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Effects of High-Pressure Processing on Rheological and Physical Properties of Artisanal Ice Cream Mixes

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ABSTRACT

High-pressure processing (HPP) is a nonthermal technology increasingly applied to food systems as a mild alternative to conventional heat treatments. The present study investigated the effects of HPP (600 MPa for 300 s) on the rheological, physical and thermal properties of three liquid gelato mixtures differing in composition: two water-based (lemon and mango) and one milk-based (pistachio) formulations. Rheological characterization was performed by oscillatory amplitude tests and temperature sweep analyses to evaluate viscoelastic behaviour and structural stability of the mixes. Differential scanning calorimetry (DSC) was used to assess thermal transitions related to ice crystallization and melting behaviour. The physical properties of the resulting ice creams were evaluated in terms of overrun, texture parameters and melting resistance. HPP treatment did not induce significant modifications in the rheological or physical properties of the water-based formulations. In contrast, the pistachio mixture exhibited marked changes in structural properties, suggesting pressure-induced rearrangements of dairy proteins and fat globules. These structural modifications were reflected in the final product, which showed enhanced melting stability (reduction of weight loss of 88.49% on average after 30 min at 20°C) and improved mechanical resistance (higher hardness values and crossover of moduli G′ and G″ shifted to higher percentage of strain for HPP-treated pistachio samples). This study provides insight into the compositional factors governing pressure-induced modifications in ice cream systems and their technological implications. The findings highlight the potential of high-pressure processing applied on ice cream production process, in order to optimize artisanal gelato production by extending shelf life of liquid mixtures while enabling improved safety and quality retention.

1 | Introduction

Ice cream is one of the most commonly consumed dairy products [1–3]. It is well described as a complex, multiphase colloidal food system, in which air bubbles, ice crystals and fat droplets coexist in a continuous phase made by sugar solution [4–6].

Artisanal gelato is slightly different compared to the worldwide known ice cream: gelato is produced daily with fresh ingredients, typically with natural flavourings (present in fresh fruit) instead of synthetic flavourings. It contains less fat and total solids, although this is offset by a higher sugar content. Glucose, dextrose and invert sugar are used to lower and control the freezing point. Typically, the process involves the use of a batch freezer and maturation times that allow the gelato to reach the right consistency. All this results in a lower overrun, which gives the ice cream its distinctive heavier density and body [7].

Different categories of artisanal gelato are based on the formulation of the mixture [8]: milk- or cream-based are referred to as ice creams in which fats act in the final texture and structure, whereas proteins contribute to emulsion and water-holding capacity; sorbets and granitas are water/fruit puree-based frozen desserts, in which a lower overrun is due to the absence of proteins that generally play the main role on air cell stability in ice cream [7].

The production process for artisan ice cream is discontinuous [9], consisting of steps and ripening intervals that limit artisanal productivity compared to industrial production. For this reason, it is important to support artisan ice cream manufacturers in optimising production by extending the shelf life of liquid gelato mixtures. Traditional pasteurisation methods are currently used in the traditional production process of liquid ice cream base mixes, typically on 'raw' bases without the addition of flavouring ingredients, which could be damaged by heat treatment.

It is therefore important to find possible alternative solutions to reduce the microbial load without compromising the nutritional and sensory characteristics after adding all the raw material used in the production of ice cream mixes.

High-pressure processing (HPP), also known as high hydrostatic pressure processing, is an increasingly adopted nonthermal food preservation technique that inactivates vegetative microorganisms [10]. Compared with conventional thermal pasteurisation, which relies on elevated temperatures to achieve microbial lethality, HPP achieves significant reductions in pathogenic and spoilage bacteria without exposing foods to high heat, thus minimising detrimental effects on heat-sensitive nutrients, flavours, and other quality attributes [11, 12]. HPP inactivates microorganisms by breaking down noncovalent bonds and irreversibly damaging microbial cell membranes [13–15].

Packaged foods (solid or liquid) are placed inside a hyperbaric chamber, which is then filled with a pressure-transmitting medium, typically water (or water-based fluid) [16, 17]. Once immersed, isostatic pressure is applied, transmitted uniformly throughout the entire food mass [18]. Typical pressures used are in the range of 100–600 MPa; many commercial applications operate around 400–600 MPa, often at low or moderate temperatures in order to avoid heat-induced damage.

In this study, three different liquid mixtures of artisanal ice cream were chosen: lemon, mango (water- or fruit-based) and pistachio (milk-based) flavours. Those liquid mixtures were treated with HPP and stored at fridge temperature for 2 weeks of storage. After a predefined period of time, the mixtures were whipped and analysed by physicochemical perspective in order to evaluate the eventual consequences of the treatment on the structural stability of the products immediately after treatment and during storage. The objective of this research was to apply HPP treatment to pasteurise liquid ice cream mixtures and extend their shelf life, in order to meet the operational needs of manufacturing companies and to develop strategies aimed at optimising productivity and enhancing management flexibility the production process of ice creams.

2 | Materials and Methods

2.1 | Raw Materials

Mango mixture followed the same formulation adopted in a previous study in which mango mixtures were evaluated by microbiological and sensory perspective [19]. Glenn variety mango fruits were purchased from the agricultural company Il Filo Tropicale (Balestrate, Italy), whereas Primofiore variety lemons were purchased from Cooperativa Agricola La Torre (Palermo, Italy). Pistachio paste and the stabilisers used were sold by Fugar Produzione S.p.a. (Verrucchio, Italy). The gelato mixtures were formulated with the compositions reported in Table 1.

TABLE 1 | Formulations of the mixtures of the three different ice creams: mango, lemon and pistachio.

Mango		Lemon		Pistachio	
Ingredient	**Percentage (%)**	**Ingredient**	**Percentage (%)**	**Ingredient**	**Percentage (%)**
Mango puree	65.0	Lemon juice	27.5	Whole milk (3.5% fat)	65.5
Sucrose	12.6	Water	41.7	Sucrose	12.0
Water	11.7	Sucrose	25.8	Pistachio paste	9.0
Dextrose	4.2	Dextrose	3.5	Skimmed powdered milk	4.0
Inulin	4.2	Glucose syrup (38DE)	1.0	Cream (35% fat)	4.0
Glucose syrup (38DE)	2.4	Stabiliser (carob and guar seed flour)	0.5	Dextrose	3.0
Stabiliser (carob seed flour)	0.1			Maltodextrins	2.0
Stabiliser (guar seed)	0.07			Stabiliser (carob and guar seed flour)	0.5

2.2 | Gelato Preparation and HPP Treatment

The formulation of the ice cream base and the subsequent preparation process were carried out at the Cappadonia Gelati S.r.l. factory, located in Cerda (Palermo, Italy). The raw materials were first blended using a Tafec Turbo mixer (Tafec Gelato GmbH, Norderstedt, Germany) and subsequently processed through a Carpigiani Pasteurizer 60HE (Carpigiani Group—Ali S.r.l. Anzola dell'Emilia, Italy) at 85°C for 5s. Pasteurisation is generally applied to the ice cream mix before flavourings are added to prevent them from being adversely affected because of heat. To extend the shelf life of liquid mixes, it is therefore necessary to protect them from spoilage after the new ingredients have been added. The gelato bases were then portioned into vacuum packaging bags (dimensions 400×500mm, ByVac S.r.l., Ghivizzano, Italy), with atmospheric gases eliminated using an Orved Cuisson 41 vacuum apparatus (Orved Spa, Musile di Piave, Italy) to achieve up to 99% vacuum level, after which the bags were hermetically sealed and maintained at 4°C–6°C temperature. The experiment was conducted on samples liquid mixes seven times: each ice cream (mango, lemon and pistachio) was divided in 14 vacuum bags, seven of which subjected to HPP and the other seven without HPP processing used as untreated control, and then all the bags were divided into three storage periods, according to their predefined whipping time (after 0, 7 and 15 days). Coding of gelato samples are reported in Table 2, whereas Figure 1 shows flow chart of gelato preparation and research activities.

The HPP was conducted using an AV-50X HPP apparatus (Avure Technologies, Ohio, United States). The hermetically sealed vacuum packages were placed within the hyperbaric vessel equipped with dual lateral compression pistons and filled with thermostatically regulated water maintained at 4°C. The samples underwent pressure application with gradual increase (rate of 40bar/sec) reaching 6000 bars (600MPa) maintained for 300s prior to the decompression phase. Following the HPP treatment, the vacuum packages were maintained at 4°C–6°C for varying storage durations (0, 7 and 15 days) until the whipping step. To obtain the final gelato structure, a Tafec Geltronic batch freezer was used (Tafec Gelato GmbH, Norderstedt, Germany). Upon completion, the final processed product was preserved in polystyrene foam containers at −20°C until analytical procedures were conducted. The microbiological and sensory analyses of these same samples are reported in another article that has not yet been published.

2.3 | Texture Analysis

Firmness of whipped gelato was evaluated according to [20] with slight modification. Ice creams were stored at −20°C after whipping until analysis. A Stable Micro System texture analyser (TA-XT2 Stable Micro Systems Co. Ltd., Surrey, UK) equipped with a stainless steel 0.5cm of diameter cylindrical probe was used to penetrate the ice cream up to the 50% of the volume depth, with a penetration rate of 0.1cm/s. Exponent Lite software was used to calculate hardness values expressed in N. Analyses were conducted in triplicate for each sample for each time of analysis (0, 7 and 15 days of storage before whipping).

2.4 | Melting Rate

Ice cream was shaped into 5cm diameter and 2cm high cylinders and left hardening at −20°C for 24h. Then, ice cream cylinders were placed upon a mesh in which each hole was 0.5 cm^2 and let them drop inside a plastic petri dish in a room at controlled temperature of 20°C. Weight of dropped ice cream was measured every 15min for 1h, according to the modified method of [4]. Analyses were conducted in triplicate for each sample for each time of analysis (0, 7 and 15 days of storage before whipping).

2.5 | Overrun

To measure the overrun, both final ice cream and liquid mixture were weighed in a plastic container used as reference fixed volume. Over run was calculated according to the following equation:

$$OR\% = \frac{W_M - W_{IC}}{W_{IC}}\, x\, 100$$

In which W_M is the weight of the liquid mixture, W_{IC} is the weight of the final ice cream, according to the method of [21]. Values are expressed as percentage and analyses were conducted in duplicate at each time previously defined (0, 7 and 15 days of storage before whipping).

2.6 | Rheological Measurement

Before rheology tests, ice cream samples were kept stored at −20°C until analyses were performed. The ice cream product was then carefully spread with a spatula—as gently as possible to avoid altering its structure—into a mold made of Teflon material, 2mm thick and 30mm of diameter, and cored with a hollow steel cylinder to produce 20-mm-diameter discs. The resulting coin-shaped discs were flash-frozen to −80°C and kept at that temperature until analysis. Viscoelastic behaviour

TABLE 2 | Coding of samples based on flavour (L for lemon, P for Pistachio and M for Mango), on treatment (HP or C) and storage time (0, 7 and 15 days).

	Lemon		Pistachio		Mango	
	Treated with HP	**Untreated**	**Treated with HP**	**Untreated**	**Treated with HP**	**Untreated**
Time 0	L-HP-T0	L-C-T0	P-HP-T0	P-C-T0	M-HP-T0	M-C-T0
Time 7	L-HP-T7	L-C-T7	P-HP-T7	P-C-T7	M-HP-T7	M-C-T7
Time 15	L-HP-T15	L-C-T15	P-HP-T15	P-C-T15	M-HP-T15	M-C-T15

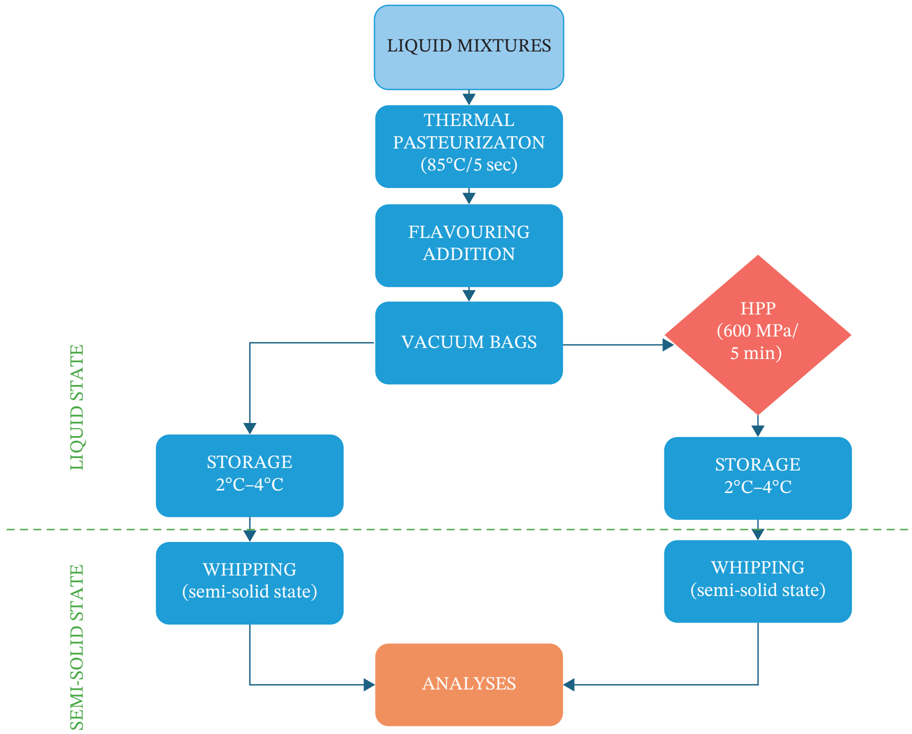

FIGURE 1 | Flowchart of gelato preparation and research activities conducted on this study.

was evaluated following oscillation amplitude and temperature ramp analysis using a Discovery Hybrid Rheometer (TA Instruments, New Castle, United States) equipped with plate–plate geometry and a chilling system to perform analysis at controlled temperature.

For both analyses, the parallel plates were conditioned at −20°C, and the ice cream samples, previously produced on a Teflon mold and maintained at −20°C, were detached and loaded onto the lower geometry. The gap was set to 2000 μm. To minimise water evaporation, an insulating thermal cover was placed around the geometry. The samples were equilibrated with an axial force of 1 N (±0.1 N).

For the oscillation amplitude analysis an increasing strain% was applied starting from $2 \cdot 10^{-4}$% to 10^3% with a constant angular frequency of 10 rad/s and at constant temperature of $-20^{\circ}C \pm 1$.Temperature ramp analysis was performed increasing temperature from −20°C to +15°C with a heating rate of 1°C/min, applying constant strain of $5 \cdot 10^{-4}$% and angular frequency of 10 rad/sec.

Analyses were performed at least in triplicate.

2.7 | Differential Scanning Calorimetry

Setaram DSC 131 EVO (Setaram, Laurel, New Jersey, United States) was used for calorimetry analysis according to [22] with modifications as following: ice cream was weighed in a range between 20 and 30 mg in aluminium 120 μL pan placed in container filled with liquid nitrogen to avoid melting during sampling. The filled pan was immediately placed in the DSC oven already prepared with nitrogen chiller at −50°C. Two separated thermal procedures were carried out in duplicate. The first temperature ramp was performed with the following steps: first isothermal equilibration at −50°C for 600 s, from −50°C to +20°C with increment of 2°C/min and another isotherm at +20°C for 300 s. The second temperature ramp was carried out immediately after the isotherm at +20°C, with a falling to −50°C with a rate of 20°C/min, then an isotherm at −50°C for 600 s to equilibrate the sample and again an increasing from −50°C to +20°C with a rate of 2°C/min. The different ramps were performed to identify differences in the calorimetric profiles between ice cream analysed starting from an unmelted state, in its original frozen form (sampling and analysis followed for the first ramp started at −50°C), compared to ice cream analysed starting in its melted state (procedure used for the second ramp started at +20°C). Calisto Thermal Analysis software from Setaram (Setaram, Laurel, New Jersey, United States) was used to integrate curves and export data. Glass transition temperature (Tg–°C) and enthalpy of meltdown (ΔH–J/g) were used as calorimetric parameters for comparison between ice creams treated with HPP and control samples.

2.8 | Statistical Analysis

For statistical analysis, Student's *t*-test was performed with R 4.1.3 (R Foundation for Statistical Computing) to evaluate comparison between treated ice cream sample (HP) and untreated control sample (C) at any stage of storage taken into consideration. A two-way ANOVA (treatment HP/C or storage times T0/T7/T15 as factors) was used to test the influence on enthalpy variation (ΔH) and glass transition temperature (Tg) evaluated in DSC analysis. Tukey HSD test was used to group samples without any statistically significant differences in the same letter groups.

3 | Results and Discussion

3.1 | Texture, Overrun and Melting Rate Analyses

Lemon and mango ice creams did not show significant differences in meltdown rate between treated HP and untreated control samples. In contrast, for pistachio ice creams, the HP samples show lower rate of melting compared to the C samples, displaying significant higher resistance to melt down [23]. The most significant difference is shown after 30 min of observation, when P-HP showed minimal melting compared to the P-C sample at all stages of assessment (T0, T7 and T15), highlighting the higher stability of the pistachio mixture treated with HPP. P-HP showed higher hardness than P-C in texture analysis, that could be related to the presence of proteins (caseins and whey proteins of milk) and fats (from milk and pistachio) that can be influenced, with a formation of protein network induced by HPP treatment [24]. Interestingly, for lemon and mango, the hardness is higher at T0 for C samples, but after 7 days this higher hardness of control samples significantly collapses, showing that the HP samples are more stable during storage. It emerges that for mango and pistachio ice creams the longer is the storage time, the higher is the melting rate, showing that the storage influences the capability of the system to retain the water. Although for lemon this trend is in reverse, probably due to the different composition that clearly affects conservative capacity of the ice creams.

For the same reasons, the overrun results show P-HP has lower air volume (T0:18.48%, T7:21.75%, T15:16.77%) compared to the P-C (T0:28.31%, T7: 26.09%, T15: 14.45%) for the first two times of analysis, due to observable changes in the consistency of the milk-based liquid mixture after treatment that inhibits the capability of the system to trap air bubbles during whipping [23]. Mango ice cream, at the contrary, shows a percentage of overrun higher for HP samples (T0:17.12%, T7:16.86%, T15:18.21%) compared to the controls C (T0:16.31%, T7:14.39%, T15:14.47%), suggesting that the pectin content contained in the mango fruit, that is able to affect the viscosity of mango puree [25] can be affected by HP treatment [26] leading to a better molecular interaction and a greater water binding capacity [13]. Another interesting result is that overrun values of lemon ice cream are significantly higher for L-HP (T0:25.02%, T7:23.82%, T15:31.65%) compared to the L-C sample (T0:18.35%, T7:20.34%, T15:20.10%) at all stages of observation (T0, T7 and T15). In the case of lemon mixture, it is not the chemical composition influencing the increasing in volume during whipping. The chemical environment represented by the lemon mixture is characterised by very acidic conditions, mainly due to the citric acid contained in lemon fruit. The low pH (2.2 ± 0.01 for L-HP and L-C), in combination with the treatment with high pressures, may have a possible influence on the hydrocolloids used as ice cream stabilisers, as reported in other study [27], enhancing the interaction among those hydrocolloidal polysaccharides and thus the foaming abilities of the mixture during whipping, even in absence of proteins or fats.

Hardness and Melting rate values are reported, respectively, in Tables 3 and 4.

3.2 | Viscoelastic Properties

Oscillation amplitude analyses were performed to define the linear viscoelastic region (LVR) and determine the elastic (G′) and viscous (G″) moduli as a function of applied strain%. Analyses of pistachios revealed that all samples, both P-HP and P-C, exhibited G′ values higher than G″, indicating gel-like behaviour. All samples exhibited both linear and nonLVRs as a function of oscillatory strain. LVR is related to sample stability as it accounts for the formation of physical entanglements between fat–protein aggregates [28]. The slight shift of the crossover point (where G′ and G″ reverse), shown in Table 5, towards higher % strain values observed in P-HP samples compared to P-C for all times (T0, T7, 715) suggests a greater resistance to deformation before structural failure [29].

The slight shift of the crossover point towards higher strain% values, observed in P-HP samples compared to P-C for all times (T0, T7, 715), suggests greater resistance to deformation before structural failure [30].

TABLE 3 | Hardness values (N) for ice cream samples expressed as mean and standard deviation. Significant difference has been measured between treated (HP) and untreated sample (C) of each ice cream.

Sample	Hardness (N)	Sign.	Sample	Hardness (N)	Sign.	Sample	Hardness (N)	Sign.
P-HP-T0	20.55 ± 0.53^a	**	L-HP-T0	5.04 ± 0.24^b	**	M-HP-T0	3.64 ± 0.08^b	***
P-C-T0	18.18 ± 0.25^b		L-C-T0	16.13 ± 4.07^a		M-C-T0	17.70 ± 1.30^a	
P-HP-T7	21.08 ± 0.58^a	***	L-HP-T7	7.90 ± 0.61^a	*	M-HP-T7	6.24 ± 0.28^a	***
P-C-T7	9.75 ± 0.27^b		L-C-T7	6.20 ± 0.58^b		M-C-T7	1.54 ± 0.07^b	
P-HP-T15	27.01 ± 5.94^a	Ns	L-HP-T15	7.57 ± 0.98^a	**	M-HP-T15	3.86 ± 0.03^a	***
P-C-T15	20.83 ± 1.76^a		L-C-T15	2.69 ± 0.71^b		M-C-T15	1.97 ± 0.04^b	

Note: Data are reported as mean plus and minus standard deviation. 'ns' = ANOVA $p > 0.05$. Regarding the asterisks as follow are reported the intervals of ANOVA p: 0.01 ≤ '*' < 0.05; 0.001 ≤ '**' < 0.01; 0 ≤ '***' < 0.001. Superscript different letters are related to significant difference (if present) between treated (HP) and control (C) samples on the same column.

TABLE 4 | Melting rate values recorded for ice cream samples every 15 min for 1 h of observation at room temperature (20°C) expressed in weight loss (g) as mean and standard deviation. Significant difference has been calculated between treated (HP) and untreated sample (C) of each ice cream.

	After 15 min	Sign	After 30 min	Sign	After 45 min	Sign	After 60 min	Sign
P-HP-T0	0.00 ± 0.00[b]	*	4.41 ± 3.38[b]	***	38.49 ± 4.50[b]	*	41.51 ± 5.66[a]	Ns
P-C-T0	5.14 ± 2.90[a]		37.72 ± 3.06[a]		49.84 ± 1.50[a]		49.63 ± 1.36[a]	
P-HP-T7	0.29 ± 0.28[a]	Ns	4.60 ± 3.78[b]	***	37.21 ± 13.18[a]	Ns	44.09 ± 5.05[b]	*
P-C-T7	4.87 ± 3.57[a]		41.08 ± 3.77[a]		55.87 ± 0.76[a]		55.87 ± 0.84[a]	
P-HP-T15	0.06 ± 0.10[b]	***	4.96 ± 6.06[b]	***	30.47 ± 3.55[b]	***	49.47 ± 4.40[a]	Ns
P-C-T15	7.41 ± 0.06[a]		58.08 ± 3.80[a]		58.14 ± 3.84[a]		58.14 ± 4.00[a]	
	After 15 min		After 30 min		After 45 min		After 60 min	
M-HP-T0	0.00 ± 0.00[a]	Ns	1.46 ± 1.53[a]	Ns	14.94 ± 9.90[a]	Ns	21.02 ± 10.15[a]	Ns
M-C-T0	0.00 ± 0.00[a]		1.71 ± 0.40[a]		14.26 ± 6.36[a]		22.59 ± 9.34[a]	
M-HP-T7	0.00 ± 0.00[a]	Ns	7.99 ± 5.51[a]	Ns	25.61 ± 3.60[a]	Ns	35.01 ± 5.76[a]	Ns
M-C-T7	0.00 ± 0.00[a]		2.31 ± 1.45[a]		33.65 ± 15.25[a]		40.12 ± 16.61[a]	
M-HP-T15	0.14 ± 0.15	Ns	26.14 ± 8.03[a]	Ns	44.22 ± 4.22[a]	Ns	45.73 ± 4.04[a]	Ns
M-C-T15	0.00 ± 0.00[a]		30.35 ± 6.12[a]		42.21 ± 6.03[a]		43.17 ± 6.20[a]	
	After 15 min		After 30 min		After 45 min		After 60 min	
L-HP-T0	24.37 ± 5.92[a]	Ns	49.13 ± 3.72[a]	Ns	58.28 ± 3.74[a]	Ns	58.12 ± 3.78[a]	Ns
L-C-T0	27.98 ± 2.85[a]		52.70 ± 1.98[a]		58.73 ± 1.92[a]		57.20 ± 3.87[a]	
L-HP-T7	8.15 ± 3.16[a]	Ns	39.68 ± 3.62[a]	Ns	57.97 ± 2.57[a]	Ns	61.62 ± 1.72[a]	Ns
L-C-T7	8.81 ± 1.50[a]		42.29 ± 1.97[a]		59.84 ± 1.95[a]		63.64 ± 4.00[a]	
L-HP-T15	0.00 ± 0.00[a]	Ns	26.68 ± 5.61[a]	Ns	64.58 ± 3.66[a]	Ns	65.64 ± 2.45[a]	Ns
L-C-T15	0.00 ± 0.00[a]		33.01 ± 2.85[a]		65.56 ± 2.32[a]		66.15 ± 3.17[a]	

Note: Data are reported as mean plus and minus standard deviation. 'ns' = ANOVA $p > 0.05$; Regarding the asterisks as follow are reported the intervals of ANOVA p value: 0.01 ≤ '*' < 0.05; 0.001 ≤ '**' < 0.01; 0 ≤ '***' < 0.001. Superscript different letters are related to significant difference (if present) between treated (HP) and control (C) samples on the same column.

TABLE 5 | Starting G′ and G″ moduli (Pa), crossover point (strain%) values from oscillation amplitude analyses of pistachio ice cream.

Sample	T0			T7			T15		
	G′	G″		G′	G″		G′	G″	
P-HP	$9.7 \cdot 10^5$	$4.7 \cdot 10^5$	G′ > G″	$1.3 \cdot 10^6$	$5.4 \cdot 10^5$	G′ > G″	$2.4 \cdot 10^6$	$1 \cdot 10^6$	G′ > G″
Crossover point (strain% value)	0.45%			1.05%			0.57%		
	G′	G″		G′	G″		G′	G″	
P-C	$2.2 \cdot 10^6$	$1.2 \cdot 10^6$	G′ > G″	$1.3 \cdot 10^6$	$6.9 \cdot 10^5$	G′ > G″	$1.9 \cdot 10^6$	$8.9 \cdot 10^5$	G′ > G″
Crossover point (strain% value)	0.35%			0.18%			0.28%		

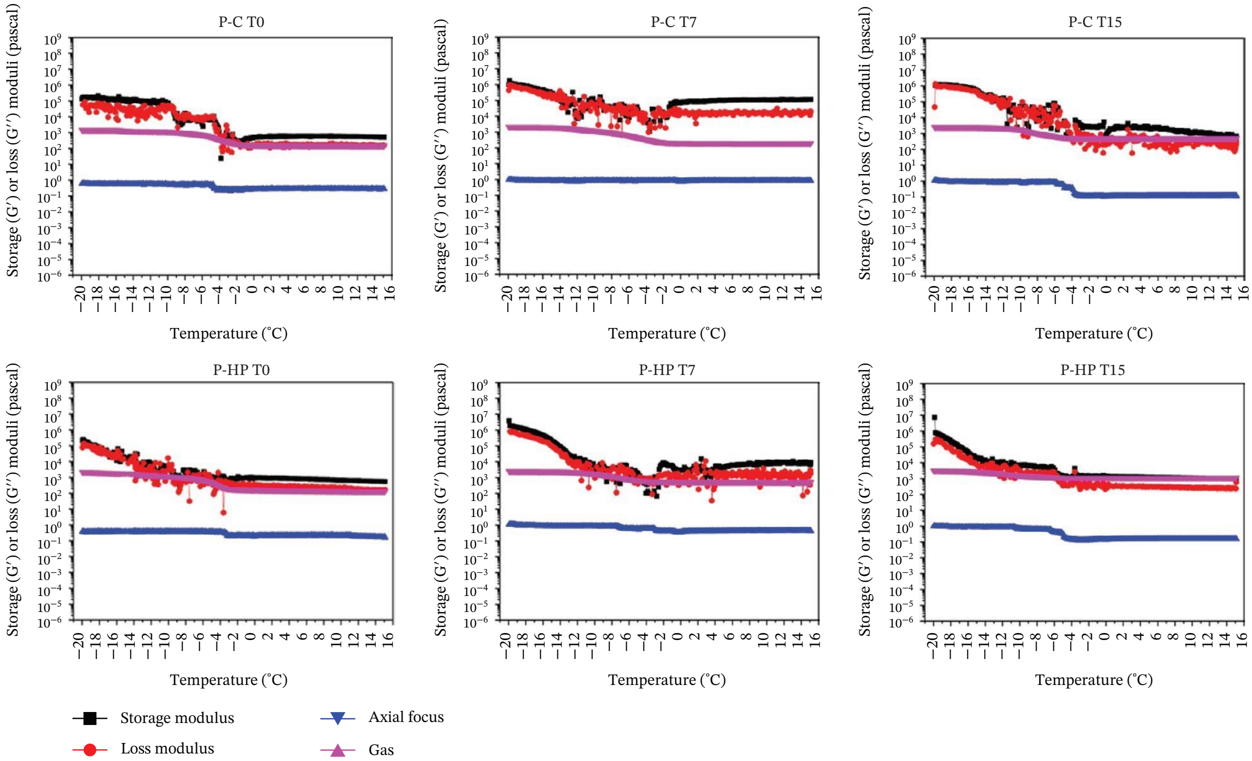

FIGURE 2 | Temperature ramp analyses of pistachio ice cream samples (treated P-HP and control P-C) whipped after different period of storage (0, 7 and 15 days) displayed in the figure: P-C-T0, P-HP-T0, P-C-T7, P-HP-T7, P-C-T15 and P-HP-T15.

TABLE 6 | Starting G′ and G″ moduli (Pa), crossover point (strain%) values from oscillation amplitude analyses of mango ice cream.

Sample	T0			T7			T15		
	G′	G″		G′	G″		G′	G″	
M-HP	7.9·10^5	1·10^6	G′ < G″	3.8·10^5	2.4·10^5	G′ > G″	7,2·10^5	4,3·10^5	G′ > G″
Crossover point (strain% value)	N/A			3.02%			0.35%		
	G′	G″		G′	G″		G′	G″	
M-C	4.7·10^5	9.4·10^5	G′ < G″	1.0·10^6	5.4 ·10^5	G′ > G″	4.1·10^5	5.6·10^5	G′ < G″
Crossover point (strain% value)	N/A			0.87%			N/A		

This is consistent with the textural and melting properties assessed in this study. This result could confirm the influence of HPP on the stability of the pistachio complex systems.

Temperature ramp analyses (Figure 2) were conducted to observe the rheological behaviour during heating. In all samples (lemon, mango and pistachio) and at all stages of analysis (T0, T7 and T15) a collapse of the axial force was recorded due to the complete meltdown of the ice cream. The pistachio ice cream, indicated as P, presents similar response comparing HP (sample treated with HPP) and C (control) samples, showing a decreasing in moduli starting around −12°C and an axial force falling at −5°C due to the melting of the ice cream.

Interestingly, starting values of G′ and G″ moduli, read at −20°C for HP samples, followed a clear increase from ~10^5 up to ~10^6 Pa, for samples immediately whipped (HP-T0) or kept for 7 and 15 days prior to whipping. This is possibly due to increased time of networking of molecules (fats and proteins) present in the formulation, influencing rheological properties of ice cream.

Oscillation amplitude analysis carried out on mango ice creams (Table 6), showed a fluctuating behaviour: at T0 both M-HP and M-C show liquid-like behaviour (G″ > G′), with a G′ always falling with a steeper slope. At T7 both M-HP and M-C show G′ higher compared to G″, highlighting solid-like behaviour. The crossover point, indicating the complete breakdown of the

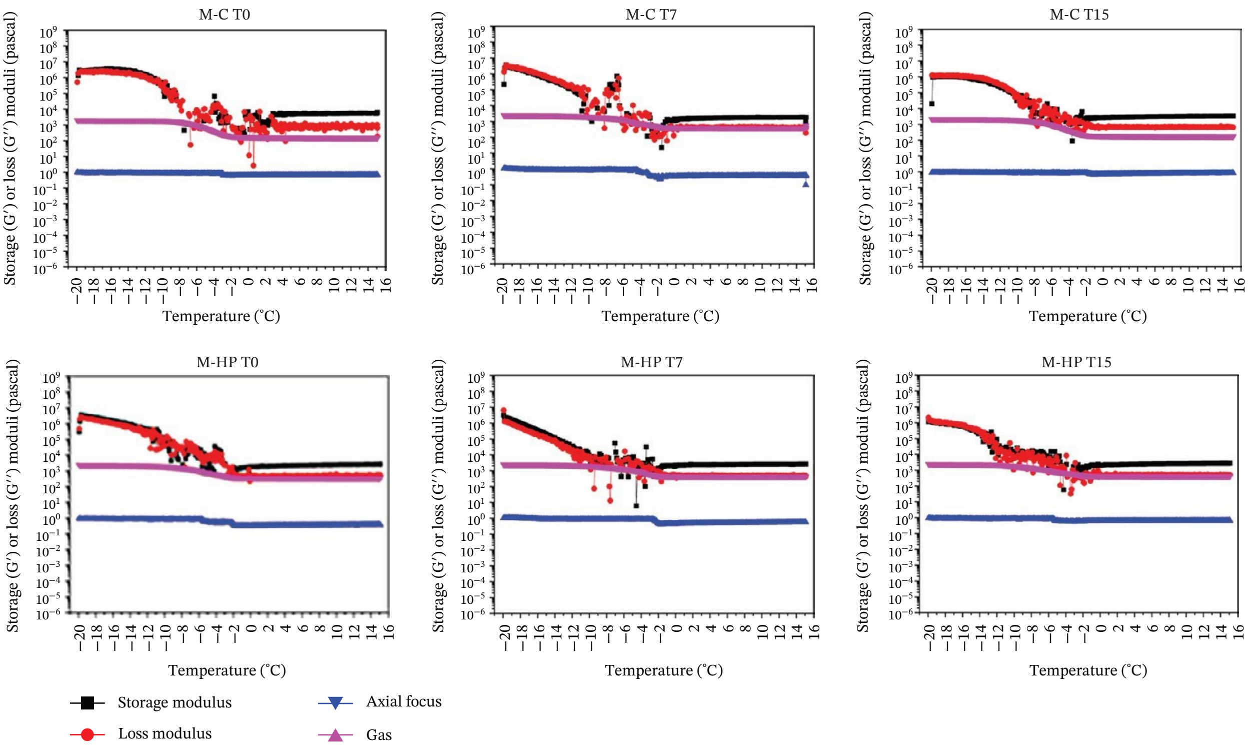

FIGURE 3 | Temperature ramp analyses of mango ice cream samples (treated M-HP and control M-C) whipped after different period of storage (0, 7 and 15 days) displayed in the figure: M-C-T0, M-HP-T0, M-C-T7, M-HP-T7, M-C-T15 and M-HP-T15.

TABLE 7 | Starting G′ and G″ moduli (Pa), crossover point (strain %) values from oscillation amplitude analyses of lemon ice cream.

Sample	**T0**			**T7**			**T15**		
	G′	**G″**		**G′**	**G″**		**G′**	**G″**	
L-HP	$7.2 \cdot 10^4$	$1.8 \cdot 10^5$	G′ < G″	$3.8 \cdot 10^5$	$9.9 \cdot 10^5$	G′ < G″	$1,6 \cdot 10^6$	$2,7 \cdot 10^6$	G′ < G″
Crossover point (strain% value)	N/A			N/A			N/A		
	G′	G″		G′	G″		G′	G″	
L-C	$7.2 \cdot 10^4$	$9.8 \cdot 10^4$	G′ < G″	$9.2 \cdot 10^5$	$1.5 \cdot 10^6$	G′ < G″	$1.6 \cdot 10^6$	$2.7 \cdot 10^6$	G′ < G″
Crossover point (strain% value)	N/A			N/A			N/A		

microstructure, shifts from ~$8 \cdot 10^{-1}$% strain for M-C to ~$3 \cdot 10^{0}$% strain for M-HP. At T15 M-C changes again the rheological behaviour, showing G″ slightly higher than G′ without any crossover point; whereas M-HP maintains the solid-like behaviour with crossover point at ~10^{-1}%.

Temperature ramp analysis of mango ice cream showed similar behaviour in treated (M-HP) and untreated (M-C) samples (Figure 3). In both cases, the G′ and G″ moduli gradually decreased with increasing temperature up to −12°C, dropping from ~10^6 Pa to ~10^4 Pa. A slight decrease of axial force is visible at −4°C for M-C samples at the three stages of observation, whereas M-HP showed the decrease of axial force shifted to −2°C at T0 and T7, falling to −5°C at T15, sign that the first higher resistance to meltdown shown by the HP samples is no more maintained after 15 days of storage. This behaviour is probably due to the presence of mango pectins [25], which initially adsorb and stabilise water. Furthermore, pectin is reported to be particularly affected by HPP enhancing its dissolution in water, resulting in increased apparent viscosity and stability [26].

However, during storage, these pectins may compete for water molecules with added sugars and those naturally present in mango because sugars are strongly hygroscopic and they preferentially bind water [31].

Viscoelastic properties assessed on lemon ice cream revealed liquid-like behaviour (G″ > G′) at any stage of analysis and for both L-HP and L-C samples.

Interestingly, the oscillation amplitude analysis (Table 7) presents a gradual increase of the G′ and G″ moduli with the increase of time of storage before whipping: at T0 C and HP samples have both moduli set at $< 10^5$ Pa, increasing up to ~10^6 Pa after 7 days of storage (T7) and after 2 weeks (T15).

This finding can be explained by the fact that lemon mixture had not water-retaining compound such as fibres or proteins: during fridge storage, phase separation may occur leading to formation of nonuniformly shaped and distributed ice crystals within the mass after whipping and freezing. Larger or more connected ice crystals in ice cream increase the resistance to deformation, implying higher G′ and G″ moduli [32–34].

Temperature ramp analysis shows a pronounced falling of axial force, which occurs at −5°C, for sample. Samples T7 and T15 showed a gradual decrease in both G′ and G″ moduli beginning at −12°C in both L-C and L-HP samples (Figure 4).

3.3 | Differential Scanning Calorimetry

The thermograms obtained by the first temperature ramp using DSC clearly show the different chemical compositions of the three ice cream mixtures. The curves consist of multiple endothermic peaks, especially for mango and pistachio ice creams, among which it is possible to distinguish the variable melting behaviour of all the compounds present in the matrices. The lemon mixture (water-based), with the sole addition of sugars and stabilisers, shows a simple and intense endothermic peak, due to the melting of the sugar solution and free water (between −2°C and −1°C).

Pistachio is the most complex matrix: milk provides proteins and mainly saturated fatty acids, whereas pistachio provides unsaturated fatty acids, each of which has specific melting points.

The thermograms recorded from the pistachio ice cream samples show transition curves with multiple peaks, composed of individual peaks relating to water first bound and then released by milk proteins (a broad, low-intensity peak, like a side shoulder, around −10°C/−15°C), to the sugar solution and ice crystals (close to zero), unsaturated fatty acids and saturated fatty acids (positive temperatures between +2°C and +15°C, approximately).

In order to compare the treated HP with the untreated C, have been taken into account the Tg, to understand whether the ice cream would be stable in its final frozen state [35] and the transition enthalpy ΔH (J/g), which refers to the total energy required to complete melting. A shift in Tg and a variation in enthalpy values would mean a significant change resulting from HPP treatment on the microstructure of the mixture and therefore on the physical stability of the ice cream. The recorded Tg and ΔH values were analysed with statistical multifactorial analysis to understand which factor (treatment HP/C or storage time T0/T7/T15) has the main influence on the results of the evaluated parameters (enthalpy ΔH and Tg). These results are shown in Table 8.

Significant reduction in the ΔH of the pistachio mixtures was recorded at all stages of the analysis for mixtures treated with HPP, so the storage time has notably influence on the energy acquired during transition phase. According to Hwang et al.

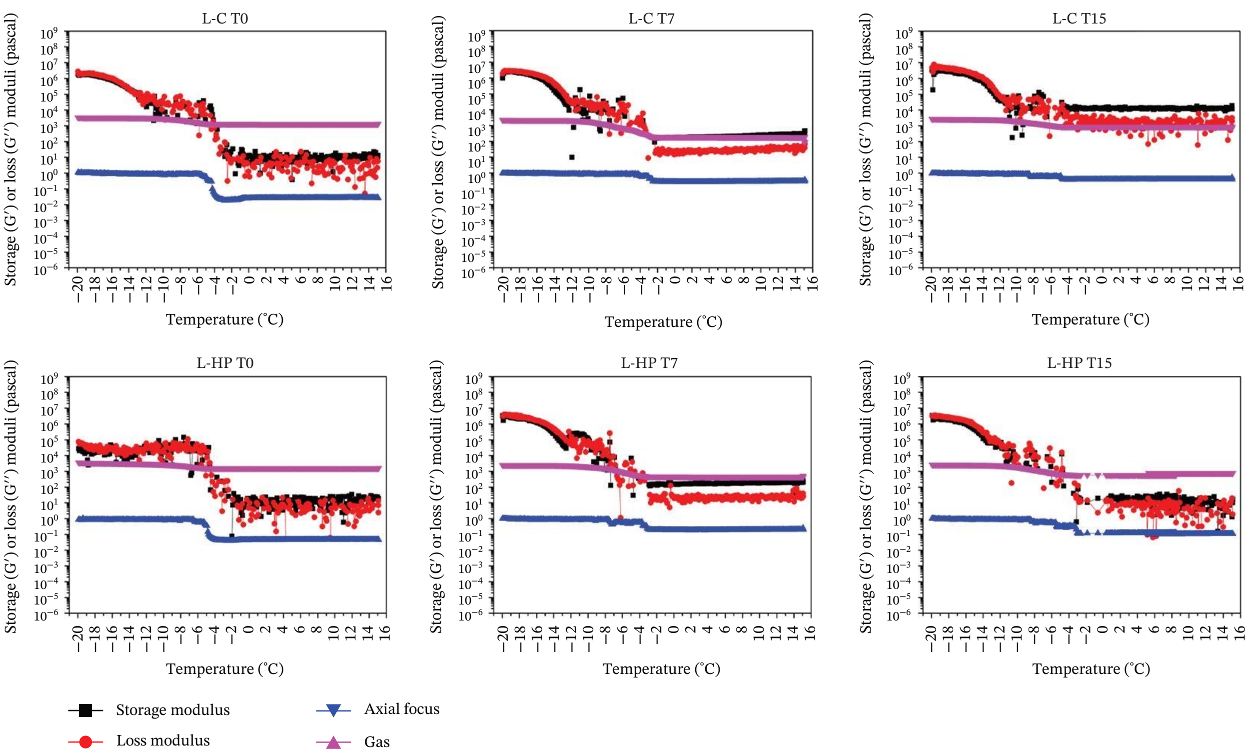

FIGURE 4 | Temperature ramp analyses of lemon ice cream samples (treated L-HP and control L-C) whipped after different period of storage (0, 7 and 15 days) displayed in the figure: L-C-T0, L-HP-T0, L-C-T7, L-HP-T7, L-C-T15 and L-HP-T15.

[36] the decreased enthalpy is related to the lower amount of frozen water within the system, due to the protein content able to hold the water. Thus, the longer contact time among components during prolonged storage probably led to higher absorption and retention phenomena within the system, with consequent less formation and growing of ice crystals in the ice cream.

This is particularly evident for P-HP that showed significantly lower enthalpy value compared to the P-C, showing the HPP treatment as a pillar factor influencing the resulting enthalpy values. The change in the energy required to melt down the pistachio ice cream samples is related to the change in microstructure after HPP. This interpretation is consistent with the results obtained from the melting rate and texture analyses, shown in Paragraph 3.1. The protein network and fat globules are particularly affected by HPP treatment depending on pressures and holding times

TABLE 8 | Glass transition temperature (Tg) and transition enthalpy (ΔH) for all the ice cream samples (L for lemon, P for Pistachio and M for Mango), differing for treatment (HP for treated samples or C for control samples) and storage time (0, 7 and 15 days) related to the first temperature ramp performed during DSC analysis. Results are reported as mean and standard deviation of statistical multifactorial analysis performed using the type of treatment (HP or C) and the time (T0, T7 and T15) as factors.

		Enthalpy (J/g)	Tg (°C)
Lemon	HP	144.349 ± 11.068^a	-30.770 ± 0.330^a
	C	148.403 ± 17.319^a	-28.456 ± 2.072^a
	Sign.	Ns	Ns
Lemon	T0	131.737 ± 0.5247^a	-30.105 ± 1.478^a
	T7	159.821 ± 8.7264^a	-28.350 ± 3.111^a
	T15	147.569 ± 0.3995^a	-30.385 ± 0.318^a
	Sign.	Ns	Ns
		Enthalpy (J/g)	**Tg (°C)**
Pistachio	HP	134.992 ± 23.091^b	-25.183 ± 1.161^a
	C	162.377 ± 17.882^a	-25.550 ± 0.953^a
	Sign.	*	Ns
Pistachio	T0	171.932 ± 14.840^a	-25.375 ± 1.803^a
	T7	140.668 ± 22.570^b	-25.685 ± 1.025^a
	T15	133.453 ± 20.681^b	-25.040 ± 0.000^a
	Sign.	*	Ns
		Enthalpy (J/g)	**Tg (°C)**
Mango	HP	159.129 ± 6.916^a	-28.770 ± 0.439^a
	C	165.215 ± 9.044^a	-29.053 ± 0.733^a
	Sign.	Ns	Ns
Mango	T0	163.640 ± 0.146^a	-28.445 ± 0.233^a
	T7	168.799 ± 8.635^a	-28.775 ± 0.177^a
	T15	154.077 ± 4.129^a	-29.515 ± 0.544^a
	Sign.	Ns	Ns

Note: Data are reported as mean plus and minus standard deviation.'ns' = ANOVA $p > 0.05$; Regarding the asterisks as follow are reported the intervals of ANOVA p: 0.01 ≤ '*' <0.05; 0.001 ≤ '**' <0.01; 0 ≤ '***' <0.001. Superscript different letters are related to significant difference (if present) between treated (HP) and control (C) samples on the same column.

TABLE 9 | Glass transition temperature (Tg) and transition enthalpy (ΔH) for all the ice cream samples (L for lemon, P for Pistachio and M for Mango), differing for treatment (HP for treated samples or C for control samples) and storage time (0, 7 and 15 days) related to the second temperature ramp performed during DSC analysis. Results are reported as mean and standard deviation of statistical multifactorial analysis performed using the type of treatment (HP or C) and the time (T0, T7 and T15) as factors.

		Enthalpy (J/g)	Tg (°C)
Lemon	HP	140.893 ± 6.437^a	-33.473 ± 0.906^a
	C	146.266 ± 3.700^a	-33.310 ± 0.502^a
	Sign.	Ns	Ns
Lemon	T0	141.948 ± 0.114^a	-33.840 ± 0.962^a
	T7	141.440 ± 10.486^a	-33.420 ± 0.636^a
	T15	147.353 ± 0.798^a	-32.915 ± 0.021^a
	Sign.	Ns	Ns
		Enthalpy (J/g)	**Tg (°C)**
Pistachio	HP	140.331 ± 2.5381^a	-31.97 ± 0.670^a
	C	133.981 ± 0.769^a	-31.13 ± 0.322^a
	Sign.	Ns	Ns
Pistachio	T0	136.142 ± 1.885^a	-32.005 ± 0.714^a
	T7	137.809 ± 6.392^a	-31.545 ± 0.8980^a
	T15	137.518 ± 5.195^a	-31.100 ± 0.170^a
	Sign.	Ns	Ns
		Enthalpy (J/g)	**Tg (°C)**
Mango	HP	170.994 ± 6.271^a	-31.613 ± 0.468^a
	C	170.747 ± 1.111^a	-31.853 ± 0.510^a
	Sign.	Ns	Ns
Mango	T0	171.343 ± 1.247^a	-31.730 ± 0.537^a
	T7	173.183 ± 4.775^a	-31.775 ± 0.841^a
	T15	168.087 ± 5.496^a	-31.695 ± 0.205^a
	Sign.	Ns	Ns

Note: Data are reported as mean plus and minus standard deviation. 'ns' = ANOVA $p > 0.05$; . Regarding the asterisks as follow are reported the intervals of ANOVA p: 0.01 ≤ '*' <0.05; 0.001 ≤ '**' <0.01; 0 ≤ '***' <0.001. Superscript different letters are related to significant difference (if present) between treated (HP) and control (C) samples on the same column.

applied [24, 37], which improves the interfacial properties, emulsifying capacity and overall melting stability of the final ice cream obtained. The pressurisation can reduce fat globule size or can induce reaggregation after treatment due to coalescence of smaller particles, with an increasing in viscosity [37].

Proteins treated with high pressure tend to partially denature, exposing hydrophobic regions that were not accessible before treatment. This facilitates the adsorption of proteins at the oil-water interface, improving the formation and stability of the protein film, which is key to good emulsifying properties. Following HPP pasteurisation, therefore, alterations in the composition of the fat globule membrane and additional protein associations (e.g. β-lactoglobulin, caseins in milk) to the membrane itself are observed [17, 38, 39]. This has effectively improved the stability of the entire system of pistachio mixture of ice cream treated with high pressures.

Lemon and mango ice creams reported no significant differences in transition enthalpy and Tg values; therefore, neither the HPP treatment nor the storage time are influencing factors. For these two kinds of mixtures, consistently with the other structural analyses performed in this study, the HPP treatment does not affect the calorimetric properties of the final ice cream products.

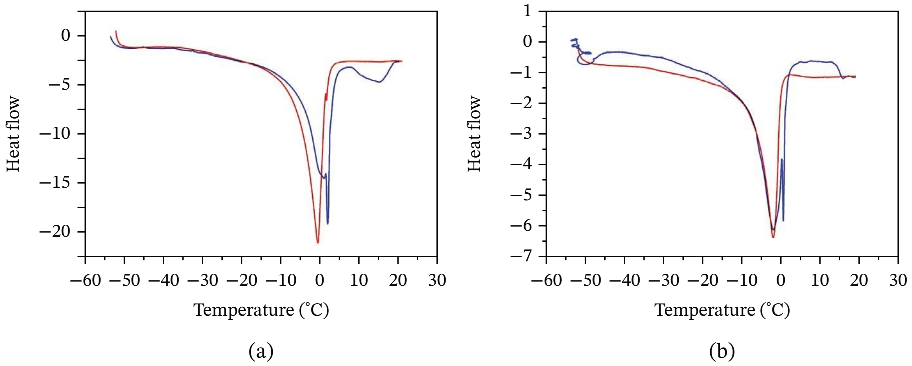

FIGURE 5 | Thermograms related to calorimetric analysis performed on pistachio ice creams at Time 0, comparing first (blue line) and second (red line) temperature ramp performed for treated HP (a) and control C (b) samples.

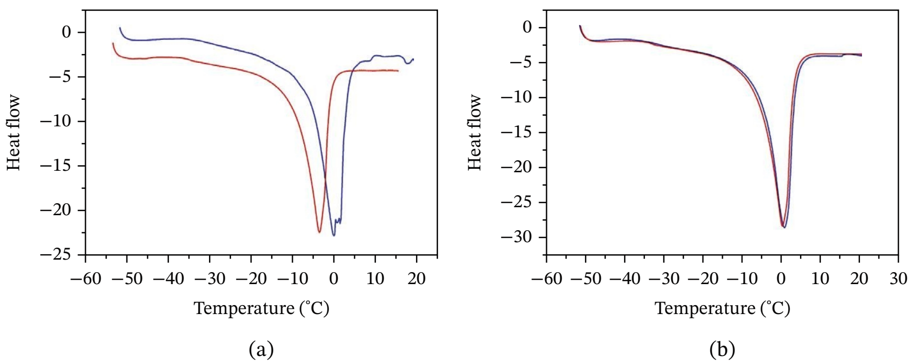

FIGURE 6 | Thermograms related to calorimetric analysis performed on mango ice creams at Time 0, comparing first (blue line) and second (red line) temperature ramp performed for treated HP (a) and control C (b) samples.

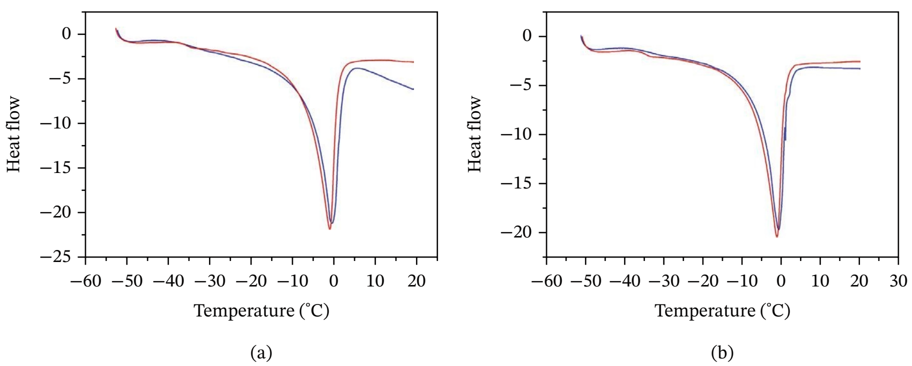

FIGURE 7 | Thermograms related to calorimetric analysis performed on lemon ice creams at Time 0, comparing first (blue line) and second (red line) temperature ramp performed for treated HP (a) and control C (b) samples.

The second temperature ramp was performed immediately after the final isothermal equilibration of the first temperature ramp, when the ice cream was left to melt down at +20°C for 600 s. Results are reported in Table 9.

Results showed that no significant differences among samples after the complete melting of the ice creams. The first ramp showed the transition phase of ice cream considering all factors, including air volume and production manipulation, whereas the second ramp shows just the interaction among components. The endothermic curves of the second temperature ramp, in fact showed one single peak, with a unique transition enthalpy curve (as shown in Figure 5, Figures 6 and 7). Multiple peaks are completely lost or became minimally evident, after sample melt-down during the first ramp. Thus, the first ramp performed is the most representative of an artisan ice cream product, instead of the second ramp in which all the air bubbles and the original structure of the ice cream are lost during melting.

4 | Conclusions

HPP can be a viable tool to prolong shelf life of ice cream mixtures (as confirmed by microbiological and sensory reported in another paper not yet been published). Lemon and mango ice creams (water-based) did not show significant differences by structural point of view, whereas pistachio ice cream (milk-based) treated with HPP showed improving of structural properties compared to the control pistachio sample due to influences of the treatment on chemical composition of the mixture. So, this cold pasteurisation technique it is useful to optimise the production process of artisan ice cream, without relevant modifications on the physical properties of the fruit-based ice creams, indeed enhancing melting stability of milk-based products.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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