Received: 3 February 2022 | Revised: 7 June 2022 | Accepted: 2 July 2022

DOI: 10.1111/jfpp.16908

Journal of Food Processing and Preservation | Institute of Food Science + Technology | ifst | WILEY

ORIGINAL ARTICLE

Use of dry ice as innovative technology to preserve the chemical and microbial characteristics of burrata cheese

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Funding information
P.S.R. Puglia 2014/2020 - Misura 16 - Cooperazione - Sottomisura 16.2 "Sostegno a progetti pilota e allo sviluppo di nuovi prodotti, pratiche, processi e tecnologie" project BURRATA (CUP: B89J20000130007; DDS 94250034439)

Abstract

Burrata cheese demand is sharply increasing on international market, but its shelf life is very short being limited to a few days due to its high perishability. A possible way to improve preservation is to promptly lower the temperature during the manufacturing process. This study aimed to obtain an immediate drop of the temperature of the product using a cryogenic agent (dry ice) during the filling phase in two different ways: by a dosing funnel (DI) or by direct addition of a flake of dry ice inside the product (INS). The chemical results showed that this technique tended to slow down the alteration processes: INS had lower content of lactic and acetic acid content, as for VOC content; lipolysis was also delayed since the free fatty acid content was less abundant in the experimental samples; the microbial counts showed better microbiological characteristic of experimental samples, in particular, *Pseudomonas* population; under the sensory point of view INS had better characteristics after 21 days.

Novelty impact statement

In this study, we used dry ice as cryogenic agent to immediate drop the temperature during burrata manufacturing, aiming to improve the product preservation. Study results revealed that this technique was able to delay the decaying process. On the chemical point of view, the arise of microbial-derived metabolites was slowed down, as for the Pseudomonas growth, finally the sensory characteristics of the experimental samples was better than control.

1 | INTRODUCTION

Burrata is a typical fresh cheese of Apulia region (Southern Italy) that presents a characteristic "double structure," consisting of a mozzarella cheese pouch containing a creamy paste made of mozzarella strips and double cream (the so-called "stracciatella"). It is easy to see that the two main ingredients of Burrata are mozzarella and cream: the mozzarella pouch and strips account for about 60% of the total cheese weight, the remaining part is the cream (Trani et al., 2016). As for all pasta filata cheeses, the production process involves stretching of the acidified curd in hot water: through this operation, a mozzarella ball is obtained that is turned, while still warm, into a pouch. The pouch is immediately filled with previously prepared stracciatella, closed with a string, and cooled down in chilled water. Once cold, the cheese is transferred into a plastic tray, covered with a governing liquid (pot water or diluted brine), and sealed with a plastic laminated film. The production process can be performed both manually and mechanically (Di Cerbo et al., 2020; Faccia et al., 2013). Mechanical manufacturing involves the use of the so-called "blower machine," made of a dosing funnel (the hopper to load stracciatella) connected to a compressed air insufflation device that injects stracciatella into the mozzarella ball; this way, the ball inflates and becomes a pouch.

For a long time, Burrata was only marketed in Italy, but in the last decade its demand abroad has sharply increased. Unfortunately, the short shelf life represents an important obstacle to the export for many dairy companies. The high perishability depends on the high moisture and fat content, availability of lactose, and mild pH. Under these conditions, the spoilage microorganism can easily grow causing loss of texture and formation of undesired flavors that, together with possible fat oxidation, rapidly lead to unacceptability of the cheese (Conte et al., 2011; Gammariello et al., 2009; Kilcast & Subramaniam, 2011). Several studies have been conducted to improve the quality characteristics of this cheese. Trani et al. (2016) and Costantino et al. (2020) tried to produce a reduced-fat type: the former used carob seeds flour to partially replace the fat and fortified the obtained cheese with polyunsaturated-fatty acids (PUFAs) to obtain a healthier product; the latter used exopolysaccharide-producing lactic acid bacteria and natural fibers as fat-replacers. Other authors applied different approaches and techniques to extend shelf life. Minervini et al. (2017) obtained interesting results by fortifying the cheese with protective lactobacilli to slow the growth of spoilage microorganisms (staphylococci, coliforms, and *Pseudonomas* spp.). Such a strategy also improved the flavor of the cheese, whereas poor results were obtained when the same protective culture was also added into the governing liquid. Other studies obtained a shelf life extension by using modified atmosphere conditions (MAP) having low concentration or lack of oxygen and high carbon dioxide content, also combined with N_2: this approach improved the microbial stability and sensory acceptability, and reduced lipid oxidation (Costa, Lucera, et al., 2016; Daniels et al., 1985; Dermiki et al., 2008; Gammariello et al., 2009). Other researchers studied the effect of Na_2-EDTA, lysozyme and lactoperoxidase, essential oils, nanoparticles (copper and silver) or active coatings on cheese shelf life, obtaining interesting results (Belewu et al., 2012; Conte et al., 2007, 2009, 2011, 2013; Costa, Conte, et al., 2016; Costa et al., 2012, 2017; De Azeredo 2009; Del Nobile et al., 2012; Fernandez et al., 2009; Gammariello et al., 2011; Incoronato et al., 2011; Kennedy et al.,2000; Martins et al., 2010; Mastromatteo et al., 2015, 2010; Sinigaglia et al., 2008; Vannini et al., 2004).

From the above studies, it clearly appears that the major issue for Burrata is keeping under control the activity of the spoilage microorganisms. In this perspective, the application of low temperatures throughout the entire processing phase could be a useful tool to improve shelf life. The aim of the present investigation was to study the effect of a sudden drop of the temperature during manufacturing, using solid carbon dioxide (dry ice) as a cryogenic agent.

2 | MATERIAL AND METHODS

2.1 | Sample preparation

The cheese samples used in the investigation were prepared as reported in Figure 1 in a dairy located in the province of Bari (Apulia, Southern Italy), specialized in Burrata production. Two different methods were used to apply dry ice (Generally Recognized as Safe- GRAS status bought in a local market) during manufacturing: one involved the use of a modified blowing machine (Alpico Srl, Gioia del Colle, Italy), the other one contemplated a manual procedure. The blowing machine had the hopper modified, since it was made of two concentric funnels that created a gap to contain dry ice for continuously refrigerating stracciatella during the filling phase (samples coded DI). The manual method involved an operator manually adding a single flake of dry ice into the pouch after the filling phase for each sample, just before closing (samples coded INS). A control burrata (CTR), without the use of dry ice, was also prepared in each trial. All trials were done in duplicate, in which each sample was made by using the same bulk milk. After preparation, the cheese samples (about 100 g each) were packaged and suddenly transported under refrigeration to the laboratory where they were stored under refrigerated condition (4 ± 2°C) for 21 days. Samples were taken for the analyses at day 0, 7, 14 and 21.

2.2 | Chemical analyses

2.2.1 | Lactose and organic acids

These compounds were used as indices of alteration caused by microbial activity Natrella, Difonzo, et al. (2020). Lactose was analyzed following the method reported by Kabakcı et al. (2020) with slight modifications. In short, 10 g of homogenized burrata were added with 20 ml of water, shacked for 1 h and centrifuged at 6000 RCF for 10 min at 4°C. The supernatant was filtered using a 0.2 mm syringe filter, then a 10 µl aliquot was injected into an HPLC system (Agilent technologies 1260 Infinity) with refractive index detector (Agilent technologies, Palo Alto, CA) equipped with a Rezex RCM-monosaccharide Ca^+ column (300 × 7.8 mm, Phenomenex, Torrance, CA, USA) heated at 80°C. Deionized water was used as mobile phase at 1 ml min^{-1} flow rate (isocratic condition). The detector temperature was held at 40°C. Lactose identification was done by using a reference standard and quantification was performed with an external calibration curve. Organic acids were extracted as reported by Buffa et al. (2004) and analyzed as reported in a previous paper (Natrella, Difonzo, et al., 2020). The analysis was done using a Waters HPLC apparatus composed of 600E pumps and a 996-diode array detector (Waters Corporation, Milford, CT, USA). The system was equipped with a Synergy Hydro RP column 80 Å, 4 µm, 250 mm × 4.6 mm (Phenomenex, Torrance, CA, USA). The analytes were separated by using 0.1% orthophosphoric acid in water (eluent A) and acetonitrile (eluent B) as mobile phase. The gradient was 0–18 min 100% A at 1 ml min^{-1} flow rate, then 18–18.3 min from 100% to 20% A; 18.3–19.5 min increasing flow rate to 1.4 ml min^{-1}, then 19.5–22.5 isocratic and 22.5–23 min from 20% to 100% A and 23–43 min isocratic. Detection was done at $\lambda = 214$ nm. Results were expressed as mg g^{-1} of sample.

2.2.2 | Free fatty acids

Free fatty acids (FFAs), whose concentration is an index of lipolysis, were extracted from the cheese according to the procedure reported

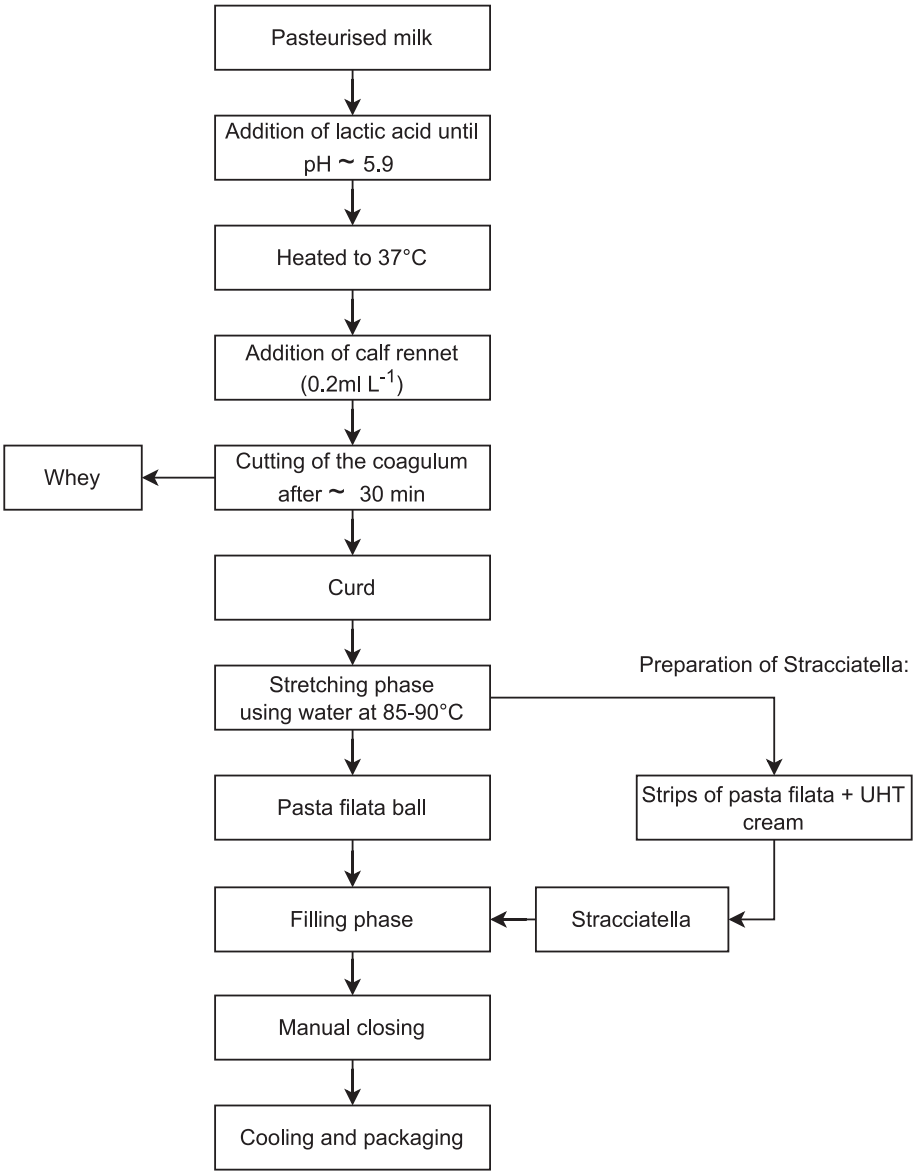

FIGURE 1 Scheme of burrata manufacturing.

by McCarthy et al. (2017). Two microliters of FFA extract were injected into a GC-FID apparatus 7890A GC-System (Agilent Technologies, Palo Alto, CA, USA) operating in splitless mode. The injector port temperature was held at 260°C, and the analytes separation was performed using a HP5 column (30 m × 0.32 mm × 0.25 μm; Agilent Technologies). The oven conditions were as follows: starting temperature 35°C held for 1 min, then 15°C min^{-1} until 75°C, once reached the temperature was held for 1 min, 3°C min^{-1} until 90°C, 20°C min^{-1} until 180°C, finally isothermal temperature for 5 min. FID temperature was set at 220°C. Results were expressed as mg g^{-1} of sample.

2.2.3 | Volatile organic compounds

The volatile compounds were analyzed by solid phase micro-extraction gas chromatography with mass spectrometry detection (SPME GC–MS). The homogenized cheese samples were weighed (1 ± 0.05 g) into 20 ml vials, closed by a silicone/PTFE septum and an aluminum cap, then were added with internal standard (81.3 ng 3-pentanone) for semi-quantitation. The vials were loaded in a refrigerated Triplus RSH autosampler, equipped with a divinylbenzene/carboxen/polydimethylsiloxane 50/30 mm SPME fiber assembly (Supelco, Bellefonte, PA, USA). Before extraction, stabilization of the headspace in the vial was obtained by equilibration for 10 min at 37°C, then the extraction was carried out at 37°C for 15 min. The fiber was desorbed at 220°C for 2 min in the injection port of the gas chromatograph, operating in splitless mode. The GC–MS analyses were performed using a Trace1300 gas chromatograph equipped with a mass spectrometer ISQ Series 3.2 SP1 (Thermo Fisher Scientific, Rodano, Italy). The compounds were separated on a Thermo capillary column VF- WAX MS thermo capillary column (60 m, 0.25 μm i.d., 0.25 mm, Agilent J&W), under the following conditions: injection port temperature, 220°C; oven temperatures,

50°C for 0.1 min then 13°C min^{-1} to 180°C, 18°C min^{-1} to 220°C final isothermal for 1.5 min. Mass detector was set at the following conditions: detector voltage, 1700 V; source temperature, 250°C; ionization energy, 70 eV; scan range 33–200 amu. Peak identification was done by means of Xcalibur V2.0 software, in particular Qual Browse, by matching with the reference mass spectra of NIST library.

2.3 | Microbiological analysis

For the microbiological analyses, 10 g of cheese sample was weighted in a Stomacher bag, added of 90 ml of Butterfield's phosphate-buffered water (Difco, Sparks, MD, USA), and homogenized using a BagMixer stomacher (Interscience, St Nom, France). Subsequently, homogenate was serially diluted (10-fold) and plated on the appropriate media in Petri dishes following the standard methods: *Enterobacteriaceae* (ISO 21528-2:2017; ISO, 2017b); yeasts and molds (ISO 21527-1:2012; ISO, 2012b); *Pseudomonas spp*. (ISO/TS 11059:2009; ISO, 2017d) (IDF/RM 225:2009); *Escherichia coli* (ISO 16649-3:2015; ISO, 2015); coagulase-positive Staphylococci (UNI EN ISO 6888-1:2018; ISO, 2018); *Salmonella spp*. (ISO 6579-1:2017; ISO, 2017a); and finally *Listeria Monocytogenes* (ISO 11290-1:2017; ISO, 2017c).

2.4 | Sensory analysis

A qualitative sensory analysis was performed by a trained panel composed of five experts belonging to the Italian Association of Cheese Tasters (ONAF) with more than 3 years of experience in cheese tasting. They had been selected following international standards (ISO 8586:2012; ISO, 2012a), had followed two courses for the evaluation of cheese texture, taste and aroma (20 h in total), and had been specifically trained to evaluate Burrata (three sessions of 2 h each). Each assessor received a form in which a series of odor and taste descriptors, taken from the ONAF vocabulary (Gambera, 2018), were indicated. The panelists were asked to mark with an "X" the descriptors they perceived. The samples were analyzed at day 0, 7, 14, 21. From day 14, panelists were asked to smell, taste, spat out the samples and wash their mouth for safety reasons, since manufacturer gave 10 days of shelf life.

2.5 | Statistical analysis

All chemical, sensory and microbiological analyses were carried out in duplicate. The analysis of variance (ANOVA) and temporal check-all-that-apply analysis were performed by XLstat software (Addinsoft, Paris, France).

3 | RESULTS

Table 1 shows the lactose and organic acid contents in the samples. During storage, lactose showed a decreasing trend, as expected: CTR and DI samples had the same trend (from 13 to 0.71 mg g^{-1}), whereas the concentration in INS at the end of storage was under the limit of detection. As to the organic acids, lactic and acetic acid underwent to an uptrend, whereas citric acid decreased during storage. In particular, the lactic acid content at T0 was very low, ranging from 0.05 to 0.08 mg g^{-1}, but it raised to 4.92–4.83 mg g^{-1} for CTR and DI, respectively, and to 2.8 mg g^{-1} for INS at T21. CTR from T7 to T21 (along with DI) had the highest lactic acid content. Also, the increasing trend of acetic acid was different among samples. In fact, its concentration at day 0 was very low for all samples, ranging from 0.04 to 0.07 mg g^{-1}, then it raised to reach at the end of storage the concentration of 0.36 and 0.34 mg g^{-1} for CTR and DI, respectively, and 0.18 mg g^{-1} for INS. Citric acid concentration at T0 was around 1 mg g^{-1} in all samples, but after 7 days of storage CTR had the lowest level (0.13 mg g^{-1}), whereas both DI and INS had a statistically higher content than CTR (0.47 and 0.37 mg g^{-1}, respectively). After 21 days this compound was almost totally degraded, with no differences among the three types of sample.

Figure 2 shows the total FFA amounts found during storage. No differences were found at day 0, being FFA present at very low concentration. After 7 days lipolysis started, and the highest amounts were found in DI, followed by CTR and INS (13.85, 7.05 and 5.10 mg g^{-1}, respectively). After 14 days of storage, CTR showed a greater increase compared to DI and INS, the latter being the sample with the lowest concentration. At the end of the storage period, CTR still was the sample with the highest total FFA content (10.15 mg g^{-1}), followed by DI and INS, which were very similar (8.62 and 8.4 mg g^{-1}, respectively). Table 2 shows the evolution of the single FFA during the experimentation. As observed for the total amounts, no differences were found at day 0, but after 7 days DI showed higher amounts of butyric, caproic, capric, lauric, myristic, palmitic, stearic, and oleic acid than the other two samples. After 14 days the short- and medium-chain FFA showed a decreasing trend, whereas the opposite happened for almost all the long-chain FFA (except for linolenic acid). At the end of storage, CTR was characterized by higher levels of capric, lauric, palmitic, and oleic acid; DI by greater amounts of butyric and caproic, and INS by higher level of linoleic acid. In general, for all cheeses the most abundant FFA found was palmitic, followed by stearic and myristic. The former reached the highest level in DI and CTR after 7 days of storage (5.2 and 3.82 mg g^{-1}, respectively), whereas its formation was slower in INS, reaching the highest concentration at the end of the storage (2.5 mg g^{-1}). Stearic progressively increased over time, except for the value at T14 in the two experimental samples, and the final concentration was not much different among samples, even though INS reached a lower level. Myristic acid was highest in DI at T7, with a three-fold higher concentration than CTR and INS, but during the successive period of storage, a reduction was observed until reaching a value of 0.96 mg g^{-1}. Differently, CTR and INS had a less marked variation almost constant level until T21, with CTR content slightly higher than INS.

The microbiological counts in the samples during storage are reported in Table 3. CTR had higher counts of *Enterobacteriaceae* and

TABLE 1 Lactose and organic acid content in burrata samples. Data expressed as mg g^{-1} of sample

	Storage (days)	CTR	DI	INS
Lactose	T0	$13.77 \pm 0.52^{A,a}$	$13.45 \pm 0.14^{A,a}$	$12.68 \pm 0.01^{A,a}$
	T7	$7.62 \pm 0.01^{B,a}$	$6.80 \pm 0.01^{B,b}$	$5.89 \pm 0.24^{B,b}$
	T14	$2.06 \pm 0.04^{C,b}$	$2.77 \pm 0.01^{C,a}$	$2.05 \pm 0.01^{C,b}$
	T21	$0.71 \pm 0.5^{C,a}$	$0.71 \pm 0.5^{D,a}$	$0.00 \pm 0.00^{D,b}$
Lactic acid	T0	$0.05 \pm 0.00^{C,a}$	$0.09 \pm 0.02^{D,a}$	$0.08 \pm 0.01^{D,a}$
	T7	$1.98 \pm 0.01^{B,a}$	$2.22 \pm 0.02^{C,a}$	$1.81 \pm 0.03^{C,b}$
	T14	$4.82 \pm 0.01^{A,a}$	$4.20 \pm 0.00^{B,b}$	$4.32 \pm 0.02^{A,b}$
	T21	$4.92 \pm 0.03^{A,a}$	$4.83 \pm 0.01^{A,a}$	$2.80 \pm 0.04^{B,b}$
Acetic acid	T0	$0.07 \pm 0.00^{B,a}$	$0.04 \pm 0.01^{C,a}$	$0.04 \pm 0.01^{C,a}$
	T7	$0.34 \pm 0.01^{A,a}$	$0.23 \pm 0.01^{B,b}$	$0.16 \pm 0.00^{B,c}$
	T14	$0.38 \pm 0.00^{A,a}$	$0.36 \pm 0.02^{A,ab}$	$0.32 \pm 0.00^{A,b}$
	T21	$0.36 \pm 0.00^{A,a}$	$0.34 \pm 0.04^{AB,a}$	$0.18 \pm 0.02^{B,b}$
Citric acid	T0	$1.13 \pm 0.00^{A,a}$	$1.10 \pm 0.04^{A,a}$	$1.00 \pm 0.05^{A,a}$
	T7	$0.13 \pm 0.01^{B,b}$	$0.47 \pm 0.04^{B,a}$	$0.37 \pm 0.06^{B,a}$
	T14	$0.02 \pm 0.00^{C,a}$	$0.09 \pm 0.05^{C,a}$	$0.05 \pm 0.01^{C,a}$
	T21	$0.08 \pm 0.05^{BC,a}$	$0.03 \pm 0.00^{C,a}$	$0.03 \pm 0.00^{C,a}$

Note: Uppercase letter shows the statistical difference of a single analyte during the shelf life considering samples separately; lowercase letter shows the statistical differences of a single analyte in a specific T among samples. $p < .05$.

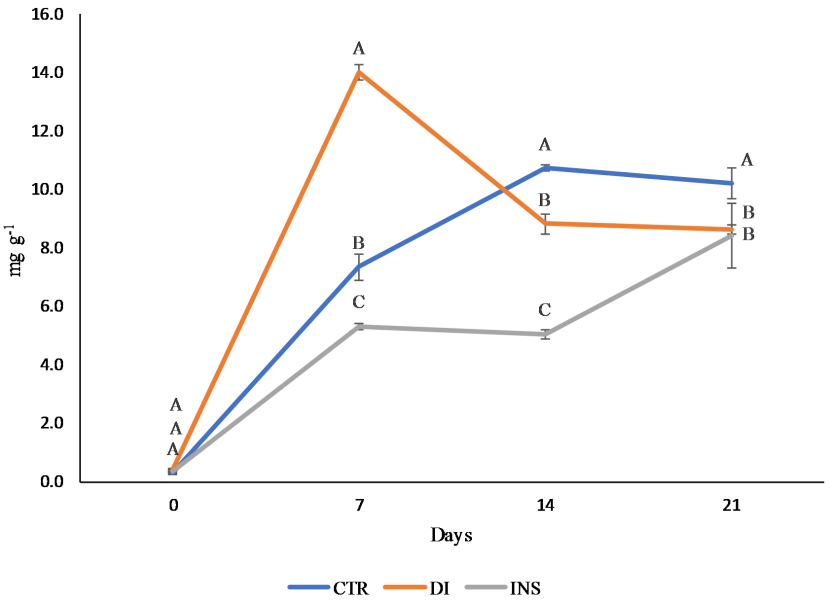

FIGURE 2 Total free fatty acid content in burrata samples during storage. Different letters indicate significant differences at $p < .05$. CTR, control; DI, dry ice; INS, dry ice inside. Results expressed as mg g^{-1}.

coliforms than treated samples from 7 days of storage until the 21st day. *E. coli* and *Staphylococcus aureus* showed no differences among samples during the whole storage time. On the other hand, the counts of *Pseudomonas ssp.* were higher in CTR than treated samples from T7 until the end of the storage, in which was very high with respect to the treated samples.

Table 4 shows the VOC profile of the samples (compounds by chemical classes). At the beginning of the storage period, the total amount was not different, but aldehydes and sulfur compound were more abundant in DI and INS than CTR, whereas CTR contained the highest level of esters, followed by DI and INS. After 7 days of storage, the total amount became different, with CTR and INS having higher abundance of VOC than DI. In particular, CTR had higher amount of sulfur compounds, ketones, aldehydes and alcohols than DI, whereas esters reached the highest concentration in INS. Total amount of VOC at T14 showed a rapid increase from about 700 to 4100 μg kg^{-1} in CTR sample, and from about 400–600 μg kg^{-1} to 2700–2300 μg kg^{-1} for DI and INS, respectively. Highest amount of acids, alcohols, and alkane were found in CTR; on the other hand, esters were highest in DI and INS. At the end of storage, CTR still

TABLE 2 Free fatty acid content in burrata samples. Data expressed as mg g^{-1} of sample

	Storage (days)	CTR	DI	INS
Butyric acid (C4:0)	T0	$0.04 \pm 0.00^{C,a}$	$0.03 \pm 0.00^{C,a}$	$0.05 \pm 0.00^{B,a}$
	T7	$0.24 \pm 0.02^{B,b}$	$0.40 \pm 0.01^{A,a}$	$0.17 \pm 0.02^{A,b}$
	T14	$0.37 \pm 0.02^{A,a}$	$0.23 \pm 0.03^{B,ab}$	$0.19 \pm 0.01^{A,b}$
	T21	$0.21 \pm 0.00^{B,b}$	$0.28 \pm 0.01^{B,a}$	$0.16 \pm 0.01^{A,c}$
Caproic acid (C6:0)	T0	$0.03 \pm 0.00^{C,a}$	$0.04 \pm 0.00^{C,a}$	$0.02 \pm 0.00^{C,a}$
	T7	$0.23 \pm 0.01^{A,b}$	$0.30 \pm 0.01^{A,a}$	$0.20 \pm 0.01^{A,b}$
	T14	$0.25 \pm 0.00^{A,a}$	$0.21 \pm 0.02^{AB,a}$	$0.19 \pm 0.01^{A,a}$
	T21	$0.14 \pm 0.01^{B,b}$	$0.19 \pm 0.01^{B,a}$	$0.13 \pm 0.01^{B,b}$
Caprylic acid (C8:0)	T0	$0.00 \pm 0.00^{B,b}$	$0.02 \pm 0.00^{C,a}$	$0.01 \pm 0.00^{B,a}$
	T7	$0.00 \pm 0.00^{B,b}$	$0.24 \pm 0.00^{A,a}$	$0.20 \pm 0.03^{A,a}$
	T14	$0.24 \pm 0.03^{A,a}$	$0.17 \pm 0.01^{B,a}$	$0.19 \pm 0.02^{A,a}$
	T21	$0.22 \pm 0.06^{A,a}$	$0.20 \pm 0.01^{AB,a}$	$0.14 \pm 0.03^{A,a}$
Capric acid (C10:0)	T0	$0.00 \pm 0.00^{C,b}$	$0.05 \pm 0.00^{C,a}$	$0.03 \pm 0.00^{B,a}$
	T7	$0.10 \pm 0.07^{BC,b}$	$0.70 \pm 0.04^{A,a}$	$0.17 \pm 0.00^{A,b}$
	T14	$0.49 \pm 0.02^{A,a}$	$0.40 \pm 0.03^{B,a}$	$0.24 \pm 0.02^{A,b}$
	T21	$0.40 \pm 0.08^{AB,a}$	$0.30 \pm 0.02^{B,b}$	$0.17 \pm 0.01^{A,b}$
Lauric acid (C12:0)	T0	$0.02 \pm 0.00^{B,b}$	$0.05 \pm 0.00^{D,a}$	$0.02 \pm 0.00^{C,b}$
	T7	$0.18 \pm 0.13^{AB,b}$	$0.83 \pm 0.03^{A,a}$	$0.28 \pm 0.03^{AB,b}$
	T14	$0.61 \pm 0.04^{A,a}$	$0.49 \pm 0.01^{B,a}$	$0.34 \pm 0.02^{A,b}$
	T21	$0.41 \pm 0.01^{AB,a}$	$0.35 \pm 0.01^{C,b}$	$0.23 \pm 0.01^{B,c}$
Myristic acid (C14:0)	T0	$0.04 \pm 0.00^{C,a}$	$0.06 \pm 0.00^{D,a}$	$0.05 \pm 0.00^{C,a}$
	T7	$0.68 \pm 0.02^{B,b}$	$2.03 \pm 0.02^{A,a}$	$0.58 \pm 0.02^{B,b}$
	T14	$1.60 \pm 0.06^{A,a}$	$1.33 \pm 0.05^{B,a}$	$0.75 \pm 0.03^{A,b}$
	T21	$1.24 \pm 0.1^{A,a}$	$0.96 \pm 0.06^{C,ab}$	$0.72 \pm 0.05^{AB,b}$
Palmitic acid (C16:0)	T0	$0.06 \pm 0.00^{B,a}$	$0.06 \pm 0.00^{C,a}$	$0.05 \pm 0.00^{C,a}$
	T7	$3.82 \pm 1.19^{A,ab}$	$5.20 \pm 0.11^{A,a}$	$1.76 \pm 0.07^{B,b}$
	T14	$3.63 \pm 0.02^{A,a}$	$3.26 \pm 0.07^{B,b}$	$1.54 \pm 0.08^{B,c}$
	T21	$3.68 \pm 0.12^{A,a}$	$3.35 \pm 0.03^{B,b}$	$2.5 \pm 0.06^{A,c}$
Stearic acid (C18:0)	T0	$0.04 \pm 0.00^{B,a}$	$0.03 \pm 0.00^{C,a}$	$0.03 \pm 0.00^{D,a}$
	T7	$0.88 \pm 0.14^{A,ab}$	$1.43 \pm 0.05^{A,a}$	$0.78 \pm 0.03^{B,b}$
	T14	$1.11 \pm 0.01^{A,a}$	$0.82 \pm 0.03^{B,b}$	$0.53 \pm 0.04^{C,c}$
	T21	$1.44 \pm 0.06^{A,ab}$	$1.72 \pm 0.06^{A,a}$	$1.27 \pm 0.04^{A,b}$
Oleic acid (C18:1)	T0	$0.01 \pm 0.00^{B,a}$	$0.03 \pm 0.00^{D,a}$	$0.01 \pm 0.00^{C,a}$
	T7	$0.36 \pm 0.25^{B,b}$	$2.23 \pm 0.05^{A,a}$	$0.55 \pm 0.03^{B,b}$
	T14	$1.67 \pm 0.03^{A,a}$	$1.34 \pm 0.05^{B,b}$	$0.5 \pm 0.02^{B,c}$
	T21	$1.77 \pm 0.05^{A,a}$	$0.84 \pm 0.01^{C,b}$	$1.61 \pm 0.19^{A,ab}$
Linoleic acid (C18:2)	T0	$0.01 \pm 0.00^{B,a}$	$0.01 \pm 0.00^{B,a}$	$0.01 \pm 0.00^{C,a}$
	T7	$0.26 \pm 0.01^{A,a}$	$0.32 \pm 0.02^{A,a}$	$0.20 \pm 0.03^{B,a}$
	T14	$0.30 \pm 0.06^{A,a}$	$0.30 \pm 0.06^{A,a}$	$0.22 \pm 0.01^{B,a}$
	T21	$0.52 \pm 0.07^{A,b}$	$0.23 \pm 0.02^{A,b}$	$1.07 \pm 0.13^{A,a}$
Linolenic acid (C18:3)	T0	$0.02 \pm 0.00^{C,a}$	$0.00 \pm 0.00^{B,b}$	$0.02 \pm 0.00^{A,a}$
	T7	$0.30 \pm 0.01^{AB,a}$	$0.17 \pm 0.02^{A,a}$	$0.20 \pm 0.03^{A,a}$
	T14	$0.46 \pm 0.03^{A,a}$	$0.27 \pm 0.01^{A,b}$	$0.33 \pm 0.03^{A,b}$
	T21	$0.18 \pm 0.02^{B,a}$	$0.20 \pm 0.01^{A,a}$	$0.36 \pm 0.03^{A,a}$

Note: For each free fatty acid, uppercase letter indicates the statistical difference during storage (CTR, DI, and INS samples were considered separately); lowercase letter indicates statistical differences among samples (sampling times were considered separately). $p < .05$.

had the highest total amount of VOC (8879.2 $\mu g kg^{-1}$), followed by DI and INS having 7099.28 and 5509.68 $\mu g kg^{-1}$, respectively. Almost all the chemical classes, except for ketones and esters, were more abundant in CTR cheese. Regarding the differences in the single VOC (data not shown), at day 0 dimethyl sulfide was higher in treated samples than CTR, whereas dimethyl sulfone was found only in DI sample; 3-hydroxy-2-butanone and nonanal were found only in treated samples; ethyl acetate was the only ester found in CTR and DI, whereas INS had no esters. After 7 days, ethyl acetate underwent to a rapid increase, more markedly in CTR sample, as well as ethanol and 3-methylbutanol (from 6 to 399 $\mu g kg^{-1}$ and from 3.48 to 136.16 $\mu g kg^{-1}$ in CTR). Moreover, other newly formed alcohols were found (i.e., 1-butanol; 2-ethyl-1-hexanol, 2-propanol). The most represented aldehyde in all samples was 3-methylbutanal, with the highest amount in CTR (9.25 $\mu g kg^{-1}$ vs. 4.15 and 1.76 $\mu g kg^{-1}$ for CTR, DI, and INS, respectively). Highest value of hexane, heptane, 2-heptanone, 2-nonanone, 2-undecanone, hexanal, isopropyl alcohol, ethanol, 2-pentanol, and many other were found in CTR sample at T14. On the other hand, DI and INS had highest concentration of esters, such as ethyl acetate, ethyl butanoate, ethyl hexanoate and ethyl octanoate. At the end of the storage period, some compounds had a major contribution to each single chemical classes' abundance. Hence, hexane was the highest alkane found in CTR and INS, heptane for DI. Ethyl acetate was the highest ester found in INS and DI (1855.26 and 500.50 $\mu g kg^{-1}$, respectively), followed by CTR with more than 70-fold lower amount. Among alcohols, ethanol increased in all samples at different rate, CTR had the highest amount followed by DI and INS (5027.09, 3540.44 and 2031.60 $\mu g kg^{-1}$, respectively). The same was for acetic acid (1793.65, 1252.62, 476.77 $\mu g kg^{-1}$ for CTR, DI, and INS, respectively).

Figure 3 shows the multivariate statistical analysis of the panel test results. All the fresh cheeses samples had a mild and delicate aroma of cream and milk, sweet taste and an elastic texture. After 7 days, the cheese still had mild notes and no differences were found among samples. After 14 days, texture, aroma, and taste changed: the texture lost consistency and became more soluble, the aroma got worst and evidenced animal or boiled vegetable note, and an acid taste was perceivable, mostly on CTR sample. At the end of the storage, CTR was very different from the two treated samples, and was characterized by the worst descriptors and clearly had unpleasant odors (i.e., sulfuric and fermented note). Unlike CTR, the best sample was INS, with buttery note as predominant odor and no off-flavors were perceived.

4 | DISCUSSION

The results obtained in the experimentation allow to make a series of considerations. As reported by Izco et al. (2002) the lactose and organic acid contents are very useful to monitor the fermentation processes in cheese during storage/ripening. In the cheese under study, lactose reduction during storage was expected, but the drop was faster in control burrata during the first week. This finding

TABLE 3 Cell numbers (log CFU g^{-1} ±SD), average values of three triplicates of *Enterobacteriaceae*, coliforms, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas spp.*, yeasts, and molds during burrata storage

Log CFU g^{-1} ± SD	CTR	DI	INS	CTR	DI	INS	CTR	DI	INS	CTR	DI	INS
Storage (days)	T0			T7			T14			T21		
Enterobacteriaceae	2.1 ± 0.44^{a}	2.1 ± 0.32^{a}	2.0 ± 0.41^{a}	4.1 ± 0.10^{a}	3.7 ± 0.09^{b}	3.8 ± 0.11^{b}	5.4 ± 0.1^{a}	5.2 ± 0.07^{b}	5.0 ± 0.19^{b}	>6.0	5.9 ± 0.4^{a}	5.9 ± 0.12^{a}
Coliforms	2.0 ± 0.21^{a}	2.1 ± 0.3^{a}	2.0 ± 0.18^{a}	4.0 ± 0.15^{a}	3.6 ± 0.21^{a}	3.7 ± 0.13^{a}	5.3 ± 0.12^{a}	5.0 ± 0.15^{a}	5.0 ± 0.08^{a}	>6.0	5.6 ± 0.34^{a}	5.7 ± 0.44^{a}
E. coli	<1	<1	<1	<2	<2	<2	<2	<2	<2	<3	<3	<3
S. aureus	<1	<1	<1	<2	<2	<2	<2	<2	<2	<3	<3	<3
Pseudomonas	4.0 ± 0.25^{a}	3.8 ± 0.31^{a}	3.9 ± 0.18^{a}	5.8 ± 0.14^{a}	5.5 ± 0.1^{b}	5.4 ± 0.24^{b}	6.8 ± 0.2^{a}	6.0 ± 0.34^{b}	6.0 ± 0.54^{b}	>7	6.9 ± 0.48^{a}	6.8 ± 0.59^{a}
Yeasts	1.7 ± 0.34^{a}	1.7 ± 0.19^{a}	1.6 ± 0.22^{a}	2.9 ± 0.28^{a}	3.0 ± 0.51^{a}	2.9 ± 0.43^{a}	3.6 ± 0.54^{a}	3.8 ± 0.47^{a}	3.2 ± 0.3^{a}	3.9 ± 0.22^{a}	4.4 ± 0.3^{a}	3.8 ± 0.53^{a}
Molds	<1	<1	<1	<2	<2	<2	<2	<2	<2	<2	<2	<2

Note: Lowercase letter indicates statistical differences among samples (sampling times were considered separately). $p < .05$.

TABLE 4 Volatile compounds concentrations grouped by chemical classes. Results are expressed as μg kg^{-1}

	CTR	DI	INS	CTR	DI	INS
Storage (days)	T0			T7		
Alkanes	4.49 ±1.07[a]	2.96 ±0.44[a]	2.83 ±0.44[a]	4.62 ±0.1[a]	6.31 ±0.91[a]	3.73 ±0.11[a]
Sulfur compounds	1.13 ±0.1[b]	3.44 ±0.82[a]	1.76 ±0.84[ab]	3.85 ±0.14[a]	4.74 ±0.23[a]	1.55 ±0.04[b]
Ketones	108.92 ±7.66[a]	100.50 ±2.0[a]	93.59 ±10.1[a]	94.55 ±0.53[a]	80.39 ±7.98[ab]	66.00 ±3.47[b]
Esters	1.02 ±0.04[a]	0.67 ±0.04[b]	0.00 ±0.00[c]	11.19 ±0.09[c]	21.74 ±0.025[b]	392.65 ±32.52[a]
Aldhydes	0.96 ±0.07[b]	6.36 ±2.72[a]	5.41 ±2.35[a]	10.30 ±0.53[a]	5.95 ±1.38[b]	3.80 ±1.46[b]
Alcohols	10.87 ±0.76[a]	9.75 ±0.67[a]	8.88 ±0.27[a]	541.75 ±1.86[a]	187.85 ±6.23[b]	96.65 ±0.35[c]
Acids	5.59 ±0.90[a]	5.98 ±1.54[a]	7.01 ±1.2[a]	105.60 ±2.55[b]	128.78 ±2.21[a]	58.27 ±3.16[c]
Total	132.98 ±5.59[a]	129.65 ±3.58[a]	119.49 ±10.25[a]	771.85 ±88.4[a]	435.76 ±10.97[b]	622.64 ±81.1[a]

Note: Lowercase letter indicates statistical differences among samples (sampling times were considered separately). $p < .05$.

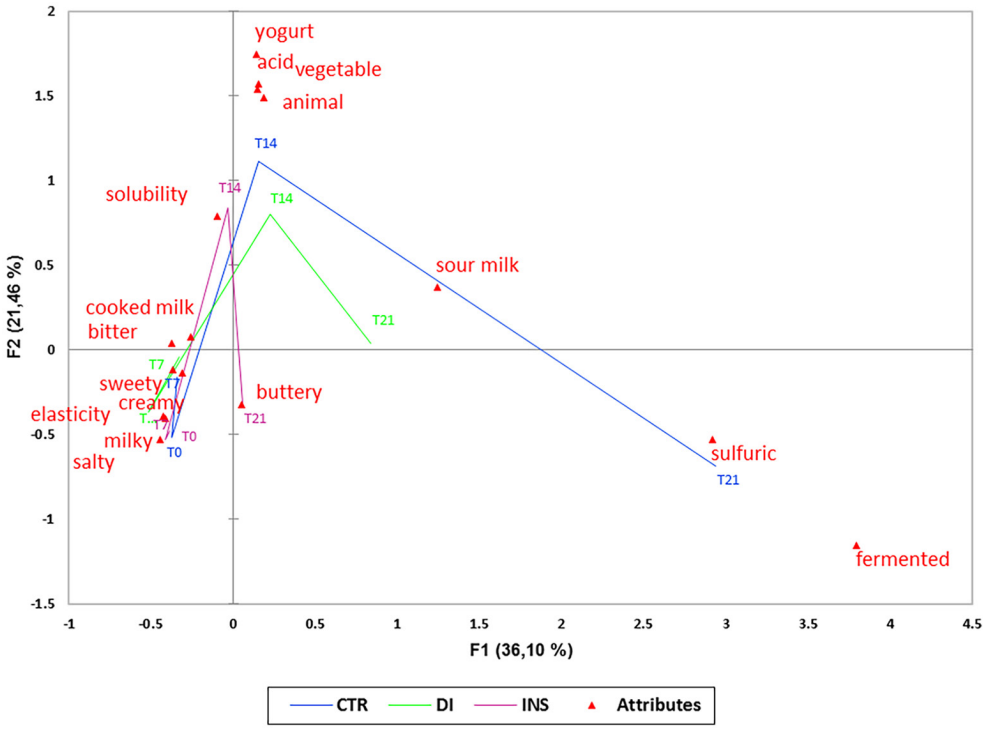

FIGURE 3 Multivariate statistical analysis (temporal check-all-that-apply) of the panel test results. CTR, control; DI, dry ice; INS, dry ice inside.

suggests an antimicrobial effect obtained by the application of dry ice, in particular when it was inserted into the cheese. Besides the lactose decrease rate, it was confirmed by the lower content of newly formed organic acids and the persistence of citric acid (deriving from milk). Regarding lipolysis, it is known that the formation of FFAs has an increasing trend during storage. This biochemical event occurs due to the presence of lipase and esterase from the raw matter, added with rennet or arising from starter and adventitious microflora, and is influenced by the storage/ripening conditions (Caboni et al., 1990; Cadwallader & Singh, 2009; Malacarne et al., 2006; Perotti et al., 2005; Poveda et al., 2000; Sandri et al., 1997; Sihufe et al., 2007). According to Cadwallader and Singh (2009) the short chain fatty acids (SCFA) have a positive role in cheese aroma, both directly (rancid, animal and pungent odors) and indirectly, as precursors of volatile organic compounds such as aldehydes, ketones, lactone, alcohols and esters. In burrata, FFAs play a different role with respect to ripened cheeses, and their formation is undesired for sensory reasons, as this cheese is characterized by poor and mild aroma and flavor (Natrella, Difonzo, et al., 2020). For this reason, no starter is used in burrata cheesemaking. As to the single FFA found, palmitic acid was the most abundant compound in all burrata samples: unfortunately, poor information is available in the scientific literature about lipolysis in fresh cheeses, but our finding is in agreement with the results reported by Evert-Arriagada et al. (2014) in a sterter-free fresh cheese. As far as the effect of dry ice treatment is concerned, it allowed to lower lipolysis, mostly

CTR	DI	INS	CTR	DI	INS
T14			T21		
118.04 ± 14.53^{a}	75.23 ± 4.69^{b}	78.99 ± 2.98^{b}	271.87 ± 6.5^{a}	188.44 ± 6.5^{b}	160.24 ± 9.9^{c}
5.22 ± 0.62^{a}	4.83 ± 0.14^{a}	4.58 ± 0.40^{a}	16.42 ± 0.58^{a}	15.83 ± 0.2^{b}	11.23 ± 0.25^{c}
27.26 ± 3.01^{a}	30.62 ± 1.43^{a}	22.00 ± 4.2^{a}	48.92 ± 1.61^{b}	87.77 ± 3.3^{a}	79.46 ± 26.95^{a}
20.16 ± 2.84^{b}	311.38 ± 18.56^{a}	311.28 ± 7.31^{a}	51.84 ± 2.92^{c}	645.43 ± 79.5^{b}	890.82 ± 88.2^{a}
6.80 ± 4.81^{a}	3.12 ± 0.5^{a}	2.48 ± 0.32^{a}	44.22 ± 7.93^{a}	30.66 ± 4.1^{b}	21.07 ± 0.25^{c}
2116.21 ± 230^{a}	1061.64 ± 55^{b}	958.20 ± 21.1^{c}	5725.24 ± 186^{a}	4068.25 ± 115^{b}	2281.64 ± 98.2^{c}
1822.50 ± 141^{a}	1239.02 ± 7.3^{b}	940.83 ± 46.4^{c}	2720.70 ± 148^{a}	2132.59 ± 182^{b}	765.23 ± 82.6^{c}
4116.18 ± 400^{a}	2725.85 ± 250^{b}	2318.38 ± 223^{b}	8879.20 ± 264^{a}	7099.28 ± 255^{b}	5509.68 ± 340^{c}

in INS sample; at the end of the storage the differences respect to the control sample regarded almost all FFA found. Considering that the treatment slowed down the growth of the most important spoilage bacteria groups such as *Enterobacteriaceae*, *Pseudomonas*, and coliforms, we can conclude that an important part of lipolysis derived from adventitious microflora. It is worth mentioning that all microorganisms (included lactic acid bacteria) in burrata are considered as spoilage agents and their presence is used as an index of the hygiene conditions of the milk and of the processing environment (Natrella, Difonzo, et al., 2020). The VOC profile can be used as an index of alteration because it is closely connected with microbial activity. In this study, the presence of some newly formed VOC matched well with the microbial counts, such as acetic acid, 3-hydroxy-2-butanone, heptane, 2-heptanone, 2-nonanone, 2-undecanone, hexanal, isopropyl alcohol, and ethanol, which were all more abundant in non-treated sample. Some other volatile compounds, such as 3-methyl-1-butanol, linear aldehydes, dimethyl sulfide, and dimethyl sulfone, which were more abundant in CTR, too, can also originate from lipidic oxidation, being burrata a fat-rich product (Chambers & Koppel, 2013). This finding suggests that the dry ice application could also slow down oxidation during storage. Dimethyl sulfide if present at concentration above 16 $\mu g\,kg^{-1}$ could confer unpleasant odor; whereas, dimethyl sulfone is a dimethyl sulfide oxidation product, suggesting a highest oxidation process in CTR followed by DI (Al-Attabi et al., 2008; Badings & de Jong, 2011; Natrella, Gambacorta, & Faccia, 2020). Linear aldehydes in cheese could originate from auto-oxidation of unsatured FFAs, such as octanal and nonanal (from oleic acid), hexanal and propanal (from linoleic and linolenic acid, respectively) (McSweeney & Fox, 2003). Among these, only nonanal followed the trend of its precursors; therefore, the pathways of formation of the other aldehydes should be different. Under the sensory point of view, the effect of the treatment appeared only after 14 days, when CTR and DI got worse while INS was the best sample and received the highest scores until the end of storage. Moreover, in accordance with VOC analysis INS had a pleasant odor, possibly in connection to the higher concentration of 3-hydroxy-2-butanone, and lesser presence of VOC responsible of off-flavors.

5 | CONCLUSION

In conclusion, the dry ice as a cryogen agent to control the microbial growth could be a promising solution to extent the shelf life of burrata. It provides for lowering temperatures during manufacturing process of burrata cheese. Between the two treated samples INS had better chemical and sensory characteristics, having lowest amount of oxidized compounds, VOC conferring pleasant odor and better sensory results even after 21 days, whereas DI maintained its good characteristics until T14, thereafter the arising of off-flavor compounds penalized the product, although it resulted better than CTR. Thus, inserting a flake of dry ice inside the burrata during manufacturing seems to delay the decay of the product, of course, if using a high-quality raw matter, the shelf life extension could be more significative. This is a non-invasive practice solution, in fact, there is no need to modify the manufacturing process; on the other hand, this solution is time-consuming and could be convenient only for a small dairy, which produces small quantity of burrata.

ACKNOWLEDGMENTS

This work was financially supported by the project PSR P.S.R. Puglia 2014/2020 - Misura 16 – Cooperazione – Sottomisura 16.2 "Sostegno a progetti pilota e allo sviluppo di nuovi prodotti, pratiche, processi e tecnologie" project BURRATA (CUP: B89J20000130007; DDS 94250034439). Open Access Funding provided by Universita degli Studi di Bari Aldo Moro within the CRUI-CARE Agreement.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

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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How to cite this article: Natrella, G., Gambacorta, G., & Faccia, M. (2022). Use of dry ice as innovative technology to preserve the chemical and microbial characteristics of burrata cheese. *Journal of Food Processing and Preservation*, *00*, e16908. https://doi.org/10.1111/jfpp.16908