Original Research Article

Microbial safety and physical chemical properties of fresh refrigerated pesto sauce ~~Behavior of *Listeria monocytogenes* during shelf-life of fresh refrigerated pesto sauce, characterized, according to regulation (EU) 2073/2005 and subsequent amendments, by chemical-physical parameters suitable for its growth.~~

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From the study you were doing; microbial safety and physical chemical properties of fresh refrigerated pesto sauce might be a suitable title

ABSTRACT

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Introduce the topic under study, the problem it tries to solve, the significance and impact of the study

State the methods used, the research finding and the conclusion based on the findings. Clearly state what was the shelf life of each of the products studied.

Aims: Ligurian pesto is one of the most popular condiments for pasta. For microbiological purposes, refrigerated pesto sauce is a great matter for concern, as it can be contaminated by microorganisms originating from both raw materials and manufacturing processes Literature data indicate that, among others, *Listeria monocytogenes* is a potential microbiological hazards for this product and that it is quite frequent. The regulatory approach, (EU) No. 2073/2005 and subsequent amendments and integrations, takes into consideration the concentration of this pathogen in foods and its growing ability. This study aimed to verify whether fresh pesto sauce can be included among the RTE that do not support the growth of *L. monocytogenes* according to this regulation.
Place and Duration of Study: Quality, Safety and Pre-Industrialization Area, Stazione Sperimentale delle Conserve Alimentari- SSICA, Parma, Italy between October 2021 and June 2023.
Methodology: a screening on 48 different types of commercially available fresh pesto sauce was carried out, evaluating physiochemical parameters (pH and a_w). For three different pesto sauces with chemical-physical characteristics suitable for the growth of L. *monocytogenes*, at the beginning and at the end of the shelf-life, microbiological analyses were carried out; the same products were inoculated with inoculum mixture of L. *monocytogenes*, to evaluate its phate during the shelf-life.
Results: From the screening emerges that there is a relevant presence of pesto sauces on the market in which the growth of the pathogen would be possible. The study, carried out at two different storage temperatures, highlighted the inability of the inoculated *L. monocytogenes* to grow in the tested pesto sauces during their shelf-life.
Conclusion: The results obtained from the study can be helpful to the food companies producing pesto sauces, to predict the behaviour of the microorganism and correctly classify the product in accordance with the reference regulation (EU) No. 2073/2005 and its subsequent amendments.

Keywords: [Refrigerated pesto sauce, Listeria monocytogenes Shelf-life, Chemicol-physical parameters, regulation (EU) No. 2073/2005}

1. INTRODUCTION

Ligurian pesto is, together with tomato sauce, one of the most popular sauces for pasta; for years there has been a constant increase in its production and exports.

The shelf-life of such product is ~~entrusted to the controlled application of the~~ depended on intrinsic and extrinsic chemicol-physical parameters ~~such as (~~pH, water activity~~) (a_w)~~, storage temperature and heat treatment (1). Since ~~the~~ heat ~~exerts a harmful action on basil, especially on its~~affects the aroma and colour of the product, ~~several~~ some food companies that produc~~e~~~~ing~~ pesto sauce ~~have chosen the~~prefers refrigeration as ~~the only conservation system~~an alternative preservative method. Nevertheless, the method is not satisfactitorily in term of product~~, with a consequent reduction in the~~ shelf- life of ~~this product~~extension. ~~For microbiological purposes,~~ For example refrigerated pesto sauce is ~~sometimes a great matter for concern, as it can be~~ contaminated by microorganisms originating from ~~both~~ raw materials and ~~manufacturing processes~~during refrigeration storage (2). Literature data indicate that among others, *Listeria monocytogenes* is ~~a potential~~the most prevalent microbiological hazard for this associated with pesto sauce product., in addition to the fact that it has been quite frequently isolated from this matrix (3,4). In fact, it is a microorganism of great adaptability and resistance, that proved able to survive in hostile environments and in food matrices either of animal or vegetable origin. Listeriosis can cause bacteremia, septicemia, meningitis, brain abscesses, local and pregnant female infections, miscarriage, stillbirth, prematurity, and neonatal infection (5). Generally, the risk of contracting listeriosis is influenced by several factors such as: host sensitivity, microorganism distribution and concentration in foods, and possible growth during shelf-life at refrigeration temperature before the opening of the packed food (5). For these reasons, the European legislation (EU Regulation No. 2073/2005 and subsequent amendments and integrations) (6) takes into consideration the concentration of *L.monocytogenes* in foods and its growing ability, based on the determination of factors such as pH, a_w, presence of preservatives and storage conditions, intended as time/temperature ratio (7).

Such factors influence the possibility for *Listeria* spp. to survive or to grow in food and are essential for a correct classification of Ready To Eat foods (RTE). Since it is known that the foods involved in the most serious poisonings are typically those in which not only *L.monocytognes* is present, but it can grow to concentrations which represent a risk for the consumer (8), in accordance with the European legislation (6), RTE products other than those for children and for special medical purposes should not have a concentration of *L.monocytogenes* greater than 100 CFU/g at the end of their shelf-life.

In particular, for RTE products, a distinction must be made on the basis of chemical-physical parameters, between those in which *Listeria* spp. growth is possible and those in which it is not possible. Products with pH ≤ 4.2 or with a_w<0.92, products with pH ≤ 5.0 and a_w ≤ 0.94, products with shelf-life of less than 5 days are automatically included in the second group and therefore *L.monocytogenes* presence is allowed at values ≤ 100 CFU/g.

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Why pursuing that topic and you should include latest scientific facts?
Why gap are you trying to fill in the scholarly body?
Provide cases most prevalent associated with the topic under study?
What problem statement and justification is your study aligning to?
And of what significance is this study? Is it worthy researching?

These should come out clear in the introductory part.

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First of all, the authors must choose one language either British or American.

The opening sentense must be revise

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Since pesto sauce does not always fit in this group, on the basis of the chemical-physical parameters, as indicated in article 3 of European Regulation No. 2073/2005, the operators of the food sector must carry out appropriate scientific studies known as Microbial Challenge Tests (MCT) in order to demonstrate that under the marketing conditions there can be no growth of *Listeria* spp. ~~on their~~in the product.

The European Union Reference Laboratory for *Listeria monocytogenes* (EURL Lm) has recently published the new revision of the "EURL Lm TECHNICAL GUIDANCE DOCUMENT on challenge tests and durability studies for assessing shelf-life of ready-to-eat foods related to *Listeria monocytogenes*" (9): such document covers the technical aspects related to *L. monocytogenes* assessment in RTE foods.

In this perspective and based on both the increase in the consumption of fresh refrigerated pesto and the recent recalls of some types of this product due to the presence of the above mentioned pathogen, this study was aimed to verify whether fresh pesto sauce can be included among the RTE foods that do not support the growth of *L. monocytogenes* according to Regulation (EU) No. 2073/2005 and subsequent amendments.

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2. MATERIALS AND METHODS

2.1 Screening of the market

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A screening was carried out on 48 different types of commercially available fresh pesto sauce, selected by their physico-chemical parameters (pH and aw). The pH was measured using a Mettler Toledo™ S210 SevenCompact™ pH meter (Thermo Fisher Scientific Instrument, Waltham, MA, USA) equipped with a glass, ceramic electrode (Hamilton, Franklin, MA, USA), whereas water activity (aw) was measured at 25 °C by means of the aw meter AquaLab, series 4, Model TE (Decagon Devices, Inc., Pullman, USA) in accordance with the ISO 18787 (10).

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1. how did you sample the 48 samples from the market, what criteria sampling techniques did you implore?
2. were the aseptic techniques followed?
3. were the physical chemical parameters be measured right at the market?, where exactly?
4.did you bring the samples to the laboratory? If so, how did you carry them to the laboratory...
5. what was the weight of each sample that you collected
6. what study design did you use?

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2.2 Microbiological analyses

2.2.1 Fresh pesto sauces

Tests were carried out on three different fresh commercial pesto sauces without garlic selected by the screening of the market (section 2.1), produced by sector companies which supplied three different batches just after production.

The three pesto sauces, named A, B and C, had the following composition and shel-life:

A: Protected designation of origin (PDO) Genoese basil 30%, olive oil, PDO "Grana Padano" cheese, extra virgin olive oil, salt, pine nuts. Shelf- life: 9 days between +1°C and + 4°C.

B: PDO Genoese basil 31%, extra virgin olive oil, PDO "Parmigiano Reggiano" cheese, pine nuts 17%, PDO "Pecorino Romano", sea salt, ascorbic acid. Shelf- life: 21 days between 0°C and +4°C.

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C: Ligurian basil, pine nuts, PDO “Parmigiano Reggiano” cheese, olive oil, salt, ascorbic acid. Shelf- life: 33 days between 0°C and +4°C.

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2.2.2 Non-inoculated samples

For the initial and final characterization of the non-inoculated pesto sauces, all analyses were conducted in accordance with International Organization for Standardization (ISO); 25 g of samples for three batches of the three different pesto sauces A, B, C, were aseptically transferred into a plastic one-chamber filter stomacher bag (Interscience bagfilter, Macerata, Italy) and homogenized in 225 ml of peptone salt solution according to ISO 6887-1 (11) for 3 min using a Stomacher400 circulator (Seward, London, UK). After decimal serial dilution, 1 ml of each sample was transferred onto Plate Count Agar (Oxoid, Cambridge, UK) to carry out the total microbial count at 30°C (ISO 4833-1:2013/Amd 1: 2022) (12), onto Violet Red Bile Lactose Agar (Oxoid, Cambridge, UK) for *Enterobacteriaceae* count (ISO 21528-2:2017/EC 1:2018) (13), onto DeMan, Rogosa and Sharpe Agar (Oxoid, Cambridge, UK) for Mesophilic Lactic Acid Bacteria count (ISO 15214:1998 (E) (14). Yeasts and moulds count was performed by means of a SSICA method which involves direct surface plating on Malt Exctract Agar plates (Oxoid, Cambridge, UK) incubated at 25°C for 5 days; presence or absence of *Listeria* spp. was performed by adding 225 ml of Fraser broth base (Oxoid, Cambridge, UK) at 25 g of sample according to ISO 11290-1 (15). Physico-chemical parameters (pH and a_w) have also been evaluated as described in section 2.1.

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Better say 25 g of each sample was aseptically transferd into a stomacher bag and mixed with 225 ml of and homogenized by a stomacher circulator.

By the way why did you use pepton salt solution? Because you were concurrently doing shelf life study. So was this a representative? Because the salt solution generally inhibit non-halophiles for the example *L. monocygenes* in this case.

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2.2.3 Microorganisms

The inoculum mixture was prepared by combining six strains of *L. monocytogenes*, two belonging at two international collections (*L. monocytogenes* ATCC 13932 and *L. monocytogenes* Scott A), one isolated from a vegetable product and belonging to SSICA collection (*L. monocytogenes* SSICA 36) and three belonging to the EURL Lm European collection (European Union Reference Laboratory for *Listeria monocytogenes*), isolated from food products and selected for their ability to grow at low a_w, pH and temperature values (*L. monocytogenes* 12MOB047LM, *L. monocytogenes* 12MOB050LM and *L. monocytogenes* 12MOB051LM).

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2.2.4 Inoculum preparation

The selected strains were grown individually from cryobeads stored at -20°C in 10 ml of non-selective Brain Heart Infusion (BHI; Oxoid, Cambridge, UK) Broth at 37°C for 24 hours. Then they were transferred into 10 ml of BHI and incubated at 7°C for 3 days. The bacterial cultures were prepared in order to obtain for each strain a concentration of about 8 Log CFU/ml; the concentration of bacterial cultures was verified by counting them on Agar Listeria Ottaviani & Agosti (Biolife, Milan, Italy)

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plates incubated at 37 °C for 24 hours under aerobic conditions according to ISO 11290-2:2017 method (16). Before their use, the individual strains cultures were combined in equal volumes, in order to obtain a final concentration varying from 50 to 200 CFU/g. Each batch of pesto sauce was microbiologically and chemically characterized and aseptically divided in sterile jars containing 25 g of product. The samples were then inoculated with the mixture of *L. monocytogenes* strains; three samples for each batch were analysed in order to verify the initial concentration of the pathogen in the product (T_0). Samples were incubated either at 7°C or 10°C as required by the guidelines (9) and ~~analysed at increasing storage times, depending on the shelf -life of the different pesto sauces investigated.~~the shelf life was determined.

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2.2.5 Inoculated samples

The analyses to evaluate *L. monocytogenes* phate during the shelf-life of inoculated pesto sauces were carried out on three sample for each batch at the corresponding incubation temperature of pesto sauces A, B, C. *Listeria* spp count was performed by serial dilution and direct surface plating on Agar Listeria Ottaviani & Agosti (ALOA; Biolife, Milan, Italy) plates (16), for a total of 72 analyses.

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For each batch, the growth potential (δ) was calculated according to the formula: $\delta = Log_{max} - Log_{i}$, where Log_{max} is the highest average value recorded during the shelf-life and Log_i is the average value at time 0 (9).

The growth potential obtained for the three lots is the highest value observed.

3. RESULTS AND DISCUSSIONS

3.1 Screening of the market

The evaluation of different types of fresh pesto sauces available on the market, showed that most references had the same recipe (the only exception being the presence of garlic), two of the types tested were defined as "Bio", the most used cheese was "Grana Padano" and basil was predominantly “PDO” certified. Most of the investigated pesto sauces had pH values between 5.00 and 5.40 (44%), or between 4.60 and 4.90 represented (30%), whereas sauces with pH range between 4.20 and 4.50 were the 26%.

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1. results or research findings generated during the study
2. discuss what does those results mean to you?
3. contextualize the results with the literature
   a) who else found similar results with yours
   b) what factors led to the similarity of the results? Is it because you used same methods, season, nuture of the sample?
   c) who else found different results with yours
   d) what factors led to the differences of the results? Is it because of methods used, season, nuture of the sample?
4. how does your research findings add to the body of literature?
5. did the research findings answer the research questions or hypotheses?

This makes your discussion well balanced, contexualized

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The most detected a_w values were between 0.90 and 0.92 (46%), pesto sauces with a_w range between 0.93 and 0.95 were the 40%, whereas products with a_w values between 0.86 and 0.89 represented the 12%. Only few samples had values equal to 0.96 (2%).

On the basis of the chemicol-physical parameters, the processing of the data allowed to make a distinction between the pesto sauces in which the growth of *L. monocytogenes* is possible and those in which it is not, according to regulation (EU) No. 2073/2005 and subsequent amendments and integrations (7).

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From this screening it clearly emerges that there is a discrete number (11/48) of pesto sauces in which the growth of the researched pathogen would be possible (Figure 1). Among them, in five samples the presence of *L. monocytogenes* was detected in 25 g (in one samples the microorganism was not only detectable, but present at a concentration of 20 CFU/g); it should be underlined that two of these pesto sauces had chemical-physical characteristics suitable for the growth of the pathogen.

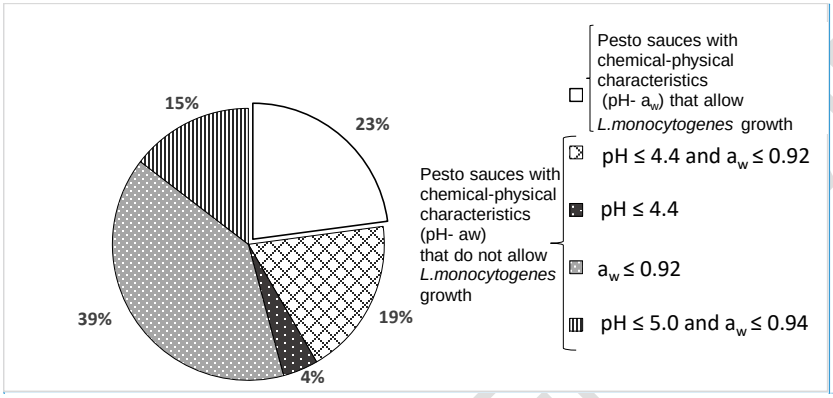

Figure 1. Percentages of pesto sauces from the market in which *L. monocytogenes* is able to grow or not, based on their chemical-physical characteristics.

3.2 Non inoculated samples

The results of the analyses carried out on the batches of pesto sauces (A, B, C) tested for their initial and final microbial characterization after storage at 7°C and 10°C are shown in Figures 2-4. The studied pesto sauces showed an initial microbial count ~~of approximately~~ranging between 5 ~~–~~ 6 Log CFU/g; at 7°C temperature storage., at the end of the storage, the concentration was substantially unchanged for pesto sauce A, whereas an increase of about 1 and 2 Log CFU/g was observed for pesto sauces B and C, respectively; at 10°C, at the end of storage, an increase of about 1 Log CFU/g was detected for pesto sauce A and of about 2 and 3 Log CFU/g for sauces B and C, respectively (Figure 2).

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The methodology guides you how the results and discussion section will flow.

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The end of storage is found when the cutoff point is reached, while in your case .....what was the cutoff point of Total microbial count, *Enterobacteriaea, Listeria monocytogen* and Lactic acid bacteria according to the standards?

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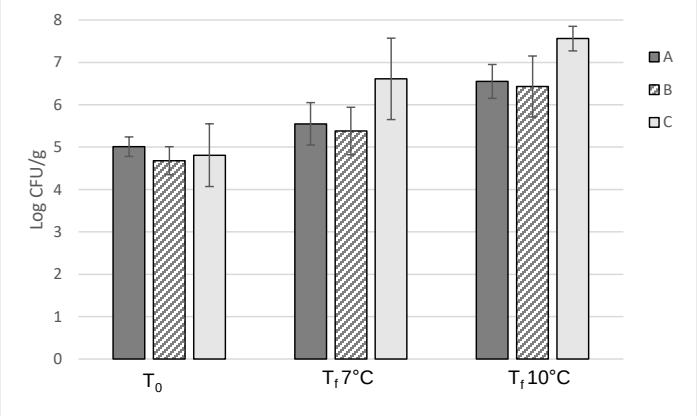

Figure 2. Total microbial count at 30°C in pesto sauces A, B, C, at time 0 (T_0) and at the end of the shelf- life after storage at 7°C (T_f 7°C) or at 10°C (T_f 10°C). The error bars indicate the Standard deviation for mean values.

Key: Sample A = , Sample B = and Sample C = .

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A very similar trend was observed for mesophilic lactic acid bacteria count (Figure 3), the main constituents of the Total microbial count at 30°C, presumably originating from cheese.

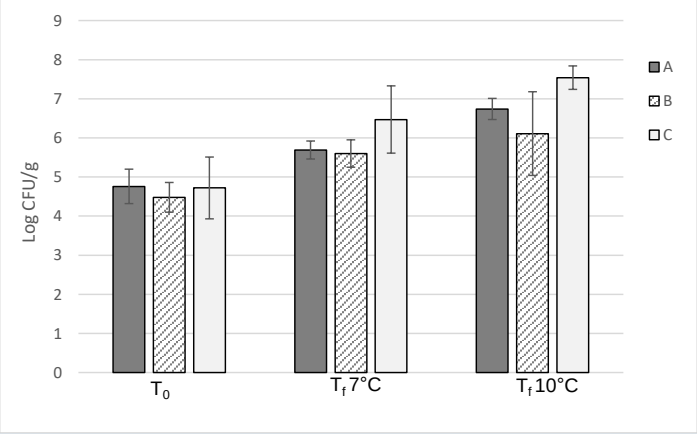

Figure 3. Mesophilic lactic acid bacteria count in pesto sauces A, B, C, at time 0 (T_0) and at the end of the shelf- life after storage at 7°C (T_f 7°C) or at 10°C (T_f 10°C). The error bars indicate the Standard deviation for mean values.

Key: Sample A = , Sample B = and Sample C = .

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Yeasts were initially (T_0) present at concentrations of about 2-3 Log CFU/g, depending on the pesto sauce; at the end of the storage, a considerable increase were observed for all sauces, maybe due to the shelf-life of the product and to the storage temperature, as reported also by Zardetto et al. (17). In pesto sauce A and B, at 7°C and at 10°C, yeasts reached a concentration of about 6 and 5 Log CFU/g, respectively. In pesto sauce C the concentration was about 5 and 7 Log CFU/g at the end of storage at 7°C and at 10°C, respectively, with a great variability between the different batches (Figure 4).

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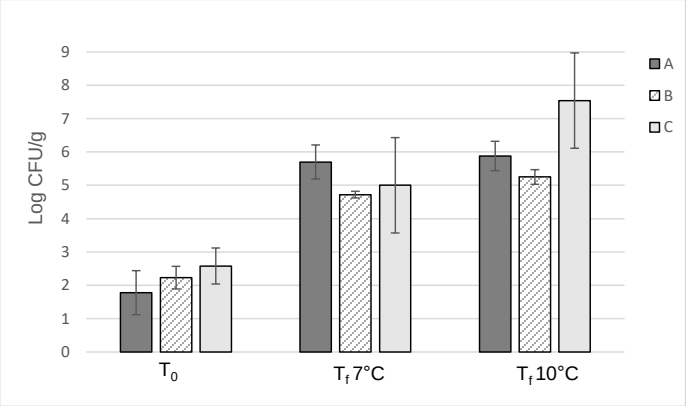

Figure 4. Yeasts count in pesto sauces A, B, C, at time 0 (T_0) and at the end of the shelf- life after storage at 7°C (T_f 7°C) or at 10°C (T_f 10°C). The error bars indicate the Standard deviation for mean values.

Key: Sample A = , Sample B = and Sample C = .

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In sauces B and C *Enterobacteriaceae* were initially present at concentrations of 1-2 Log CFU/g, depending on the batches, whereas they were below the analytical limit in product A (data not reported). At the end of the shelf-life, in all types of sauces and at both storage temperatures, they were below the analytical limit; they seemed to disappear over time, as observed by Vicini et al. (18). Moulds concentration was always below the analytical limit in all pesto sauces for both storage temperatures.

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L. monocytogenes was undetectable in 25 g of product in all sauces analysed at T_0.

The pH at T_0 was 5.50 in pesto sauce A, 5.10-5.32 in sauce B and 5.00-5.13 in sauce C. It remained unchanged in all samples at both storage temperatures, the only exception being the pesto sauce C, where it slightly decreased at the end of the shelf-life at 10°C, reaching a value of 4.92.

No changes in a_w values were observed.

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3.3 Microbial Challenge Tests

Following the study carried out about the different chemical-physical characteristics of fresh pesto sauces available on the market, that confirmed a relevant number of products in which growth of *L. monocytogenes* was possible, we assessed its behaviour in pesto sauces A, B, C, these sauces having pH and a_w values which would allow the development of the studied microorganism.

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3.3.1 Behaviour of L. monocytogenes inoculated in Pesto sauces

In pesto sauces A the initial average inoculum concentrations of *L. monocytogenes* ranged between 1.85 and 2.22 Log CFU/g, with standard deviations (sd) lower than 0.20, confirmed the inoculum correctness.

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Pesto sauce A had pH and a_w values suitable for *L. monocytogenes* growth. However, the growth dynamics of the pathogen, highlighted in the study, indicate that the sauce does not represent a favourable substrate for the development of the microorganism during the shelf-life at the tested storage temperatures (δ <0.5 log CFU/g) (9). It could be supposed a competitive action against *L. monocytogenes* from the naturally present microbiota consisting of lactic acid bacteria and yeasts which can grow in the product during its shelf-life, as reported by numerous scientific studies (19), (20), (21) (22).

The initial inoculum concentration of *L. monocytogenes* in pesto sauce B varied between 1.74 and 2.36 Log CFU/g. This pesto sauce also showed pH and a_w values suitable for *L. monocytogenes* growth, but the growth dynamics of the pathogen confirm that product does not represent a favourable substrate for the development of the bacterium during shelf-life, at the storage temperatures tested (δ <0.5 log cfu/g). An increase in the microbiota naturally present during storage was also observed for this type of pesto sauce.

The initial contamination of *L. monocytogenes* in pesto C ranged from 1.76 to 2.04 Log CFU/g, with standard deviations less than 0.20. This pesto sauce had a pH value of 5.0-5.1 and aw of 0.95, suitable for *L. monocytogenes* growth; the behaviour of the pathogen indicates that the sauce not only does not represent a suitable substrate for the growth of the bacterium during shelf-life, but determined a slight inactivation of the pathogen during storage at the temperatures tested (δ log CFU/g negative). A large number of published studies demonstrated an antibacterial activity of basil essential oil as reported many authors (23), (24), although it cannot be explained why the same inactivating action was not observed for the other types of pesto sauces tested, it could be argued a different origin of the basil used.

The results obtained expressed as Log CFU/g, and the related statistical calculations for the samples incubated at 7°C and at 10°C are reported in Table 1.

Microbial counts were converted into logarithms before means and standard deviations were computed, and counts were reported as Log CFU/g. The statistical elaboration of the results was performed using SPSS 13.0.

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Table 1. Results for *L. monocytogenes* inoculated in Pesto sauces A, B, C during shelf-life at a storage temperatures of 7°C or 10°C.

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A						
Storage temperature 7°C						
batch	T=0	T=3 days	T=7 days	T=10 days	δ batch	δ Pesto sauce
	Mean ± sd*					
1	1.85±0.14	1.7 ±0.18	1.36±0.32	1.42±0.10	1.75 -1.85 = - 0.1	0.12
2	1.97±0.12	1.94±0.06	2.09±0.12	1.99±0.13	2.09 - 1.97 = 0.12	
3	2.22± 0.08	1.7 ±0.26	1.88±0.35	2.02±0.11	2.02 - 2.22 = -0.20	
Storage temperature 10°C						
1	1.85±0.14	1.72±0.10	1.50±0.17	1.33±0.35	1.72 - 1.85= -0.13	0.27
2	1.97±0.12	2.05±0.18	2.24±0.05	2.08±0.04	2.24 - 1.97 = 0.27	
3	2.22±0.08	1.93±0.24	2.00±0.27	2.02±0.29	2.02- 2.22= - 0.20	

B							
Storage temperature 7°C							
batch	T=0	T=7 days	T=14 days	T=21 days	T=28 days	δ batch	δ Pesto sauce
	Mean ± sd*						
1	2.06±0.11	2.08±0.06	2.16±0.15	2.09±0.08	2.14±0.11	2.16-2.06=0.1	0.10
2	1.74±0.13	1.64±0.05	1.68±0.04	1.40±0.17	1.39±0.36	1.68-1.74=-0.06	
3	2.36±0.03	1.87±0.11	2.05±0.10	2.10±0.18	1.77±0.07	2.10-2.36=-0.26	
Storage temperature 10°C							
1	2.06±0.11	1.91±0.18	2.04±0.23	2.12±0.07	1.86±0.07	2.12-2.06=0.06	0.06
2	1.74±0.13	1.50±0.1	1.48±0.10	0.80±0.17	1.40±0.17	1.50-1.74=-0.24	
3	2.36±0.03	1.97±0.18	2.00±0.04	2.02±0.15	1.70±0.20	2.02-2.36=-0.34	

C							
Storage temperature 7°C							
batch	T=0	T=10 days	T=20 days	T=30 days	T=40 days	δ batch	δ Pesto sauce
	Mean ± sd*						
1	1.76±0.15	1.10±0.17	0.90±0.17	0.70±0.00	0.70±0.00	1.10-1.76= -0.66	-0.66
2	1.95±0.05	1.22±0.45	1.10±0.17	1.00±0.30	0.80±0.17	1.22-1.95= -0.73	
3	2.04±0.07	1.22±0.45	1.20±0.17	0.70±0.00	0.70±0.00	1.22-2.04= -0.82	
Storage temperature 10°C							
1	1.76±0.15	1.00±0.30	0.80±0.17	0.70±0.00	0.70±0.00	1.00-1.76= -0.76	-0.57
2	1.95±0.05	1.38±0.60	1.03±0.58	1.03±0.58	0.70±0.00	1.38-1.95= -0.57	
3	2.04±0.07	1.38±0.60	1.16±0.41	0.70±0.00	0.70±0.00	1.38-2.04= -0.66	

4. CONCLUSION

The screening carried out on 50 different types of fresh commercial pesto sauces highlighted a relevant number of pesto sauces with chemical-physical characteristics that would be suitable for the growth of *L. monocytogenes*; in five samples, the presence of the pathogen was also detected, and in one sample the microorganism was present at a concentration of 20 CFU/g.

Refrigerated uninoculated fresh pesto sauces tested in this study showed an initial (T_0) Total Microbial Count at 30°C consisting mainly of lactic acid bacteria and yeasts that were present at variable concentrations and only in some cases of *Enterobacteriaceae*. During the storage, an increase in lactic acid bacteria and a considerable increase in yeasts was

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observed for all pesto sauces, thus being linked to storage time and temperature applied. The pH was generally unchanged, slightly decreased in one of the pesto sauces tested stored at 10°C.

The study, carried out at two different storage temperatures, highlighted the inability of the inoculated *L. monocytogenes* to grow in the tested pesto sauces during their shelf-life; furthermore, in one case a slight inactivation of the pathogen was observed. It can therefore be assumed that, at the temperatures assayed, all the pesto sauces tested were unable to support the growth of *L. monocytogenes.*

The results obtained from the Microbial Challenge Tests can be helpful to the food companies producing pesto sauces to predict the behaviour of the microorganism and correctly classify the product in accordance with the regulation (EU) No. 2073/2005 with its subsequent amendments.

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