ g\ of\ ice\ cream\ at\ storage\ period}{Log_{10}\ cfu\ of\ ice\ cream\ at\ first\ day} \times 100$$

2.2.5.5. Pathogenic detection: Enumeration of Enterobacteriaceae was determined by Colony Count Technique. (ISO 21528-2). Enumeration of β-glucuronidase-positive *Escherichia coli* (ISO 4832). For detection of *Salmonella typhimurium*. (ISO 6579 -1). Enumeration of *Staphylococcus aureus* (ISO 6888-1). Bacterial pathogens during storage were in all treatments having shown negative results.

2.2.5.6. Sensory properties: A regular taste panel of 21 staff members from the National Research Centre, Dairy Science Department evaluated the frozen ice cream samples. Frozen ice cream was sensorially evaluated using the nine-point hedonic scale, ranging from "like extremely" (9 points) through "like or dislike" (5 points) to "dislike extremely" (1 point) according to the method of Fiol et al. (2017).

2.2.5.7. Statistical analysis: Statistical analysis was performed using the GLM procedure with SAS (2008) software. Analysis of variance (ANOVA, one way) and Duncan's multiple comparison procedure were used to compare the means. A probability of $P \leq 0.05$ was used to establish statistical significance. Data were expressed as means ±SE.

3. Results And Discussion

3.1. HPLC analysis of CCE

Table 1 shows the quantities of 19 phenolic components determined by HPLC analysis in concentrated coffee extract. Chlorogenic acid (CGA) was the main phenolic component in CCE, with a concentration of 7.391 mg/mL. According to Yahayu et al. (2020), CGA is the major polyphenol in both green and roasted coffee. Total CGA content in CCE (1.699% DM) was within the typical ranges reported by Nogueira and

Trugo (2003) in nine Brazilian instant coffee samples (0.6–5.9% DM). Pyro-catechol, naringenin, gallic acid, and catechin were all found in considerable amounts in CCE, with 1.865, 1.544, 1.448, and 1.394 mg/mL, respectively. Similar findings were made in both green and roasted coffee (Erskine et al. 2022). Syringic, ferulic, and ellagic acids, as well as Daidzein, were found in low amounts while coffeic acid and coumaric acid, rutin and methyl gallate, were identified in trace amounts in CCE. In contrast, there were no detectable amounts of cinnamic acid, vanillin, querectin, apigenin, kaempfero, or hesperetin. The concentrations of caffeic and vanillic acids in CCE were similar to those found by Trandafir et al. (2013), who found that the contents of coffee and vanillic acids for certain commercial coffees available in Romania ranged between 0.075-0.140 and 0.0-20 mg/g, respectively. Additionally, vitamins are essential micronutrients that an organism requires in small amounts for optimum metabolism. In the CCE, the B-complex vitamins B_1, B_2, B_6, B_9, and B_{12} are all present in considerable amounts (Table 1), especially B_9 (4.67 mg/mL) followed by B_1 (2.13 mg/mL) According to the National Institutes of Health, each 1 mL of CCE contains 1.77, 0.84, 0.39, 11.7, and 20.0 times the recommended daily intake (RDI) for vitamins B_1, B_2, B_6, B_9, and B_{12} (Wolfenden, 2019).

Table 1. HPLC analysis of phenolic compounds and B-complex vitamins in concentrated coffee extract.

Items	(mg/mL)
Phenolic compounds	
Gallic acid	1.448
Chlorogenic acid	7.391
Catechin	1.394
Methyl gallate	0.009
Coffeic acid	0.053
Syringic acid	0.682
Pyro catechol	1.865
Rutin	0.030
Ellagic acid	0.415
Coumaric acid	0.008
Vanillin	ND
Ferulic acid	0.683
Naringenin	1.544
Daidzein	0.276
Querectin	ND
Cinnamic acid	ND
Apigenin	ND
Kaempferol	ND
Hesperetin	ND
Water soluble vitamins (B-complex)	
Thiamin (B1)	2.130
Riboflavin (B2)	1.090
pyridoxine (B6)	0.510
Folic acid (B9)	4.670
Cyanocobalamin (B12)	0.480

ND, non-detected

3.2. Antimicrobial activities of CCE

The antimicrobial activity of CCE was evaluated against four gram-negative (*E. coli*, *S. typhimurium, P. aeruginosa* and *Y. enterocolitica*), two gram-positive (*Staph. aureus*, and *L. monocytogenes*) bacteria, and three fungi as shown in Table 2. The results, known as the zone of inhibition, demonstrated that CCE inhibited all of the microorganisms tested, with varying degrees of efficacy against each. The CCE had higher action against fungi, with an inhibition zone ranging from 12 to 20 mm, than bacteria, which had an inhibition zone ranging from 6.8 to 18 mm. Both *A. niger* and *A. terreus* were more sensitive to CCE than *C. albicans* in fungus, whereas *Staph. aureus* was the most sensitive to CCE in bacteria, followed by *E. coli* and *P. aeruginosa*. *S. typhimurium,* on the other hand, was more resistant to CCE. Similarly, the Arabica coffee extract exhibited a significant bacteriostatic effect against *Staph. aureus* and *Staph. epidermidis* at short exposure times and became bactericidal after prolonged exposure (Runti et al., 2015). According to several studies (Roginsky et al., 2005; Lou et al., 2011; Duangjaia et al., 2016), antibacterial activity is attributed to phenolic acids, malic acid, tannin, caffeine, and hydroxycinnamic acid (especially the hydroxyl groups in chlorogenic acid).

Table 2. Antimicrobial activities of concentrated coffee extract measured as the inhibition zone (mm)

Antimicrobial activity	(mm)
Gram-positive bacteria	
Staphylococcus aureus	18.3
Listeria monocytogenes	10.0
Gram-negative bacteria	
Escherichia coli	15.0
Pseudomonas aeruginosa	14.7
Yersinia enterocolitica	10.0
Salmonella typhimurium	6.50
Fungi	
Aspergillus terreus	20.0
Aspergillus niger	20.0
Candida albicans	12.0

3.3. Physical of coffee ice cream properties

3.3.1. pH and surface tension of coffee ice cream

Some physical properties, pH and surface tension, of probiotic and non-probiotic ice cream formulated with concentrated coffee extract (CCE) are presented in Table 3. Adding CCE significantly decreased the pH of the ice cream mixture ($p < 0.01$), which was proportional to the concentration added. The pH dropped from 6.60±0.042 in the control mixture (C) to 6.43±0.035 and 6.25±0.078 in the mixtures containing 3% (T1) and 5% (T2) CCE, respectively. El-Hadad et al. (2020) reported a similar result in ice cream containing wheat germ oil extract. Several phenolic chemicals, such as hydroxylbenzoic acid derivatives and hydroxycinnamic acid derivatives, cause a pH reduction due to their interaction with milk proteins (Colak et al., 2016; Naeem et al., 2019) and their acidic character (Sagdic et al., 2012). A similar, but less pronounced, the surface tension (dyne) decreased slightly ($P > 0.05$) when the same concentration of CCE was added. The small particle size (≤ 0.2 µm) could explain why CCE has such a minor effect on the surface tension of mixtures. According to Gmoser et al. (2016), the surface tension of the unfiltered sample (45 mN/m) was the lowest and increased as the filtration size cut-offs were reduced. During filtering, large particles and slowly diffusing soluble molecular species are removed, leaving only the less surface-active species. When probiotic bacteria such as *B. breve* Bb-12 and *L. plantarum* were added, the pH in probiotic ice cream mixtures (PC, PT1, and PT2) declined slightly ($P > 0.05$) compared to non-probiotic ice cream mixtures, but the surface tension values it remained approximately constant.

Table 3. Some physical properties of probiotic and non-probiotic ice cream formulated with concentrated coffee extract.

Ice cream mixtures	Some physical properties of mixtures	
	pH	Surface tension (dyne)
Non-probiotic ice cream formulated with CCE		
C	6.60^{a} ±0.042	50.80^{a} ±0.89
T1	6.43^{bc}±0.035	50.45^{a} ±0.33
T2	6.25^{d} ±0.078	49.37^{a} ±0.54
Probiotic ice cream formulated with CCE		
PC	6.55^{ab}±0.007	50.79^{a} ±0.33
PT1	6.36^{cd}±0.021	49.91^{a} ±0.32
PT2	6.21^{c} ±0.056	49.67^{a} ±0.32

Means (±SE) with the same letters in the same column are not significantly different at $p \leq 0.05$; C, ice cream mixture with 0.0% CCE, T1, ice cream mixture with 3% CCE; T2, ice cream mixture with 5% CCE; PC, probiotic ice cream mixture with 0.0% CCE, PT1, probiotic ice cream mixture with 3% CCE; PT2, probiotic ice cream mixture with 5% CCE.

3.3.2. Structure viscosity

In General, the viscosity of all of the ice cream mixtures decreased as the rotating velocity of the viscometer increased, indicating pseudoplastic behavior (Fig 1). At rpms ranging from 4 to 50, the viscosity of the C mixture was lowered from 25.5 to 17.9 mPa.s, while the viscosity of the T1 and T2 mixtures was reduced from 25.5 to 16.6 mPa.s and from 23.0 to 14.0 mPa.s, respectively. Such results were observed in ice cream mixtures containing tiger nut aqueous extract (El-Shenawy et al., 2016) and Nescafe (Khider et al., 2021). Despite the fact that polysaccharides make up about half of the weight of green and roasted coffee, the viscosity of the ice cream mixtures decreased as the concentration of CCF was increased, the decrease being significant only when CCE was added at 5%. This could be due to the roasting process causing the polysaccharides to lose their functional characteristics (water binding). However, the viscosity of the mixture with 3% CCF (T1) was comparable to that of the C mixture ($p > 0.05$). The inclusion of probiotic bacteria enhanced the viscosity of all of the ice cream mixtures somewhat, which is most likely due to the probiotics' ability to produce exopolysaccharides (EPS). Soni et al. (2020) reported that EPS may interact with milk protein to improve viscosity and rheological quality. *Lactobacillus, Lactococcus, Bifidobacterium, Leuconostoc, Pediococcus, Streptococcus, Enterococcus,* and *Weissella* sp. are the most common EPS producing LAB (Angelin and Kavitha, 2020). Microbial EPS have a wide range of functions in food processing, including viscosifiers, bio-thickeners, emulsifiers, stabilizers, and other compounds that are affected by temperature, pH, and ionic strength (Abid et al., 2018).

3.3.3. Overrun

Overrun of probiotic and non-probiotic coffee ice cream are presented in Table 4. According to Hashim and Al Shamsi (2016), a higher overrun improves the softness and creaminess of ice cream. The overrun of ice cream with 3% CCE (T1) was higher than that of C and T2; the difference was only significant between T1 and C ($p < 0.05$). When all ice cream samples were compared, probiotic ice cream with 3% CCE (PT1) had the highest overrun percentage (48.51±1.54%), followed by T1. This suggests that adding CCE to ice cream enhances its overrun and that this impact is magnified when probiotic bacteria are present. Muhardina et al. (2019) reported a similar finding in probiotic ice cream substituted by encapsulated LAB. The bacterial cells and/or small particles of CCE along the fat globules may be causing the increase in overrun by trapping air in the ice cream mixture and increasing the volume. Gmoser et al. (2016) reported that foam ability and stability are improved by filtration with the smallest cut-off (0.2 µm). Surface active components in instant coffee solutions, such as galactomannan and arabinogalactan protein, compete with other surface active species at the air-water interface, which has a strong stabilizing influence on foam stability.

Table 4. Physical properties of probiotic and non-probiotic ice cream formulated with concentrated coffee extract.

Ice cream treatments	Some physical properties of ice cream		
	Overrun (%)	Fat destabilization (%)	Melting rate (g/min)
Non-probiotic ice cream formulated with CCE			
C	33.89^{c} ±3.06	11.72^{b} ±1.03	0.97^{c} ±0.030
T1	41.76^{ab}±1.82	15.14^{ab}±0.54	1.19^{a} ±0.027
T2	37.90^{bc} ±3.51	13.32^{b} ±1.34	1.11^{ab}±0.066
Probiotic ice cream formulated with CCE			
PC	34.73^{bc} ±3.75	14.39^{ab}±1.42	1.05^{b}±0.042
PT1	48.51^{a} ±1.54	18.62^{a} ±1.25	0.99^{bc}±0.035
PT2	39.41^{bc}±2.50	17.57^{a} ±1.32	1.20^{a} ±0.021

Means (±SE) with the same letters in the same column are not significantly different at $p \leq 0.05$; C, ice cream with 0.0% CCE, T1, ice cream with 3% CCE; T2, ice cream with 5% CCE; PC, probiotic ice cream with 0.0% CCE, PT1, probiotic ice cream with 3% CCE; PT2, probiotic ice cream with 5% CCE.

3.3.4. Fat destabilization

As shown in Table 4, fat destabilization increased as overrun increased; a significant correlation was found ($r = 0.81$). The fat destabilization of PT1 was much higher than that of all ice cream samples ($p < 0.05$). The C sample, on the other hand, which had a lower overrun, exhibited less fat destabilization. The increasing overrun causing increased fat destabilization has been reported previously (Warren & Hartel, 2018; Wu et al., 2019). The high overrun ice cream tiny lamellae between the air cells may increase the chance of fat globule collision and adsorption to the air cells' surface, promoting partial coalescence (Wu et al., 2019). Furthermore, the probiotic ice cream showed somewhat higher fat destabilization than the non-probiotic ice cream, possibly because to the slightly higher viscosity of their mixtures. When the viscosity of the ice cream mixture increased, the shear stress increased as well during freezing, promoting shear interactions among fat globules (Warren & Hartel, 2018).

3.3.5. Melting rate

The melting rates of the PT1 sample, which has the highest overrun and fat destabilization, and the C sample, which has the least overrun and fat destabilization, were nearly comparable (Table 4). Melting rates were also higher in T1 and PT2 samples than in C and PC samples, with the differences being significant only when compared to the C sample ($p < 0.05$). These findings contradict previous findings (Bajad et al., 2016; Wu et al., 2019), which indicated that the melting rate of ice cream decreases with

increasing overrun and fat destabilization. The larger volumes of air present in high overrun products resulted in a slower rate of heat transmission, resulting in slower melting rates (Muse and Hartel, 2004; Bajad et al., 2016). The same pattern did not appear in CCE-containing ice creams with high overrun. This suggests that fat destabilization and overrun had no effect on the melting rates of CCE-containing ice creams. However, El-Hadad et al. (2020) found similar results when wheat germ oil extract was used in ice cream. As a result, adding the CCE to ice cream could change heat transfer and/or physical properties such as fat crystallization, nucleation, crystal growth, and polymorphism (Talbot et al., 2002). For example, at 0 and 10 °C, solid fat contents dropped from 58.2 to 48.7% and 46.5 to 43.8%, respectively, when 20% palm oil was added to milk fat (Abd El-Aziz et al., 2013).

3.4. Antiradical activities

The DPPH• and ABTS• radical-scavenging activity assays are widely used to evaluate the antioxidant activities of natural products. When compared to C sample, CCE-containing ice creams had higher antioxidant activity ($P < 0.01$) against the DPPH• and ABTS• radicals (Table 5). The DPPH• and ABTS• radical-scavenging activity of coffee ice creams increased as CCE concentrations increased. The DPPH• radical-scavenging activity of the T1 and T2 samples increased from 1.75±0.13 for the C sample to 37.91±1.16 and 59.62±1.46 ($P < 0.01$), respectively. The increase in antioxidant components in CCE, such as phenolic acids, including chlorogenic, gallic, ferulic, syringic, and ellagic acids, and flavonoid compounds, primarily catechin, pyro-catechol, and naringenin, could explain the high antioxidant activity of CCE-containing ice creams (Table 1). A similar effect has been observed by other studies as well (Sultana and Anwar, 2008; Geremu et al., 2016). A strong correlation ($r^2 = 0.85$) was found between phenolic content of coffee extracts and their antioxidant (Trandafir et al., 2013). Phenolic compounds can act as hydrogen donors to the lipid-free radicals formed during lipid oxidation or act as chelators of metal ions, decompose hydroperoxide into non-radical species, deactivate singlet oxygen, absorb ultraviolet radiation, or act as oxygen scavengers to slow down the rate of oxidation reactions through a variety of mechanisms (Reische et al., 2002). The Maillard reaction products, particularly melanoidins, formed during the thermal treatment can also be attributed to the higher oxygen scavenging properties of roasted coffee extract (Komes and Bui, 2014). The antioxidant results of ABTS• were higher than the results of DPPH• free radicals. Similarly, the ABTS assay showed higher antioxidant capacity than the DPPH assay in fruits, vegetables, and beverages (Dudonne et al., 2009; Floegel et al., 2011). The DPPH• is a lipophilic radical with restricted access to the hydrophilic components of brewed coffee, whereas the ABTS• assay can access both lipophilic and hydrophilic antioxidant compounds. Furthermore, unlike the DPPH assay, the ABTS can be used at a wide range of pH values (Yahayu et al., 2020).

Table 5. Antiradical activities of probiotic and non-probiotic ice cream formulated with concentrated coffee extract.

Ice cream treatments	Antiradical activities of ice cream	
	DPPH• scavenging activity (%)	ABTS• scavenging activity (%)
Non-probiotic ice cream formulated with CCE		
C	1.75^{c} ±0.13	9.56^{c} ±0.65
T1	37.91^{b}±1.16	49.92^{b}±1.32
T2	59.62^{a}±1.46	70.62^{a}±2.66
Probiotic ice cream formulated with CCE		
PC	1.87^{c} ±0.30	11.07^{c}±0.66
PT1	37.91^{b}±1.15	51.25^{b}±3.62
PT2	58.62^{a}±1.59	73.59^{a}±1.07

Means (±SE) with the same letters in the same column are not significantly different at $p \leq 0.05$; C, ice cream with 0.0% CCE, T1, ice cream with 3% CCE; T2, ice cream with 5% CCE; PC, probiotic ice cream with 0.0% CCE, PT1, probiotic ice cream with 3% CCE; PT2, probiotic ice cream with 5% CCE.

3.5. Survival of probiotic bacteria

In Table 6 shows the survival percentages of probiotic bacteria in ice cream formulated with CCE during 90 days of storage at −20°C. After aging for one day at 5±1°C, the counts of *L. plantarum* and *B. breve* in PT1 and PT2 were slightly higher ($P > 0.05$) than those of PC. The survival percentages of probiotic bacteria decreased slightly in all ice cream samples after freezing and hardening at −20 °C. Some bacterial cells may have died as a result of the mechanical stress of mixing and the incorporation of oxygen into the mixtures during the freezing process (Hagen and Narvhus, 1999). During storage at −20°C, both *L. plantarum* and *B. breve* survival percentages declined progressively, with the same rate in all ice cream samples; the decrease was significant only after 30 days ($P < 0.05$). This implies that adding CCE to ice cream has no effect on probiotic bacteria survival during storage when compared to control ice cream. The decrease in the viable count of probiotic bacteria in ice cream samples was also observed by Akın (2005) and Salem et al. (2005).

Table 6. The survival (%) of *L. plantarum* and *B. breve* in probiotic ice cream formulated with concentrated coffee extract during 90 days of storage at −20°C.

	Log_{10} cfu/g after aging	Storage periods (days)				
		1	15	30	60	90
L. plantarum						
PC	9.74^{a}±0.12	98.1^{Aa}±0.96	93.6^{ABa}±2.89	90.0^{Ba}±0.80	82.4^{Ca}±2.05	76.3^{Da}±1.80
PT1	9.97^{a}±0.11	96.7^{Aa}±0.46	93.8^{ABa}±1.37	89.1^{BCa}±1.35	85.3^{Ca}±1.29	77.6^{Da}±1.92
PT2	9.89^{a}±0.13	97.9^{Aa}±1.13	94.1^{ABa}±1.32	90.2^{BCa}±1.06	88.0^{Ca}±1.63	80.1^{Da}±1.71
B. breve						
PC	10.28^{a}±0.16	97.5^{Aa}±1.11	95.5^{Aa}±1.07	87.8^{Ba}±1.26	83.6^{Ba}±1.61	76.4^{Ca}±1.80
PT1	10.55^{a}±0.19	98.4^{Aa}±0.37	96.8^{Aa}±0.70	90.2^{Ba}±0.79	83.1^{Ca}±01.27	75.5^{Da}±1.72
PT2	10.69^{a}±0.15	98.4^{Aa}±0.82	95.0^{Aa}±0.62	90.3^{Ba}±1.66	82.7^{Ca}±1.56	$75.^{8Da}$±1.66

Means (±SE) with the same letters in the same column are not significantly different at $p \leq 0.05$; C, ice cream with 0.0% CCE, T1, ice cream with 3% CCE; T2, ice cream with 5% CCE; PC, probiotic ice cream with 0.0% CCE, PT1, probiotic ice cream with 3% CCE; PT2, probiotic ice cream with 5% CCE.

3.6. Sensory evaluation

Sensory attributes such as color, body & texture, flavor, and melting quality were evaluated to determine the best percentage of CCE addition to probiotic and non-probiotic ice cream (Fig. 2). Both probiotic and non-probiotic ice cream samples formulated with 3% CCE had a light brown color, the desired coffee flavor, and a smooth texture as compared to those made with 5% CCE. Ice cream samples with a high CCE content, on the other hand, were darker and had an unpleasant bitter flavor for the most of the judges. The bitter flavour could be attributable to an increase in caffeine with a larger percentage of addition, as well as an increase in quinic acid created by the decomposition of chlorogenic acids during the roasting process, which heightens the bitterness sensation (Perfect Daily Grind, 2017).The increase in the degree of darkening is due to the caramelization of sugar and the increase in the proportion of melanoidins produced during the roasting process (Ally Open, 2022). Adding probiotic bacteria had no significant effect on the sensory quality of the ice cream samples when compared to non-probiotic ice creams ($P > 0.05$). The judging scores were very similar between the T1 and PT1 samples.

4. Conclusion

It can be concluded that CCE can blend well with a variety of other food ingredients, giving a unique and desirable flavor as well as health advantages. CCE improved the majority of the ice cream's properties, especially overrun and viscosity, as well as antioxidant activity, while having no effect on probiotic bacteria survival during storage. In addition to their health benefits, probiotic bacteria can be used to

improve the viscosity of the mixture and increase the overrun of the resulting ice cream. Overall, the findings indicated that 3% CCE with probiotic bacteria *L. plantarum* and *B. breve* can be used successfully in the ice cream industry. Coffee ice cream is either a form of taking ice cream with the desired coffee flavor and rich in phenolic compounds and vitamins, or a form of consuming coffee in an iced state, with a sweeter taste and less sour than hot coffee.

Declarations

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Authors' contributions

Ahmed B. Shazly, Mohamed T. Fouad and Mostafa Elaaser performed the experiment conceived and improved the theory and carried out the computations. Rehab S. Sayed2 and Mahmoud Abd El-Aziz conceptualization, methodology, formal analysis, writing—review and editing, visualization, supervision. All authors contributed critical feedback and prepared shape the research, analysis, and manuscript

Data Availability The datasets generated or analyzed during the current study are available in this manuscript

Conflicts of interests the authors declare no competing interests.

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Figures

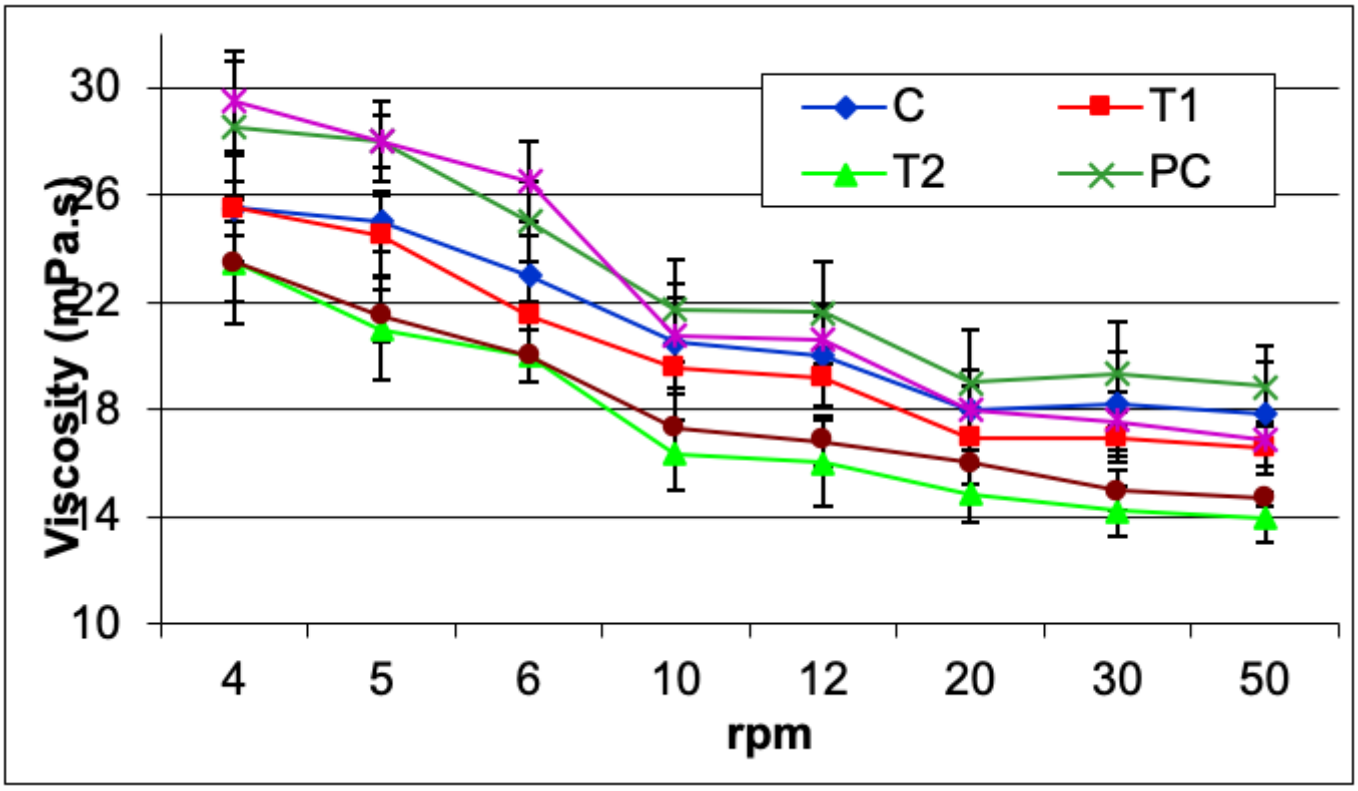

Figure 1

Structure viscosity of probiotic and non-probiotic ice cream formulated with concentrated coffee extract

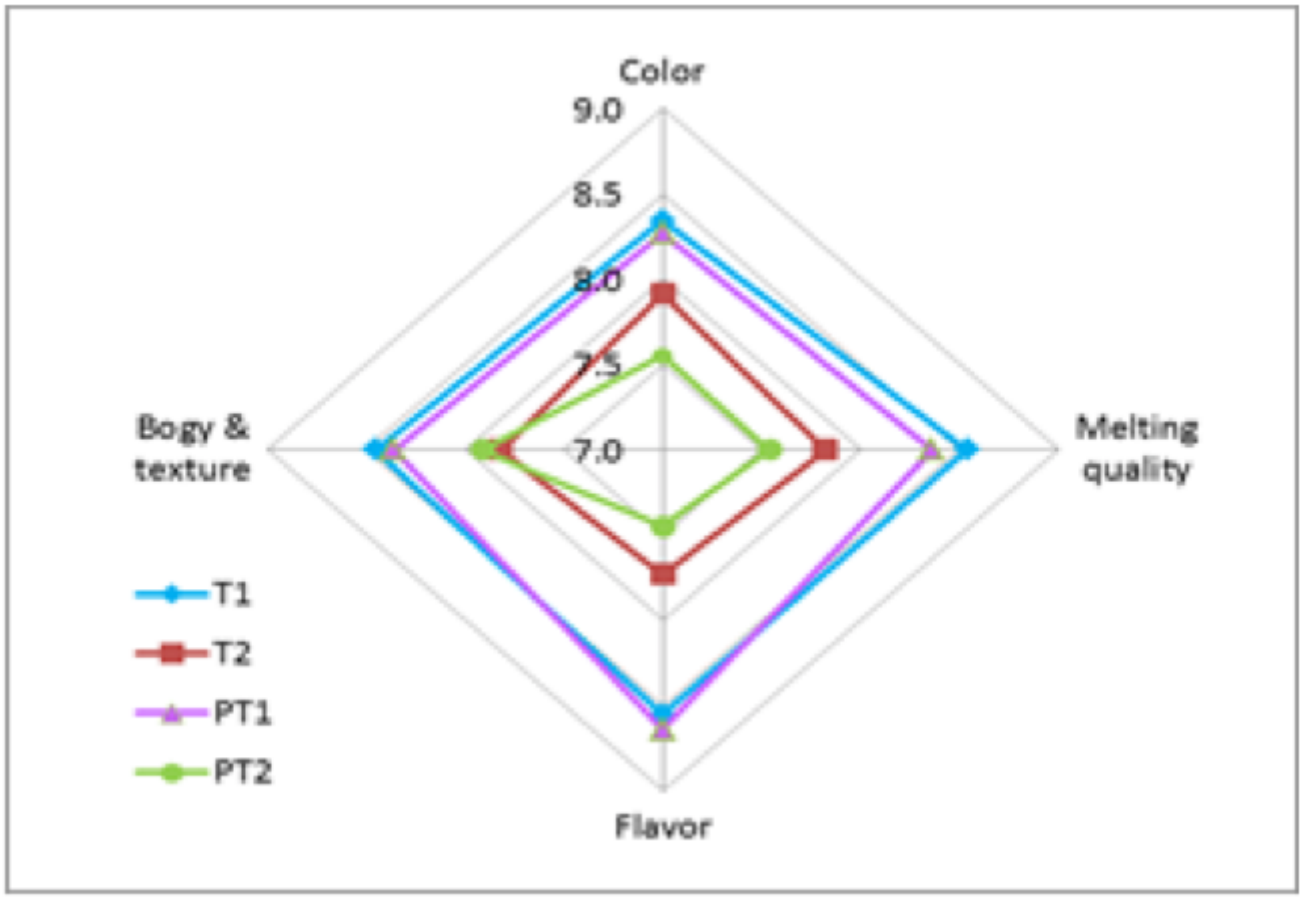

Figure 2

Sensory attributes of probiotic and non-probiotic ice cream formulated with concentrated coffee extract