

High Pressure Processing (HPP) Applied on the Production and Stabilization of Sicilian Artisanal Gelato Mixture

Federica Torregrossa*, Luciano Cinquanta, Monica Di Maria, Matteo Pollon, Giuliana Garofalo, Raimondo Gaglio, Diego Planeta

University of Palermo, Department of Agricultural Forestry and Food Sciences, Palermo.

federica.torregrossa01@unipa.it

In this study High Pressure Processing (HPP) was applied on mango gelato mixtures to evaluate the possible application as a pasteurization method to prolong shelf life of gelato. Microbiological analysis revealed that HPP treatment inhibited the growth of the main spoilage and pathogenic microorganisms throughout the entire storage period. By a sensory point of view, is not perceivable the difference between the experimental treated sample and the control untreated sample. Regarding the flavour profile, organic acids and alcohols were significantly reduced after 15 days of storage, while terpenes decreased after treatment and were more stable during the storage period. HPP can be an applicable solution to optimize gelato production and prolong shelf life of mango gelato mixture.

1. Introduction

Gelato is a worldwide spread product, due to its sweetness and refreshing sensation (Bahram-Parvar, 2015). Italian style gelato differs from the worldwide spreader gelato: it can be distinguished in ice creams, sorbets and granitas (Planeta et al., 2020) based on the composition and the production process. Differences may be evident between industrial and artisanal product because of the lower fat content, the lower overrun and a significant reduction of the use of stabilizers and emulsifiers (Sacchi et al., 2019). Furthermore, artisanal gelato is generally flavoured by raw materials instead of flavouring additives (Rinaldi et al., 2014). From a larger scale point of view, it is important to find new alternative solutions able to prolong the shelf life of the gelato mixtures to increase the productivity of artisanal processes while maintaining longer the characteristics of the final product from a qualitative perspective, since the mandatory thermal pasteurization allows the survival of microbial species, capable of altering the above mixture. Novel cold pasteurization techniques have been extensively researched to enhance food safety and longevity without affecting organoleptic properties (Zulli et al., 2023) (D'Addio et al., 2014). High pressure processing is a non-thermal treatment nowadays applied on foods that are generally stabilized by high temperature. It is a mild pasteurization technology in which, for microbial breakdown, food products are subjected to isostatic pressures ranging from 400 to 600 MPa, with holding times of few minutes (Koutsoumanis et al., 2022). In this study, we aim to evaluate the effectiveness of high pressures (HPP) on refrigerated ice cream mixtures, and assess its effect in prolonging their shelf-life. The liquid mango ice cream mixture was thus subjected to HPP as a cold pasteurization method to extend its shelf life, evaluated by microbiological and sensory analyses.

2. Materials and Methods

2.1 Raw Materials

Gleen variety mango fruit was purchased from the agricultural company Il Filo Tropicale (Balestrate, Italy). The mixture of gelato was formulated as follows: mango fruit 65%, sucrose 12.6%, water 11.5%, dextrose 4.2%, inulin 4.2%, glucose syrup 38DE 2.35% and as stabilizer carob seed flour and guar seed flour as 0.10% and 0.07% respectively.

2.2 Gelato Mixture Preparation

Preparation of gelato mixture and the final whipping step were performed in gelato productive laboratory by Cappadonia Gelati s.r.l. located in Cerda (Palermo, Italy). Ingredients were initially mixed with a Tafec Turbo mixer (Tafec Gelato GmbH, Norderstedt, Germany) then treated with Carpigiani Pasteurizer 60HE (Carpigiani Group – Ali s.r.l. Anzola dell'Emilia, Italy). After the pasteurization, necessary step applied in the worldwide production process of gelato, the mixture was divided in eight vacuum bags (400 x 500 mm size, ByVac s.r.l., Ghivizzano, Italy), air gases were removed with a Orved Cuisson 41 vacuum machine (Orved Spa, Musile di Piave, Italy) to obtain up to 99% of void and finally bags were sealed and stored at 4 – 6°C of temperature. A first set of 4 bags was subjected to the HPP treatment and directly whipped (HPP-T0) or stored for 7, 15 and 30 days before whipping (HPP-T7, HPP-T15, and HPP-T30, respectively). A second set of 4 bags was kept for the same times without the HPP treatment as controls (NoHPP-T0, NoHPP-T7, NoHPP-T15, and NoHPP-T30). The final whipping was done with batch freezer Tafec Geltronic (Tafec Gelato GmbH, Norderstedt, Germany).

2.3 HPP Treatment

HPP treatment was performed using an AV-50X machine (Avure Technologies, Ohio, USA). Sealed samples were placed in a water-filled hyperbaric chamber at 4°C and pressurized with increasing pressure (rate of 40 bar/sec) up to 6000 bars (600 MPa) maintained for 300 seconds. Post-treatment samples were stored at 4-6°C for 0, 7, 15, and 30 days before whipping. Final gelato products were stored at -20°C in Styrofoam containers until analysis.

2.4 Microbiological analysis

Mango puree as well as untreated (NoHPP) and HPP-treated (HPP) gelato mixture samples were microbiologically investigated using a culture-dependent approach. Briefly, one mL of each sample was serially diluted in a 1:10 ratio with Ringer's solution (Thermo Fisher Scientific, Waltham, MA, USA). The resulting cell suspensions were then used for the enumeration and detection of the main spoilage and pathogenic microorganisms according to the International Organization for Standardization (ISO) guidelines. Specifically, pseudomonads (ISO 11059:2009), yeasts, and molds (ISO 21527-1:2008) were assessed among spoilage microorganisms, while members of the Enterobacteriaceae family (ISO 21528-2:2017), coagulase-positive staphylococci (CPS) (ISO 6888-2:1999), *Listeria monocytogenes* (ISO 11290-1, 2017), *Escherichia coli* (ISO 7251:2005), and *Salmonella* spp. (ISO 6579-1:2017) were evaluated among pathogenic microbial groups. All samples were also subjected to the enumeration of total mesophilic microorganisms (TMM) (ISO 4833:2003) and total psychrotrophic microorganisms (TPM) (ISO 17410:2019). The analyses were performed in triplicate at each sampling time (0, 7, 15 and 30 d of cold storage). All media and supplement were purchased from Oxoid (Hampshire, United Kingdom).

2.5 Volatile Organic Compounds Profile

Volatile organic compounds characterization was analysed comparing the treated (HPP) and the untreated control sample (NoHPP) at T0 (0 days) and T15 (15 days). HS-SPME was conducted for extraction of volatile compounds according to Conurso et al. (2008): 5 g of whipped gelato were mixed in a vial with 50 µL of solution at 10% v/v of absolute ethanol in water containing 38.22 mg/L of 1-heptanol. The vial was placed at 40 °C in water bath in continuous magnetic stirring for 20 minutes to carry on the equilibration time, followed by the absorption step carried on for 30 minutes, with fiber (50/30µm DVB/Car/PDMS fiber—Supelco Inc., Bellefonte, PA) inserted in the headspace of the vial. Fiber was first activated directly in the injector port of the GC-MS for 15 min at 230 °C before the absorption of volatile compounds. Analysis of VOCs was performed with GC-MS Agilent 6890 coupled with an Agilent single quadrupole mass spectrometer 5973N (Agilent Technologies, Santa Clara, California, USA) equipped with a DB-WAX capillary column (Agilent Technologies; 30 m 0.250 mm i.d. film thickness 0.25 µm) according to Pollon et al. (2024). Analysis was performed in triplicate. Data are expressed in equivalent µg of 1-heptanol per 1 kg of gelato.

2.6 Colour Measurement

The colour analysis was performed with Konika Minolta CR300 colour spectrophotometer (Konika sensing Americas, Ramsey, New Jersey USA), comparing untreated sample to the treated sample after different storage times (0, 7 and 15 days). A plastic petri dishes of 9 cm Ø was filled with mango gelato stored at -20 °C and covered with its own lid. Collected data were evaluated according to the CIELab Color space, in which L* refers to brightness, a* refers to green (-a) and red (+a), while b* refers to blue (-b) and yellow (+b).

Difference in colorimetric values (ΔE) was evaluated according to the following equation:

$$\Delta E = \sqrt{(L_c - L_s)^2 + (a_c - a_s)^2 + (b_c - b_s)^2} \quad (1)$$

Values with subscript c (Lc, ac and bc) refer to the control untreated sample, while values with subscript s (Ls, as and bs) refer to the treated sample.

2.7 Sensory Analyses

Two sensory tests were conducted with 20 semi-trained panelists just on samples T0. The Duo-Trio Test involved the identification of the sample (A: NoHPP or B: HPP) most similar to a random reference R (A or B). While the Sensory Descriptive Analysis, based on Giudici et al. (2021) with modifications, used a 0-10 scale to evaluate eight attributes of the mango ice cream: appearance, melting speed, granularity, cold sensation, sweetness, intensity of taste, persistence of taste and presence of off-flavour (0=totally unpleasant/absent; 10=totally pleasant/present). The overall assessment was calculated by summing the averages of the values of all attributes for each sample.

2.8 Statistical Analyses

For Statistical Analysis, Student's t test was performed with R 4.1.3 (R Foundation for Statistical Computing) to evaluate comparison between treated experimental sample (HPP) and untreated control sample (NoHPP) at any stage of storage taken into consideration.

3. Results and Discussion

3.1 Microbial count evolution

The mango puree and the NoHPP and HPP gelato mixtures produced in this study were subjected to plate counts to assess the presence of the main undesired populations associated to foods (Barbosa et al., 2021). All samples did not host detectable levels of CPS, *L. monocytogenes*, *E. coli*, and *Salmonella* spp. These bacteria are key indicators of food hygiene and safety (Commission Regulation, 2073/2005). No colonies of molds responsible for microbial decay (Azad et al., 2019) were detected in any of the analysed samples. The mango puree exhibited TMM, TPM, and pseudomonad levels of 2.46, 2.39 and 2.35 Log CFU/mL respectively, suggesting that the microbial populations in this fruit puree were predominantly composed of psychrotrophic bacteria. Similar results were previously reported by Adjou et al. (2017) for mango purees soon after production. The evolution of microbiological populations in NoHPP and HPP mango gelato samples during refrigerated storage are reported in Table 1. Statistically significant differences between the trials were observed after 7 days of refrigerated storage. In the NoHPP sample, TMM and yeasts were approximately 10^4 and 10^3 CFU/mL, respectively, whereas they remained undetectable up to 15 days in the HPP-treated mixture samples.

Table 2: Microbial evolution during refrigerated storage of untreated and HPP-treated gelato mixtures.

Storage time	Samples	Microbial loads				
		TMM	TPM	Pseudomonads	Enterobacteriaceae	Yeast
0	NoHPP	2.99±0.02a	<1	<1	0	<1
	HPP	<1b	<1	<1	0	<1
	SEM	0.47	n.d.	n.d.	n.d.	n.d.
	P value	<0.0001	n.d.	n.d.	n.d.	n.d.
7	NoHPP	3.9±0.28a	<1	<2	0	2.6±0.08a
	HPP	<1b	<1	0	0	<1b
	SEM	0.62	n.d.	n.d.	n.d.	0.41
	P value	<0.0001	n.d.	n.d.	n.d.	<0.0001
15	NoHPP	4.08±0.11	4.11±0.10a	<1	3.09±0.12a	3.57±0.12a
	HPP	<1	<1b	<1	0b	<1b
	SEM	0.64	0.65	n.d.	0.49	0.56
	P value	<0.0001	<0.0001	n.d.	<0.0001	<0.0001
30	NoHPP	5.06±0.20a	5.15±0.04a	4.22±0.68a	3.39±0.12a	4.26±0.12a
	HPP	3.24±0.21b	1.85±0.21	<1b	0b	<1b
	SEM	0.29	0.52	0.68	0.54	0.67
	P value	0.001	<0.0001	<0.0001	<0.0001	<0.0001

Units are Log CFU/mL. Results indicate the mean values ± standard deviation (S.D.) of three plate counts. Data within a column followed by the same letter are not significantly different. Abbreviations: NoHPP, untreated gelato mixture; HPP, gelato mixture treated with HPP; TMM, total mesophilic microorganisms; TPM, total psychrophilic microorganisms; SEM, standard error of the mean; n.d., not detected.

Interestingly, members of the Enterobacteriaceae family, which are potentially responsible for gastroenteritis and even chronic infections (Janda et al., 2021), appeared after 15 days of storage only in the NoHPP samples, reaching values of 3.39 Log CFU/mL by the end of the trial. A similar trend was observed for pseudomonads and yeasts, which are commonly associated with the development of off-flavors and off-odors in processed foods (Miceli et al., 2019; Gram et al., 2002). The only viable microorganisms detected in the HPP-treated gelato mixture samples were TMM and TPM, but at concentrations 3 log cycles lower than those found in the untreated production. These differences are undoubtedly imputable to the ability of HPP treatment to destroy microbial spores and vegetative cells (Kaushik et al., 2014).

3.2 Volatile Organic Compounds Profile

The volatile compound profile shows at T0 that just the categories of organic acids, monoterpenes and C₁₃-norisprenoids have significant differences between treated HPP sample and untreated control sample NoHPP (Table 3). Organic acids are mainly referred to the acetic acid present only in the untreated sample. Monoterpenes are significantly present in the untreated control sample, while the experimental sample shows a concentration equal to half compared to the control sample at T0, meaning that the HPP process negatively affected in the short term the monoterpenes profile but the rate of decreasing in the long term is lower in HPP treated sample, compared to the untreated NoHPP sample. So, from a conservative perspective, the presence of monoterpenes in treated gelato is more stable during storage time compared to the untreated sample. The concentrations of sesquiterpenes are not different between the two samples, the only significant difference being related to δ -cadinene, an essential oil, which is highly present in the untreated control sample compared to the HPP-treated sample (7.50 μ g/kg and 17.13 μ g/kg respectively). Significantly relevant is also the difference in alcohols, mostly present in the untreated NoHPP sample at T0 compared to the treated HPP, while after 15 days of storage the trend is in reverse. Those alcohols are deriving from the enzymatic activity that convert in fruits aldehydes into alcohols such as 1-hexanol and Z-3-hexenol (Contreras & Beaudry, 2013), associated with an intensely green, gassy, fruity, green grass-like odour. Anyway, the odour detection threshold reported for those volatiles is 0.91 ppm and 1.6 ppm respectively (Tamura et al., 2001), so they are under the limit of detection. Finally, the difference in organic acid concentration is less significant compared to the T0 values, acetic acid is increasing but always lower compared to the untreated NoHPP.

Table 3: Main categories of volatile organic compounds for NoHPP and HPP samples at different time of analysis (0 and 15 days).

Samples	HPP-T0	NoHPP-T0	Sig.	HPP-T15	NoHPP-T15	Sig.
Organic Acids	<LOQ	19.5 \pm 4.7	**	17.1 \pm 0.01	28.1 \pm 6.0	*
Aldehydes	9.5 \pm 2.4	8.6 \pm 0.8	n.s.	25.13 \pm 4.1	13.5 \pm 6.2	n.s.
Esters	41.9 \pm 9.8	56.4 \pm 3.4	n.s.	44.5 \pm 11.2	70.1 \pm 32.3	n.s.
C ₁₃ -norisprenoids	5.5 \pm 1.5	6.2 \pm 1.8	n.s.	7.2 \pm 1.1	5.1 \pm 3.3	n.s.
Alcohols	19.9 \pm 0.4	31.4 \pm 6.6	*	72.7 \pm 6.3	23.2 \pm 4.2	***
Sesquiterpenes	183.9 \pm 24.2	249.4 \pm 50.6	n.s.	122.1 \pm 19.4	187.3 \pm 49.7	n.s.
Monoterpenes	1'615.4 \pm 252.4	3'341.1 \pm 274.1	**	1'099.3 \pm 225.6	1'608.5 \pm 171.7	*

Anova F test significativity: * = 0.01 \leq p value < 0.05, ** = 0.001 \leq p value < 0.01, *** = p value < 0.001

3.3 Colour Measurement

Table 4: NoHPP, untreated control sample; HPP: sample treated with HPP at different time of analysis (0, 7 and 15 days). L*, a* and b* refer to CIELab parameters. ΔE means the difference between reference and experimental sample, involving color parameters L*, a*, and b*, according to the eq. 1.

Samples	L*	a*	b*	ΔE
HPP-T0	70.24 \pm 0.44	-0.59 \pm 0.05	41.06 \pm 0.30	2.9414
NoHPP-T0	73.09 \pm 0.41	-1.03 \pm 0.04	41.64 \pm 0.23	
Sig.	**	***	n.s.	
HPP-T7	76.94 \pm 0.15	-0.65 \pm 0.09	36.80 \pm 0.55	5.537
NoHPP-T7	73.07 \pm 0.17	-0.37 \pm 0.04	40.75 \pm 0.14	
Sig.	***	**	***	
HPP-T15	74.04 \pm 0.40	-0.26 \pm 0.02	39.31 \pm 0.26	2.9323
NoHPP-T15	74.39 \pm 0.21	-1.10 \pm 0.29	42.10 \pm 0.27	
Sig.	n.s.	**	***	

Anova F test significativity: * = 0.01 \leq p value < 0.05, ** = 0.001 \leq p value < 0.01, *** = p value < 0.001

Treatment-dependent colour trends changed during storage: the HPP-treated sample showed increasing values of L* and a* parameters, peaking at 7 days of storage and decreasing at 15 days. The NoHPP sample showed

a more stable trend in L^* and b^* parameters over. These trends are confirmed by the calculation of ΔE , where the T7 value showed a ΔE of 5.5 related to a discernible difference between the two HPP and NoHPP samples, due to the ΔE greater than 3 (Giannoutsos et al., 2023). While T0 and T15 values showed no difference between the two samples perceptible by human eyes.

3.4 Sensory Analysis

The Duo-Trio test revealed no perceivable differences between treated and untreated samples (13/20 correct answers, $p > 0.05$), confirming HPP treatment doesn't affect sensory perception. Overall evaluations were comparable: 56/80 (NoHPP) vs 57.65/80 (HPP). HPP samples scored higher for appearance, melting rate, fresh sensation, and absence of off-flavours. The spider plot displays the means of data collected during the sensory test.

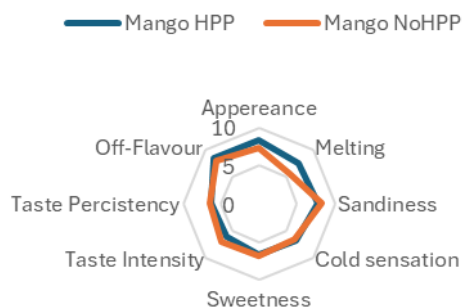


Figure 1: Spider plot related to the sensory descriptive analysis performed through 8 attributes (appearance, melting, sandiness, cold sensation, sweetness, taste intensity, taste persistence and presence of off-flavour).

4. Conclusions

The application of HPP treatment enhanced the microbiological quality of mango gelato mixtures. Specifically, the HPP trial exhibited a complete absence of undesired populations, in contrast to the NoHPP trial, while showed a slight decrease of terpenes compounds in the long-term conservation, controlling the concentration of acetic acid and maintaining the overall acceptance of final product. In general, it is possible to consider HPP as an innovative method to prolong the shelf life of mango gelato mixture up to 15 days of cold storage at positive temperature, without affecting the overall quality of the product.

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