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Microbial Risk Assessment of Industrial Ice Cream Marketed in Italy

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Featured Application: This study can be used by industrial ice cream manufacturers for the proper management of associated microbiological hazards.

Abstract: Ice cream is a frozen dairy dessert consumed worldwide. The frozen state may give a positive impression regarding microbiological safety; however, transmission of foodborne pathogens can also occur through ice cream consumption. A total of 200 samples of milk-based industrial ice cream, with and without inclusions, were purchased at different mass-market retailers in Italy and analyzed for the detection and enumeration of the aerobic colonies, *Enterobacteriaceae*, coagulase-positive staphylococci, *Salmonella* spp. and *Listeria monocytogenes*. Results were classified according to different ranges of acceptability, whose limits were set for each microbiological parameter. Unsatisfactory loads were obtained for two and nine samples as regarded the aerobic colonies and *Enterobacteriaceae*, respectively. *L. monocytogenes* was detected in 16 samples, and in three of them, the loads exceeded the legal limit of acceptability (≤100 cfu/g) during marketing. No unsatisfactory loads were obtained for coagulase-positive staphylococci and no *Salmonella* spp. was detected. The results obtained allow speculation that inclusions may be a relevant source of contamination for industrial ice cream. However, inadequate manufacturing and hygiene practices also threaten the safety of the finished product. Ice cream is a complex food matrix, and a comprehensive approach to the whole production system is required to ensure high standards of quality and safety.

Keywords: frozen dessert; inclusion; topping; candy pieces; bakery pieces; fruit pieces; manufacturing practice; hygiene practice; foodborne pathogens; *Listeria monocytogenes*

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1. Introduction

Within the frozen dairy desserts, ice cream is certainly one of the most popular and is consumed worldwide. Not by chance, although ice cream consumption is typically seasonal, the market demand is still considerable, as evidenced by the amounts of production, which reached over 2.9 billion liters in the E.U. and almost 5 billion liters in the U.S. in 2020 [1,2]. Furthermore, data reported by the International Dairy Foods Associations in 2021 show significant growth in the global ice cream market over the coming next years, estimating a turnover of USD 91.9 billion in 2027, a 30% increase from USD 70.9 billion in 2019 [3].

Ice cream can be defined as a complex food matrix consisting of a frozen multiphase mixture containing ice crystals, air bubbles and partially coalesced fat globules within an unfrozen serum phase of dissolved proteins, sugars and mineral salts [4,5].

Although there are many varieties of industrially manufactured ice cream with different formulations and presentations, it is possible to recognize common steps for all production [6–9]. Overall, the industrial production of ice cream involves the preparation of a liquid mix that is whipped and frozen to obtain a viscous semi-frozen mixture to which flavoring ingredients can be optionally added before being hardened and packaged. The liquid mix is obtained by blending the desired ingredients, which generally are water, dairy or non-dairy fats, milk solids-non-fat, sweeteners, stabilizers, emulsifiers and flavors, followed by pasteurization, homogenization and cold ageing steps. The obtained mix is pumped into a batch freezer, where it undergoes the first freezing step while incorporating air, thus obtaining a viscous mixture not yet completely frozen. Then, the mixture is dispensed into trays or pods by extruders and then hardened through a second freezing step, reducing the internal temperature between −25 and −30 °C. Flavorings or colorings continuous throughout the ice cream are added within the batch freezing step, while other discrete decorations are added just before the hardening step. The ice cream thus obtained is stored usually at −18 °C [6–9].

The shelf life of ice cream is relatively long (even over 2 years) and it is mainly related to physicochemical changes rather than microbial growth [6,10,11]. Indeed, despite the high nutritional value and the basically neutral pH, the frozen state prevents the growth of specific spoilage organisms [12,13]. However, what makes ice cream worthy of attention from a microbiological point of view is the significant amount of processing carried out after the pasteurization step [7]. This raises great concern especially for foodborne pathogens, which can contaminate the ice cream after the heat treatment from the working environments or through the addition of contaminated ingredients. Overall, only a few outbreaks of foodborne illness related to ice cream consumption have been recorded and these are linked to different pathogens, such as *Salmonella* spp., *Listeria monocytogenes*, verotoxin-producing *Escherichia coli* and coagulase-positive staphylococci [14–18]. Among these bacteria, the most severe outbreak was related to *Salmonella enteritidis*, involving 224,000 cases of infections in the U.S., as the result of cross-contamination during the transport of a pasteurized mix into inadequately sanitized tanks in which contaminated liquid eggs were previously transported [14].

From a risk assessment perspective, the health sensitivities of specific consumer categories, such as young, old, pregnant and immunocompromised (so-called YOPI), deserve particular attention since even low contaminating loads of pathogens could have fatal outcomes [19].

Against this background, although ice cream can be considered a microbiologically safe food, the manufacturing process and the wide consumption, even among consumer categories generally considered at risk, stress the importance of not underestimating the associated hazards.

Therefore, this study aimed to investigate the hygienic–sanitary quality of industrially manufactured ice creams commercially available at Italian mass-market retailers.

2. Materials and Methods

2.1. Sampling of Ice Creams

The present investigation was carried out on a total of 200 samples of ice cream in tubs, weighing 500 g and 1 kg, purchased at different stores of mass-market retailers in different regions of Northern and Southern Italy. From the label, it was possible to trace the country of production of each ice cream, which was Italy for 95.5% of the samples and Germany for the remaining 4.5% (see Table S1). All the ice cream samples contained milk ingredients and were of different flavors, with or without inclusions such as toppings or decorations (candy, bakery or fruit pieces). Briefly, 119 had inclusions (84 with both toppings and decorations, 24 with decorations only, 3 with toppings only), while 81 had neither toppings nor decorations. More in-depth details about the flavors and inclusions of the sampled ice creams are available among the supplementary files in Table S1.

The sampling was performed over 16 months, from May 2020 to September 2021. The samples were mostly collected during the summer months (between May and August), considering the increase in ice cream consumption typical of this period.

The choice of the ice creams to sample was based on the availability in the stores, collecting as many different flavors as possible. The ice cream samples with the same flavors were either from different manufacturers or from different production batches.

Once purchased, the samples collected in Southern Italy were transported inside coolers to the "Microbiology Laboratory of Food of Animal Origin" of the "Department of Veterinary Sciences" of the University of Messina (Italy), while those collected in Northern Italy were transported to the laboratory of the "Department of Health, Animal Science and Food Safety" of the University of Milano (Italy) and stored at −20 °C up to the time of analysis.

2.2. Microbiological Analyses

Samples were kept at room temperature for 10 min to ease the sampling step, paying particular attention to collecting a representative portion (including toppings and decorations) of each ice cream. From each sample, two aliquots of ~25 g were aseptically sampled and each placed into a sterile stomacher bag.

One aliquot was diluted with buffered peptone water (Biolife, Milano, Italy) in a ratio of 1:9 *w*/*v* and homogenized through a stomacher (400 Circulator; International PBI s.p.a., Milano, Italy) for 60 s at 230 rpm. Samples thus prepared were processed for the following bacteriological determinations: (i) enumeration of the aerobic colonies at 30 °C according to ISO 4833-1:2013 [20], on plates of Plate Count Agar (Biolife, Milano, Italy) incubated at 30 ± 1 °C for 72 ± 3 h; (ii) enumeration of *Enterobacteriaceae*, according to ISO 21528-2:2017 [21], on plates of Violet Red Bile Glucose Agar (Biolife, Milano, Italy) incubated at 37 ± 1 °C for 24 ± 2 h; (iii) enumeration of coagulase-positive staphylococci according to ISO 6888-1:2018 [22], on plates of Baird Parker Agar Base (Biolife, Milano, Italy) supplemented with Egg Yolk Tellurite Emulsion (Biolife, Milano, Italy) incubated at 37 ± 1 °C for 48 h; (iv) detection of *Salmonella* spp. according to ISO 6579-1:2017 [23], with enrichment in Mueller Kauffmann Tetrathionate Broth base (Biolife, Milano, Italy) and Rappaport Vassiliadis Soy Broth (Biolife, Milano, Italy), followed by a smear on plates of Chromogenic Salmonella Agar (Biolife, Milano, Italy) and Xylose Lysine Deoxycholate Agar (Biolife, Milano, Italy), both incubated at 37 ± 1 °C for 24 ± 3 h; (v) enumeration of *L. monocytogenes* according to ISO 11290-2:2017 [24] on plates of Agar Listeria according to Ottaviani and Agosti (Biolife, Milano, Italy) and Listeria Palcam Agar (Biolife, Milano, Italy), both incubated at 37 ± 1 °C for 48 ± 2 h.

The other aliquot was diluted with Fraser Broth Base (Biolife, Milano, Italy) in a ratio of 1:9 *w*/*v* and homogenized through a stomacher (400 Circulator; International PBI s.p.a., Milano, Italy) for 60 s at 230 rpm. Samples thus obtained were processed for the detection of *L. monocytogenes*, according to ISO 11290-1:2017 [25], on plates of Agar Listeria according to Ottaviani and Agosti and Listeria Palcam Agar, both incubated at 37 ± 1 °C for 48 ± 2 h.

The identification of all the isolated *Listeria monocytogenes* strains was further confirmed with Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) Mass Spectrometry (MS) [26]. A Vitek Mass Spectrometer Axima Assurance (bioMérieux, Firenze, Italy) was used with the following settings: positive linear mode, laser frequency of 50 Hz, acceleration voltage of 20 kV and extraction delay time of 200 ns. The mass spectra range was set to detect from 2000 to 20,000 Da. MALDI-TOF MS generated unique mass spectra for each tested colony, which were transferred into the SARAMIS software (Spectral Archive and Microbial Identification System, database version V4.12, software year 2013, bioMérieux, Firenze, Italy) and compared to the database of reference bacteria spectra and super spectra, obtaining identification at the genus and species levels. Only a match of at least 70% was considered reliable.

2.3. Data Analysis

The microbiological results obtained were expressed as colony-forming units per grams (cfu/g) of ice cream. The results were classified according to different ranges of acceptability, whose limits were set for each microbiological parameter according to the guidelines provided by the Interdepartmental Center for Research and Documentation on Food Safety (Ce.I.R.S.A) (Table 1) [27]. The number and percentage of samples whose values were within each range were then calculated.

Table 1. Recommended values for determining the acceptability levels of different microbiological parameters used as quality and safety indices of the ice cream according to the guidelines provided by the Interdepartmental Center for Research and Documentation on Food Safety (Ce.I.R.S.A).

Parameter	Recommended Guide Values (cfu/g)			
	Satisfactory	**Acceptable**	**Unsatisfactory**	**Potentially Harmful**
Aerobic colonies	$<10^5$	$10^5 \leq x \leq 5 \times 10^5$	$>5 \times 10^5$	–
Enterobacteriaceae	<10	$10 \leq x < 10^2$	$\geq 10^2$	–
Coagulase-positive staphylococci	$<10^2$	$10^2 \leq x < 10^4$	$\geq 10^4$	$\geq 10^5$
Listeria monocytogenes	Not detected	$\leq 10^2$	–	$>10^2$
Salmonella spp.	Not detected	–	–	Detected

Any significant differences regarding the enumeration of the aerobic colonies, *Enterobacteriaceae* and coagulase-positive staphylococci were evaluated between the ice cream samples that had at least one inclusion (toppings or decorations) and those that had none.

The normal distribution of the raw data of each microbiological parameter was tested by the D'Agostino–Pearson omnibus test, and the Mann–Whitney test was used to compare the two groups, with and without inclusions. Within each group of ice creams, any correlations between the loads obtained for each microbiological parameter were computed using the Spearman test.

The critical significance level (p) was set at 5% (0.05) and all tests were performed two-sided. All the statistical analyses were carried out using Graph Pad Prism 9 software (San Diego, CA, USA).

3. Results

The results obtained are summarized in Figure 1, while, in Tables S2 and S3, the individual values of each microbiological parameter are detailed for each ice cream sample, with inclusions and without, respectively.

The classification of the results divided according to the samples of ice cream with and without inclusions is shown in Table 2.

Overall, satisfactory values were observed in 195 (~97.5%) samples for the enumeration of aerobic colonies, in 155 (77.5%) samples for the enumeration of *Enterobacteriaceae* and 192 (96%) samples for the enumeration of coagulase-positive staphylococci. As regards the enumeration of the aerobic colonies, acceptable values were obtained in three (~1.5%) samples, while unsatisfactory values were obtained for two (~1%) samples (see, in Tables S1 and S2, the samples with ID: 77, 139), with the highest observed load of 7.1×10^5 cfu/g. As regards the enumeration of the *Enterobacteriaceae*, acceptable values were obtained in 36 (~18%) samples, while unsatisfactory values were obtained for nine (~4.5%) samples (see, in Tables S1 and S2, the samples with ID: 2, 52, 53, 56, 69, 108, 158, 182, 197), with the highest observed load of 1.6×10^4 cfu/g. As regards the enumeration of the coagulase-positive staphylococci, acceptable values were obtained in eight (4%) samples, while no unsatisfactory values were observed. All unsatisfactory values were obtained in ice cream samples with inclusions, for the enumeration of both aerobic colonies and *Enterobacteriaceae*.

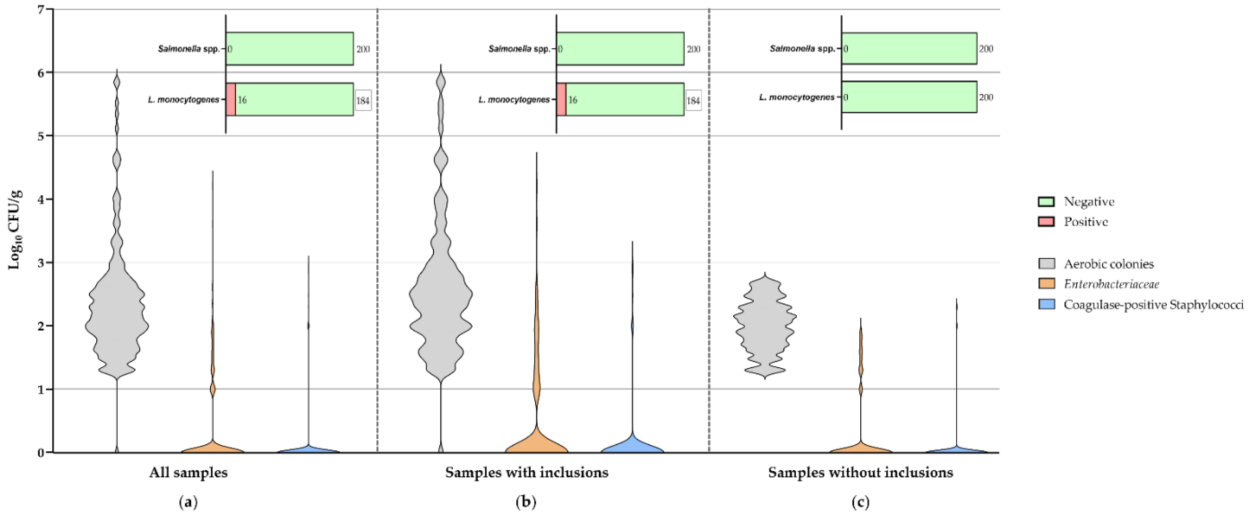

Figure 1. Distributions of the results of different microbiological parameters obtained for 200 samples of industrial ice cream collected at different mass-market retailers. The results were arranged considering (**a**) all samples, (**b**) the samples with inclusions (toppings or decorations) and (**c**) samples without inclusions.

Table 2. Classification of the results of different microbiological analyses obtained for 200 samples of milk-based industrial ice cream based on different ranges of acceptability set for each microbiological parameter according to the guidelines provided by the Interdepartmental Center for Research and Documentation on Food Safety (Ce.I.R.S.A).

Ice Cream Samples	Parameter	Number and Percentage of Samples			
		Satisfactory	Acceptable	Unsatisfactory	Potentially Harmful
With inclusions	Aerobic colonies	114 (95.80%)	3 (2.52%)	2 (1.68%)	–
	Enterobacteriaceae	89 (74.79%)	21 (17.65%)	9 (7.56%)	–
	Coagulase-positive staphylococci	113 (94.96%)	6 (5.04%)	0	0
	Listeria monocytogenes	103 (85.55%)	13 (10.92%)	–	3 (2.52%)
	Salmonella spp.	119 (100%)	–	–	0
Without inclusions	Aerobic colonies	81 (100%)	0	0	–
	Enterobacteriaceae	66 (81.48%)	15 (18.52%)	0	–
	Coagulase-positive staphylococci	79 (97.53%)	2 (2.47%)	0	0
	Listeria monocytogenes	81 (100%)	–	–	0
	Salmonella spp.	81 (100%)	–	–	0
Total	Aerobic colonies	195 (97.50%)	3 (1.50%)	2 (1.00%)	–
	Enterobacteriaceae	155 (77.5%)	36 (18.00%)	9 (4.50%)	–
	Coagulase-positive staphylococci	192 (96.00%)	8 (4.00%)	0	0
	Listeria monocytogenes	184 (92.00%)	13 (6.50%)	–	3 (1.50%)
	Salmonella spp.	200 (100%)	–	–	0

Salmonella spp. was not detected in any of the samples, while the presence of *L. monocytogenes* was detected in a total of 16 (~8%) samples (see, in Tables S1 and S2, the samples with ID: 3, 9, 15, 52, 54, 55, 56, 57, 60, 88, 108, 139, 148, 163, 182, 197), all ice creams with inclusions. Among the positive samples, the critical limit of 100 cfu of *L. monocytogenes* per g of product was exceeded in three samples (~1.5%) (see, in Tables S1 and S2, the samples with ID: 56, 60, 139) with loads of 110, 120 and 350 cfu/g, respectively. The presence of *L. monocytogens* was detected in five samples that had also unsatisfactory values of

Enterobacteriaceae (see, in Tables S1 and S2, the samples with ID: 52, 56, 108, 182, 197) and in one sample with unsatisfactory values of aerobic colonies (see, in Tables S1 and S2, the sample with ID: 139). However, no substantial correlations were found between the microbiological parameters, as shown in Figure 2.

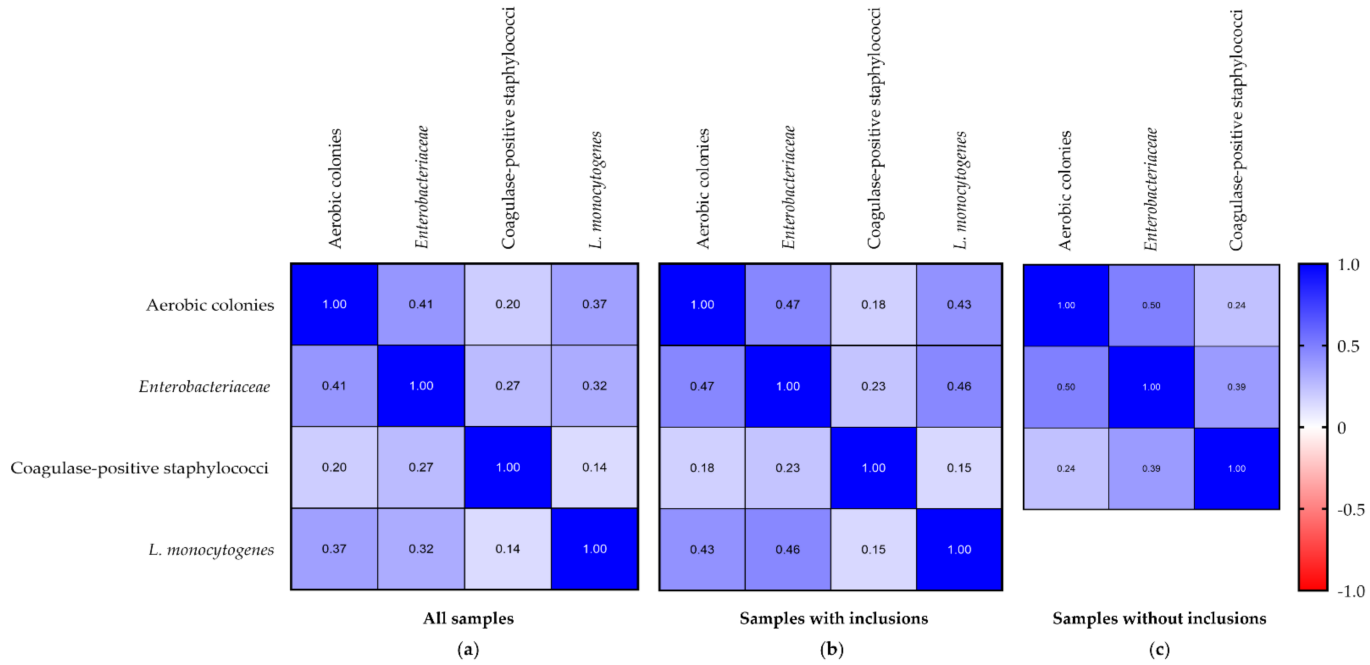

Figure 2. Correlation matrices between the results of different microbiological parameters obtained for 200 samples of industrial ice cream collected at different mass-market retailers. The value of the correlation coefficient (r) is reported inside each square. The results were arranged considering (**a**) all samples, (**b**) the samples with inclusions (toppings or decorations) and (**c**) samples without inclusions.

MALDI-TOFF MS analysis associated with SARAMIS confirmed the biochemical identification of all isolated strains of *L. monocytogenes*.

Significantly higher aerobic colony loads were observed for the ice cream samples with inclusions than for those without ($p < 0.0001$, U = 3065), while no significant differences were observed between the two groups regarding *Enterobacteriaceae* ($p < 0.2213$, U = 4460) and coagulase-positive staphylococci ($p < 0.3462$, U = 4694).

4. Discussion

Although freezing appears to ensure the high microbiological safety of ice cream, it does not exclude the fact that the transmission of foodborne pathogens to humans can also occur through its consumption [12,13,28–30]. This is stressed by the results of the present study, which revealed the presence of *L. monocytogenes* in 16 store-bought industrial ice creams, and, in three of them, the loads were even beyond the legal limit (<100 cfu/g) allowed in Europe [31]. Major concerns are associated with vulnerable people such as YOPI, for whom even low loads of pathogens can result in serious outcomes [19]. In this regard, we underline that ice cream is widely consumed by children and also recommended in postoperative recovery diets in patients undergoing various types of surgeries [32,33].

The microbiological quality and safety of industrial ice cream depend on many factors, which could be divided into three strictly interconnected groups: microbiological standards of the raw materials, the production process and the hygiene of the working environments [6]. Any ingredient used in the production of ice cream represents a source of microorganisms that can affect the composition of the microbial flora in the finished product [34]. In the first steps of production, the desired ingredients are blended to obtain a

liquid mix that will undergo a pasteurization treatment. If properly performed, pasteurization ensures the inactivation of pathogenic microorganisms and reduction of the vegetative microbial forms [35]. The use of raw materials with poor microbiological standards can negatively impact the effectiveness of the heat treatment, threatening the quality and safety of the product [6,36,37] Indeed, pasteurization treatments lose their effectiveness against spore-forms, thermoduric microorganisms, thermostable myco- and bacterial toxins and heat-resistant enzymes possibly present in the raw materials, which may spoil the product and pose risks for the consumer's health [35].

The raw materials commonly used to produce industrial ice cream are milk, water, cream, butter, sugars, dairy or non-dairy fats and additives such as thickeners, stabilizers and emulsifiers. The industrial production of ice cream involves the use of liquid or powdered milk that has already undergone a heat treatment by the supplier [38]. The water used to mix the dry ingredients together is of drinkable quality and should not pose a risk if the distribution and storage facilities are properly managed. Even cream and butter, in their production processes, are subjected to heat treatments that ensure their microbiological safety [39]. Contamination by sugars, used in powder or syrup, should be limited only to certain types of osmophilic microorganisms considering that the high sugar content and the production process make them almost sterile [34]. Moreover, fats and additives are usually produced with techniques that involve high temperatures and therefore they should not represent an important source of contamination [40,41]. In addition, the low moisture content of fats offers a further guarantee [42]. Many other ingredients, such as flavorings, colorings and inclusions, can be added to the mix after pasteurization, which, for the same reasons as the other ingredients, would not represent a real risk in themselves.

However, although most of the ingredients should not be relevant sources of microorganisms or be able to support their growth, their contamination would threaten the safety of the ice cream already during production [43,44].

The addition of ingredients post-pasteurization is of particular concern as there will be no other steps before marketing specifically designed to eliminate microbiological hazards that can contaminate the product.

We could speculate that the results of the present study stress this criticality. A significantly greater load in aerobic colony count was found for the ice cream samples that had toppings or decorations compared to those that did not. In addition, the nine samples with unsatisfactory *Enterobacteriaceae* values were all ice creams with inclusions contaminated after heat treatment, since these bacteria are usually killed by pasteurization if properly performed [45]. Against this background, the supply chain plays a key role in providing reliable ingredients. An effective supplier qualification system represents the first important instrument for manufacturers to produce high-quality and safe ice cream.

Factors other than ingredients can contribute to the microbial contamination of the finished product, such as inadequate manufacturing practices and poor hygiene of the working environments.

The manufacturing of ice cream involves several steps, some of which are particularly critical for its safety [6]. The first significant step is the storage of the raw materials, which must be kept at suitable temperatures, especially those that should be refrigerated, for which thermal abuse could favor microbial growth [46–48]. Once the ingredients have been weighed and blended, they are pasteurized. The success of the heat treatment ensures the inactivation of pathogenic microorganisms potentially present in the raw materials; therefore, accurate monitoring at this step is needed. Once pasteurized, the liquid mix is homogenized through a forced passage into small orifices, which serves to reduce the sizes of the fat globules. This process takes place after pasteurization, when the pasteurizer is of the batch type, while, in a continuous system, the homogenizer is usually placed before the heating section of the pasteurizer. The next step involves rapid cooling of the liquid mix, which is kept at ~+4 °C for a few hours up to a maximum of 24 h, to favor its ageing. Maintaining adequate temperatures during this step limits the growth of bacteria that have withstood pasteurization or recontaminated the mixture after the heat treatment. Then, the

mix is pumped into the batch freezer, where it is extensively stirred while incorporating air, responsible for the typical soft texture of the ice cream. Moreover, airborne contaminations can occur [49]; therefore, air is usually microfiltered before being introduced into the batch freezer. The mixture from the batch freezer is dispensed into the final container and subjected to the hardening step inside wind tunnels or cold rooms, where it is further frozen without stirring. Any flavorings, colorings or inclusions, combined with the mix within the batch freezer or before the hardening step, are added either manually or through separated extruders. From the batch freezing step to packaging, ice cream undergoes a gradual freezing process that stops microbial growth. Compliance with the cold chain during storage of the finished product is critical for the ice cream itself, even before its safety. Indeed, the refrigeration temperatures necessary to allow microbial growth would completely melt the ice cream.

Therefore, it is clear that the real risk is represented by contamination with high microbial loads, such as for the three ice creams contaminated by levels of *L. monocytogenes* beyond the acceptability limits, or if the contamination occurs by pathogenic microorganisms with a very low minimum infectious dose, such as some strains of *Salmonella* spp. [37].

In addition to the ingredient, contamination during production can occur through the workforce, plants and equipment; therefore, a comprehensive approach to the whole production system is needed to guarantee safe and high-quality ice cream. Appropriate training of the workforce in good hygiene and manufacturing practices is required, as well as ongoing maintenance of the plant and equipment, which should be easily cleaned and sanitized. Cleaning and sanitizing of facilities in contact with ice cream or ingredients must be carefully performed, usually at the end of each production cycle. The dirt residues could become a favorable substrate for microbial growth and lead to the formation of biofilms, compromising the hygiene of the processing at the outset [50]. Gunduz and Tuncel, investigating the presence of biofilms in an ice cream plant, detected several microbial niches with also potential pathogens such as *Listeria* spp. on different surfaces in the working environment [51]. Miettinen et al. characterized 41 *L. monocytogenes* isolates originated from an ice cream plant, molecularly identifying 12 different strains, and the dominant type was found to have persisted in the plant for seven years [52]. Nowadays, production lines are equipped with cleaning-in-place (CIP), automatic cleaning systems in which all washing and rinsing operations are managed electronically, without moving or disassembling the systems. Checking of the proper functioning of the CIPs and their constant maintenance guarantees an effective and standardized sanitization process, an indispensable prerogative for safe ice cream production [34].

5. Conclusions

The present study shows that commonly marketed industrial ice cream can represent a source of foodborne pathogens for humans. The presence of *L. monocytogenes* and high loads of aerobic colonies and *Enterobacteriaceae* were detected only in ice cream samples with inclusions, stressing the idea that inclusions could act as a relevant source of contamination. However, given the complexity of the production process, contaminations can occur by many other sources. Ice cream is a complex food matrix, and a comprehensive approach to the whole production system is required to ensure high standards of quality and safety.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/app12041988/s1, Table S1: Details of the ice cream samples, Table S2: Microbiological results of the ice cream samples with inclusions, Table S3: Microbiological results of the ice cream samples without inclusions.

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