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High Pressure Homogenization versus Heat Treatment: Effect on Survival, Growth, and Metabolism of Dairy *Leuconostoc* Strains

D. M. GUGLIELMOTTI,[1]* F. PATRIGNANI,[2] R. LANCIOTTI,[2] M. E. GUERZONI,[2] J. A. REINHEIMER,[1] AND A. QUIBERONI[1]

[1]*Instituto de Lactología Industrial (INLAIN, UNL-CONICET), Facultad de Ingeniería Química, Santiago del Estero 2829, 3000 Santa Fe, Argentina; and* [2]*Dipartimento di Scienze degli Alimenti, University of Bologna, Piazza Goidanich 60, 47023 Cesena, Italy*

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ABSTRACT

The effect of high pressure homogenization (HPH) with respect to a traditional heat treatment on the inactivation, growth at 8°C after treatments, and volatile profile of adventitious *Leuconostoc* strains isolated from Cremoso Argentino spoiled cheeses and ingredients used for their manufacture was evaluated. Most *Leuconostoc* strains revealed elevated resistance to HPH (eight passes, 100 MPa), especially when resuspended in skim milk. Heat treatment was more efficient than HPH in inactivating *Leuconostoc* cells at the three initial levels tested. The levels of alcohols and sulfur compounds increased during incubation at 8°C in HPH-treated samples, while the highest amounts of aldehydes and ketones characterized were in heated samples. *Leuconostoc* cells resuspended in skim milk and subjected to one single-pass HPH treatment using an industrial-scale machine showed remarkable reductions in viable cell counts only when 300 and 400 MPa were applied. However, the cell counts of treated samples rose rapidly after only 5 days of storage at 8°C. The *Leuconostoc* strains tested in this work were highly resistant to the inactivation treatments applied. Neither HPH nor heat treatment assured their total destruction, even though they were more sensitive to the thermal treatment. To enhance the inhibitory effect on *Leuconostoc* cells, HPH should be combined with a mild heat treatment, which in addition to efficient microbial inactivation, could allow maximal retention of the physicochemical properties of the product.

Some species of *Leuconostoc* are very relevant in the dairy industry. *Leuconostoc* bacteria use lactose and citrate, producing lactic acid, acetate, CO_2, ethanol, acetaldehyde, diacetyl, acetoin, and 2,3-butanediol, which contribute to the sensory (flavor and texture) characteristics of butter and cream and allow the openings in some soft and semihard cheeses (Edam and Gouda cheeses) *(9, 18, 30)*. However, the production of CO_2 and certain flavor compounds by *Leuconostoc* is not well received in other types of cheeses, where it leads to diminished quality of the product. The presence of *Leuconostoc* bacteria in milk and dairy products as adventitious microflora could be the consequence of contamination during milk collection and/or during the manufacture of the products. This could be favored by the great ability of *Leuconostoc* to survive on surfaces, tools, and pasteurizers for long periods of time, and in adverse environments as well. Wild-type *Leuconostoc* strains are also reported to be heat resistant and able to survive pasteurization *(17)*. Many adventitious *Leuconostoc* strains were recently isolated and found to be the main reason for blowing defects in Cremoso Argentino cheeses *(22)*. To face this problem, some dairy industries subject milk to a stronger thermal treatment. However, excessive thermal treatments can negatively affect the yield and sensory quality of cheeses as a consequence of protein denaturation, nonenzymatic browning, and loss of vitamins and volatile flavor compounds.

In recent years, the development of alternative, nonthermal processes has received considerable attention, since they combine efficient microbial reduction with a maximal retention of the chemical and physicochemical properties of products. Among them, one of the most interesting is high pressure homogenization (HPH) *(4, 11, 21, 31)*. HPH technology can be used as an alternative to pasteurization for improved safety and better microbiological quality of many products, including milk, fermented milks, and cheeses *(7, 13, 14, 19, 20, 29)*. Studies based on HPH technology have been mostly focused on the inactivation of more common spoilage and pathogenic microorganisms *(5, 16, 24, 28, 31)*. Information concerning the effect of HPH on *Leuconostoc* bacteria is almost nonexistent, probably because the genus is traditionally known for its positive role in the dairy field *(31)*.

The principal aim of this work was to evaluate the effect of HPH in comparison to the effect of traditional heat

* Author for correspondence. Tel: +54 (0342) 453-0302; Fax: +54 (0342) 457-1162; E-mail: dgugliel@fiq.unl.edu.ar.

TABLE 1. *Identification and origin of* Leuconostoc *strains used in this study*

Classification[a] and strain		Origin, isolation date (mo/yr)
L. mesenteroides subsp. *mesenteroides*	D2	Cremoso Argentino cheese, 06/2008
	D3	Pasteurized milk,[b] 07/2008
	D4	Pasteurized milk,[b] 07/2008
	D8	Whey cream,[b] 09/2008
	D10	Whey protein concentrate,[b] 10/2008
	D11	Cremoso Argentino cheese, 10/2008
L. pseudomesenteroides	D6	Pasteurized milk,[b] 07/2008
	D7	Cremoso Argentino cheese, 09/2008
	MB2	Cremoso Argentino cheese, 09/2008
	MB3	Cremoso Argentino cheese, 09/2008
	MB4	Cremoso Argentino cheese, 09/2008
L. garlicum	D1	Pasteurized milk,[b] 05/2008
	D5	Whey cream,[b] 07/2008
L. citreum	MB1	Cremoso Argentino cheese, 09/2008

[a] Classification according to sequencing of the hypervariable region (first 500 bp) in the 5′ end of the 16S rRNA gene *(2)*.
[b] Used for Cremoso Argentino cheese manufacture.

treatment on the inactivation, growth after treatment, and volatile profiles of adventitious *Leuconostoc* strains isolated from Cremoso Argentino spoiled cheeses and ingredients used for their manufacture. In particular, the inactivation effectiveness of HPH was studied in relation to the suspension media and inoculation levels and the performance of laboratory-scale and industrial homogenizers was compared.

MATERIALS AND METHODS

Strains and growth conditions. Fourteen *Leuconostoc* strains previously isolated at the Instituto de Lactología Industrial (INLAIN, UNL-CONICET, Facultad de Ingeniería Química, Santa Fe, Argentina) (Table 1) and characterized at the biochemical, genetic, and technological levels *(2)* were used in this study. They were routinely grown until stationary stage (overnight, 16 to 18 h) in de Man Rogosa Sharpe (MRS; Oxoid, Basingstoke, Hampshire, England) broth at 32°C and stored at −80°C in MRS supplemented with glycerol (15%, vol/vol). For the enumeration of *Leuconostoc* strains, MRS agar was used, and the plates were incubated in microaerophilic atmosphere at 32°C for 48 h.

HPH treatments. For laboratory-scale trials, a PANDA continuous high pressure homogenizer (Niro Soavi, Parma, Italy), previously sterilized according to the manufacturer's recommendations, was used for all the homogenizing treatments. The machine was supplied with a homogenizing pressure relief–type valve with a flow rate of 10 liters/h. The valve assembly included a ball-type impact head made of ceramic, a stainless steel large-inner-diameter impact ring, and a tungsten carbide passage head. A water refrigeration system (Niro Soavi) was used to counterbalance the temperature increase during the treatments (about 2.5°C/10 MPa). The maximum temperature reached by the samples did not exceed 25°C. For the industrial-scale trials, a prototype homogenizer (Niro Soavi) able to reach up to 400 MPa was used.

HPH inactivation of *Leuconostoc* strains in relation to suspension media and number of passes at 100 MPa. The fourteen *Leuconostoc* strains were subjected to multipass HPH treatments using the laboratory-scale equipment (Niro Soavi). Fresh *Leuconostoc* cultures (16 to 18 h at 32°C) were inoculated (10^7 CFU/ml initial cell loads) into 500 ml of MRS broth and reconstituted skim milk (Oxoid). The suspensions were subjected to HPH treatments for up to eight successive passes at 100 MPa. Viable cell counts on MRS agar (48 h at 32°C) were carried out before (control) and after one, three, five, and eight passes.

Recovery of cell viability during refrigerated storage of HPH- and thermal-treated *Leuconostoc* cells. *Leuconostoc mesenteroides* subsp. *mesenteroides* D11, selected according to the data obtained from the initial screening, was inoculated into reconstituted skim milk (Oxoid) at three different initial cell loads (10^3, 10^5, and 10^7 CFU/ml). Different aliquots of these suspensions were subjected to HPH (as described above) or to a thermal treatment performed in a water bath at 63°C for 30 min. The temperature increase of samples was monitored by using a thermal probe. Viable cell counts in MRS agar (48 h at 32°C) were carried out before (control, cells without treatment) and immediately after (time zero) treatment and at different intervals of time during refrigerated storage (8°C) of the controls and the homogenization- and heat-treated samples.

Volatile molecule profiles of HPH- and thermal-treated skim milk previously inoculated with *Leuconostoc* cells. To determine the volatile aromatic compounds produced by the survivor cells of *L. mesenteroides* subsp. *mesenteroides* D11 inoculated into skim milk at an initial level of 10^5 CFU/ml and subjected to HPH or heat treatment, a gas chromatography–mass spectrometry analysis coupled with solid-phase microextraction technique was used. For each condition, aliquots of 5 g of sample were sealed in sterilized vials. Samples were heated at 40°C for 10 min, and volatiles adsorbed for 50 min on fused-silica fiber covered by carboxen polydimethyl siloxane (75-μm Supelco, Sigma-Aldrich Chemie GmbH, Munich, Germany). Adsorbed molecules were desorbed in the gas chromatograph for 5 min. For peak detection, a gas chromatograph (Agilent 6890GC, Agilent Technologies, Milan, Italy) equipped with a mass spectrometry detector (5970 MSD, Agilent) and a fused-silica capillary column (50-m length, 0.32-mm inside diameter) coated with a 1.2-μm polyethylene glycol film (CP-Wax 52 CB, Chrompack, Middleburg, The Netherlands) as stationary phase were used. The conditions were as follows: injection temperature, 220°C; detector temperature, 220°C; carrier gas (He) flow rate, 1.5 ml/min; splitting

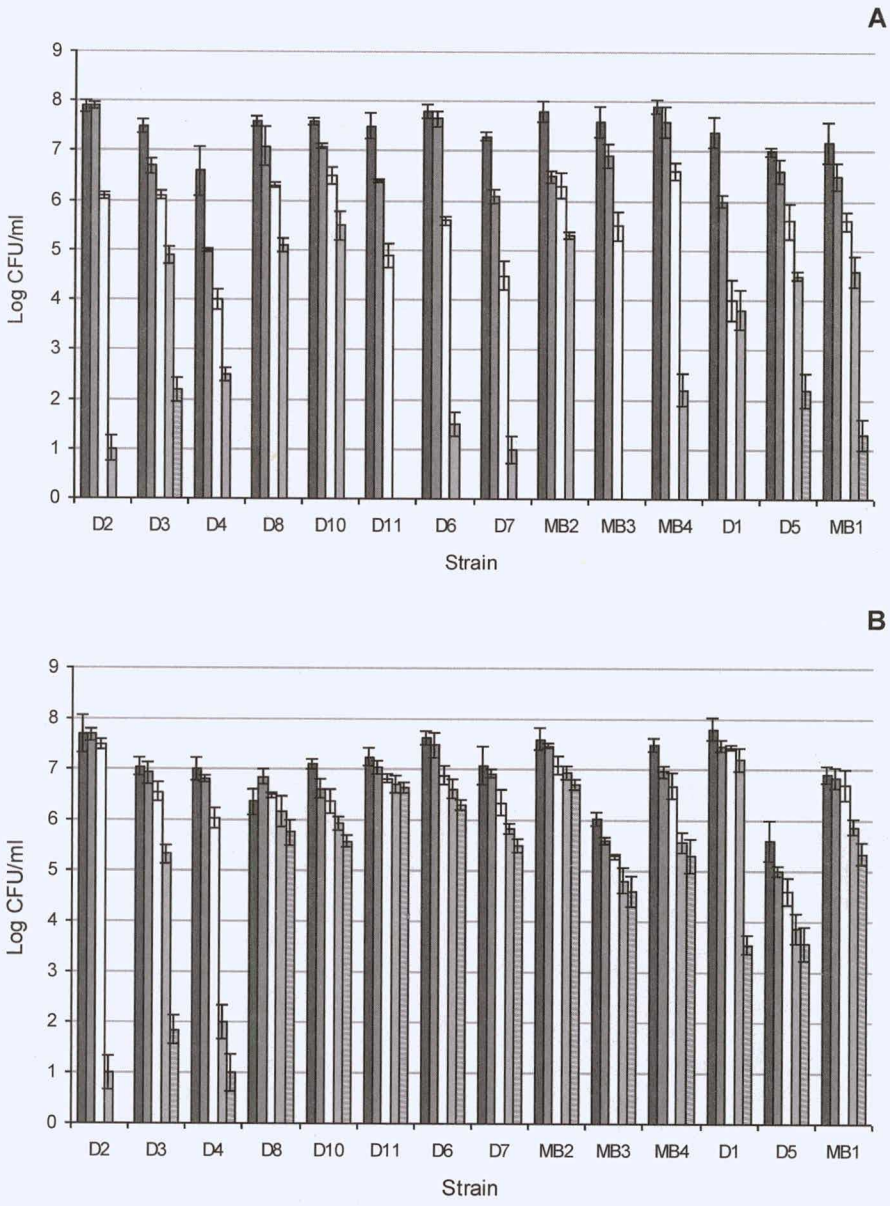

FIGURE 1. *Effect of multipass HPH treatments at 100 MPa on the viability of* Leuconostoc *strains resuspended in MRS broth (A) or reconstituted skim milk (B). Bars correspond to viable cell counts (log CFU per milliliter) on MRS agar (48 h at 32°C) of (■) untreated samples and samples after (■) one, (□) three, (■) five, and (▨) eight passes at 100 MPa.*

ratio, 1:20 (vol/vol). The oven temperature was programmed as follows: from 45°C to 100°C, increasing at 2.5°C/min; from 100 to 200°C, increasing at 6.5°C/min, and then holding for 5 min. Volatile peak identification was carried out by computer matching of mass spectral data with those of the compounds contained in the NIST '98 Mass Spectral Library (No. 1A, version 1.6, 1998, National Institute of Standards and Technology–U.S. Environmental Protection Agency–National Institutes of Health) and the Registry of Mass Spectral Data (1998, Wiley, New York).

Effect of single-pass HPH treatments up to 400 MPa using prototype industrial-scale equipment on *Leuconostoc* viability and recovery during storage. One hundred liters of commercial ultrahigh-temperature–treated skim milk (Granarolo, Italy) was inoculated with a fresh culture of *L. mesenteroides* subsp. *mesenteroides* D11 (16 to 18 h at 32°C) to an initial cell load of approximately 10^6 CFU/ml. The inoculated milk was subjected to single-pass treatments at 100, 200, 300, and 400 MPa using the prototype industrial-scale equipment (Niro Soavi). Viable cell counts in MRS agar (48 h at 32°C) were carried out before (control, cells without treatment) and immediately after (time zero) HPH treatments and at different intervals of time up to 15 days during the storage (8°C).

Statistical analysis. Experiments were replicated three times. All data were analyzed using the one-way analysis of variance procedure in Statgraphics Plus software (version 3.0, Statistical Graphics Corp., Warrenton, VA). Differences among means were detected by Duncan's multiple range test. Differences were considered significant at a P value of <0.05.

RESULTS

HPH inactivation of *Leuconostoc* strains in relation to suspension media and number of passes at 100 MPa. The fourteen *Leuconostoc* strains subjected to HPH treatment at 100 MPa for up to eight passes revealed different susceptibilities when suspended in MRS broth or skim milk. Inactivation was significantly greater when

TABLE 2. *Evolution of different initial cell loads of* Leuconostoc mesenteroides *subsp.* mesenteroides *D11 in reconstituted skim milk immediately after high pressure homogenization treatments and thermal treatment and during various times of storage at low temperature*

Days of storage at 8°C	Cell load (log CFU/ml) following indicated treatment of skim milk inoculated with initial cell load of[a]:																				
	10^7 CFU/ml						10^5 CFU/ml						10^3 CFU/ml								
	C	HPH					HT	C	HPH				HT	C	HPH				HT		
		1	3	5	8				1	3	5	8			1	3	5	8			
0	7.16 b A	6.9 b A	6.81 ab A	6.60 a A	6.49 a A		—	5.18 c A	5.09 c A	4.82 bc A	4.53 ab A	4.14 a A	—	3.98 b A	3.93 ab A	3.42 ab A	3.23 a A	2.94 a A	—		
1	7.90 c B	7.96 c B	7.92 c B	7.36 bc B	7.30 b A	3.32 a A		6.53 c B	6.45 c B	6.09 b B	5.95 b B	5.22 a B	—	5.36 c B	5.19 c B	4.58 b B	4.44 ab B	4.33 a B	—		
4	8.42 bc C	8.66 c C	8.41 bc C	8.44 bc C	8.29 b B	4.41 a B		8.34 b C	8.24 b C	8.45 b C	8.21 b C	8.94 c C	1.89 a A	7.56 c C	7.62 c C	7.41 bc C	7.22 b C	6.92 a C	—		
6	8.58 b C	8.69 b C	8.72 b C	8.80 b C	8.61 b B	7.98 a C		8.87 d D	8.57 bc C	8.41 b C	8.61 bcd C	8.72 cd C	3.34 a B	8.51 a D	8.41 a D	8.34 a D	8.53 a D	8.28 a D	—		
8	ND	ND	ND	ND	ND	8.0 C		ND	ND	ND	ND	ND	4.50 C	ND	ND	ND	ND	ND	—		
15	ND	ND	ND	ND	ND	ND		ND	ND	ND	ND	ND	6.0 D	ND	ND	ND	ND	ND	—		

[a] C, control *Leuconostoc* cells without any treatment; HT, thermal treatment of 63°C for 30 min; HPH, high pressure homogenization treatment with laboratory-scale equipment at 100 MPa for indicated number of passes; ND, not determined. Dashes indicate viable cell counts of <1 CFU/ml (detection limit). Within a row and for each initial cell load, means with different lowercase letters are significantly different ($P < 0.05$). Within a column, means with different uppercase letters are significantly different ($P < 0.05$).

bacterial cells were suspended in MRS broth, as large reductions in cell counts were observed after only three passes at 100 MPa (Fig. 1). Moreover, undetectable cell counts were obtained for most *Leuconostoc* strains tested after eight passes (Fig. 1A). In contrast, HPH treatments were remarkably less effective when *Leuconostoc* cells were inoculated into skim milk, as shown by high cell counts even after five or eight passes at 100 MPa (Fig. 1B). *Leuconostoc pseudomesenteroides* D6 and MB2 and *L. mesenteroides* subsp. *mesenteroides* D11 showed the highest resistance to HPH treatments when they were suspended in skim milk. In general, for both suspension media, higher inactivation rates were reached when the number of passes was greater.

Recovery of cell viability during refrigerated storage of HPH- and thermal-treated *Leuconostoc* cells. Immediately after eight passes at 100 MPa, the viable cell counts of *L. mesenteroides* subsp. *mesenteroides* D11 decreased by approximately 1 log with respect to the viable cell counts of controls for the three initial cell loads tested (Table 2). Following 1 day of storage at 8°C, a rise of at least 1 log was accomplished by most of the homogenized samples. The maximum viable cell loads were demonstrated after 4 days of refrigerated incubation when the initial cell loads were 10^7 and 10^5 CFU/ml and after 6 days when it was 10^3 CFU/ml. On the other hand, thermal treatment (63°C for 30 min) allowed a higher inactivation of *Leuconostoc* cells. In this case, viable cell counts of heated samples showed a drastic reduction (under the detection limit, <1 CFU/ml) immediately after thermal treatment, even for a 10^7 CFU/ml initial cell load. However, after 6 days of storage at 8°C, samples inoculated at the highest levels considered, 10^7 and 10^5 CFU/ml, reached viable cell counts of approximately 8 and 3 CFU/ml, respectively. On the other hand, viable cell counts remained undetectable after the same period and to the end of storage (15 days) when the initial cell load was the lowest (10^3 CFU/ml).

Volatile molecule profiles of HPH- and thermal-treated skim milk previously inoculated with *Leuconostoc* cells. To study the effect of HPH in comparison with the effect of the traditional thermal treatment on volatile molecule profiles, skim milk samples inoculated with *L. mesenteroides* subsp. *mesenteroides* D11 at an initial level of 10^5 CFU/ml and treated for eight passes at 100 MPa or 63°C for 30 min were analyzed after 24 and 144 h (6 days) of storage at 8°C. The gas chromatography–mass spectrometry–solid-phase microextraction profiles were compared with those obtained from uninoculated samples subjected to eight passes at 100 MPa or 63°C for 30 min. The gas chromatography–mass spectrometry–solid-phase microextraction analyses allowed the identification of 30 molecules, including acids, ketones, aldehydes, alcohols, fatty acids, sulfur hydrocarbons, and furan compounds. The data reported in Figure 2 show that after 24 h of storage, both heated and HPH-treated samples, independently of the inoculation with *L. mesenteroides* subsp. *mesenteroides* D11, had higher amounts of compounds related to thermal

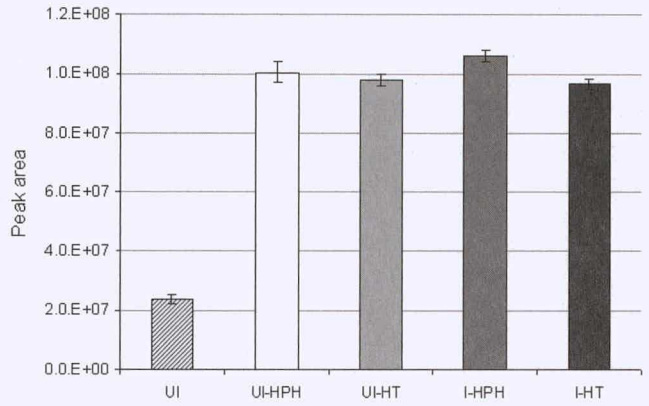

FIGURE 2. *Furan methanol and aldehydes (expressed as peak area) detected in uninoculated untreated skim milk (UI), uninoculated HPH (UI-HPH) and heat-treated (UI-HT) skim milk, or HPH (I-HPH) and heat-treated (I-HT) skim milk inoculated with* Leuconostoc mesenteroides *subsp.* mesenteroides *D11 after 24 h of storage at 8°C.*

damage (i.e., hexanal, nonanal, furfural, heptanal, decanal, and 2-furanmethanol) with respect to the amounts in untreated/uninoculated skim milk. Among heat-treated or HPH-treated skim milk samples, no significant difference in oxidation compounds was detected. In addition, HPH-treated samples revealed the presence of high levels of ethanol, while heated ones were characterized by higher content of short-chain fatty acids (data not shown). The levels of alcohols, such as ethanol, hexanol, heptanol, octanol, and nonanol, as well as sulfur compounds, increased over time (6 days) in the inoculated HPH-treated samples (Fig. 3). In contrast, higher amounts of aldehydes and ketones characterized the samples obtained from inoculated heat-treated skim milks. According to the data shown in Figure 3, the uninoculated HPH- and heat-treated skim milk samples were characterized by the same volatile molecules detected after 24 h at 8°C. However, the storage time increased the accumulation of ketones, furanes, and to a minor extent, aldehydes.

Effect of single-pass HPH treatments up to 400 MPa using prototype industrial-scale equipment on *Leuconostoc* viability and recovery during storage at 8°C. After HPH treatments at 100 and 200 MPa, no reduction in viable cell loads was observed (Fig. 4). However, when *L. mesenteroides* subsp. *mesenteroides* D11 cells were subjected to 300 and 400 MPa, reductions of more than 5 log in comparison to the viable cell count in the control (cells without treatment) were observed. After 2 days of storage of the homogenized samples at 8°C, a rise of about 2 log was observed for those subjected to 100 and 200 MPa, and the increase was greater than 3 log for those subjected to 300 and 400 MPa. For all HPH-treated samples, the maximum viable cell loads were demonstrated after 5 days of refrigerated storage.

DISCUSSION

Adventitious *Leuconostoc* strains can cause commercial losses in many food industries, including dairy fermentation. In our region, some *Leuconostoc* species induced spoilage by producing excessive amounts of CO_2, which caused blowing defects in Cremoso Argentino cheese *(22)*. *Leuconostoc* species have been identified as resistant to thermal treatments applied to milk, but they are also capable of growing at temperatures as low as 8°C *(2)* and 4°C *(8)*, allowing them to grow during cheese ripening and causing early blowing by gas production. This fact makes it relevant to study their behavior at refrigeration temperatures. The control of adventitious *Leuconostoc* by cheesemakers is generally achieved by intensifying thermal treatments applied to the raw milk, which also can destroy heat-labile components of the milk and decrease the quality of the product. As a result, there has been high interest in nonthermal processes, which combine efficient microbial reduction with a maximal retention of the chemical and physicochemical properties of the product *(31)*. In the present work, the effectiveness of nonthermal HPH technology was compared with that of a traditional thermal process, focusing on cell viability and recovery during refrigerated storage and differences in the volatile profiles of HPH- and heat-treated samples inoculated with spoilage *Leuconostoc* strains.

Fourteen *Leuconostoc* strains were subjected to HPH treatments at 100 MPa for up to eight passes, using MRS

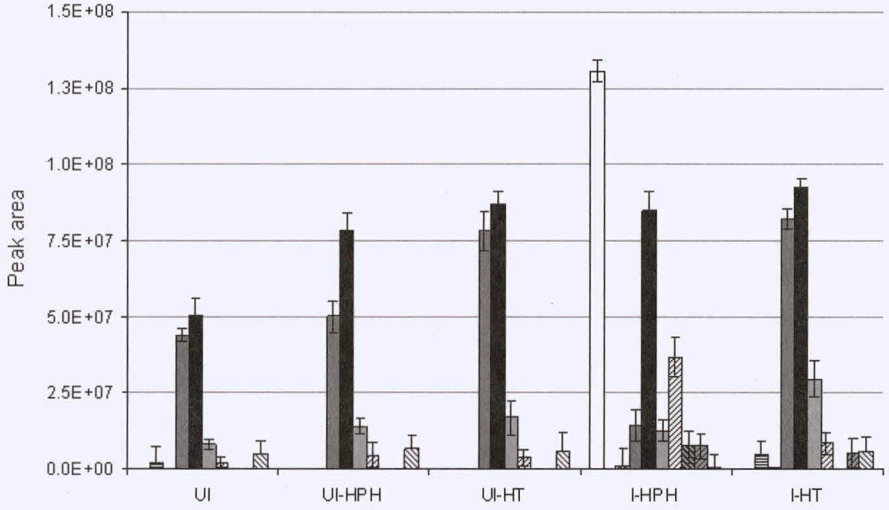

FIGURE 3. *Aroma compounds (expressed as peak area) detected in uninoculated skim milk (UI) or HPH (I-HPH) and heat-treated (I-HT) skim milk inoculated with* Leuconostoc mesenteroides *subsp.* mesenteroides *D11 after 144 h of storage at 8°C. Bars correspond to (□) ethanol, (▤) diacetyl, (▨) acetic acid, (■) ketones, (■) furan, (■) aldehydes, (▨) alcohols, (▩) sulfur compounds, (▨) octanoic acid, and (▩) benzaldehyde.*

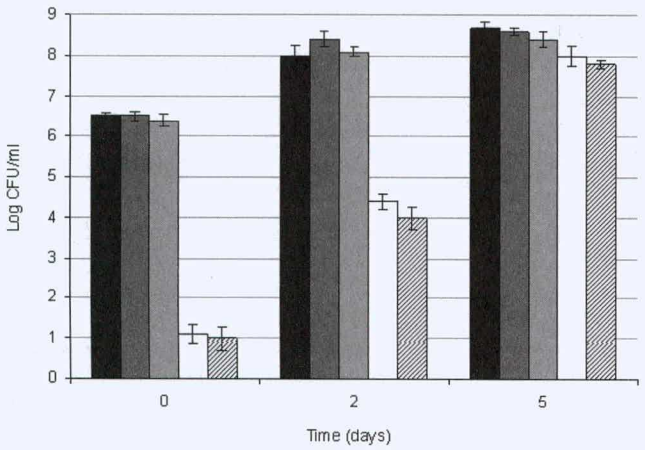

FIGURE 4. *Viable cell counts (log CFU per milliliter) obtained immediately after single-pass HPH treatments at 100, 200, 300, and 400 MPa using industrial-scale equipment on* Leuconostoc mesenteroides *subsp.* mesenteroides *D11 cells suspended in commercial skim milk (Granarolo, Italy) and after 2 and 5 days during refrigerated storage (8°C). Bars correspond to (■) untreated sample and samples treated at (■) 100, (■) 200, (□) 300, and (▨) 400 MPa.*

broth and reconstituted skim milk as suspension media. According to our results, the inactivation of *Leuconostoc* cells was dependent on the suspension medium used, as it was remarkably greater in MRS broth than in skim milk. As can be expected and as was also noticed by other authors *(3, 27, 28),* the inactivation was greater when the number of passes was higher (from one to eight). The resistance of *Leuconostoc* cells in skim milk may be due to some milk constituents which would provide a protective effect against high pressure treatments. In this regard, Vachon and colleagues *(28)* studied the inactivation of foodborne pathogens and concluded that, regardless of the high pressure treatment applied, the bacteria tested were more resistant in milk than in phosphate buffer.

Among the fourteen *Leuconostoc* strains tested, *L. mesenteroides* subsp. *mesenteroides* D11 was chosen to perform the second stage of our experiments. According to our results, thermal treatment was more effective than the HPH treatment used for all the initial cell loads tested, as shown by undetectable cell counts immediately after thermal treatments and delayed growth during refrigerated storage as well. Likewise, Kheadr and coworkers *(10)* reported that milk pasteurization produced the greatest reduction in viable cell counts of *Listeria innocua* and total bacteria in a comparison to high pressure–treated milk. Our study showed that during the refrigerated storage (8°C), HPH-treated samples reached maximum cell counts in a shorter period than heat-treated ones. Even though heat treatment remained more effective than HPH to inactivate *Leuconostoc* cells, it could not assure the total inactivation of cell populations, as demonstrated by the maximum viable cell counts after a short period of storage at 8°C for samples with 10^7 and 10^5 CFU/ml initial cell loads. The cell counts of heated samples remained undetectable during 15 days only for the lowest initial inoculum tested (10^3 CFU/ml). Still, undetectable counts do not assure irreversible destruction of bacterial cells but could be a consequence of reversible damage that keeps the cells in a noncultivable state on the usual growth media. If this was the case, some special enriched medium should be necessary in order to observe bacterial growth.

The volatile profiles showed the occurrence of high levels of aldehydes and hydrocarbon- and furan-derived compounds both in HPH- and heat-treated samples. These compounds are originated mainly from lipid oxidation *(26),* indicating that the thermal damage is similar for both treatments applied. Although HPH is regarded as a nonthermal technology, during this treatment, the temperature increases due to frictional heating in the homogenization valve *(4).* The temperature rise depends on several factors (inlet temperature, pressure level, number of passes, matrix, valve geometry, and temperature exchanger). In the present work, the use of a thermal exchanger limited the temperature rise, and after eight passes, the outlet temperature did not exceed 25°C. However, repeated passes at 100 MPa seem to induce, independent of inoculation, a matrix of thermal stress able to increase the levels of furans and aldehydes. The data obtained after 24 h showed that HPH-treated samples also had fatty acids, although in lower amounts than heat-treated ones. These fatty acids, absent in the uninoculated controls, can be attributed to the release of fatty acids from the disrupted microbial cells, while the increase over time is probably due to the increased milk fat susceptibility to lipolysis and lipid oxidation *(26)* of skim milk, characterized by a fat content of 0.2%. Moreover, HPH treatment is reported to activate microbial enzymes, including lipase *(15),* contributing to the observed increase of fatty acids during storage. After 6 days of storage at 8°C, HPH-treated samples were characterized by metabolites such as ethanol, hexanol, heptanol, and octanol due to faster microbial cell recovery and the microbial detoxification system. In fact, hexanol is the detoxification product of hexanal which has antimicrobial activity *(12).* The high amounts of ethanol after 24 h can be attributed to its extraction, due to cavitation phenomena, from the heterofermentative microbial cells. The ability to strip ethanol from yeasts has been reported previously *(6).* In the present study, the aldehyde content was similar in HPH-treated and heated samples after 24 h, while a marked increase in their content was observed for heated samples during storage, confirming the data reported by Rerkrai and colleagues *(23).* These authors reported a general increase in aldehyde content during storage of thermal-treated milk, attributing this behavior to oxygen availability and storage temperature. After 6 days of storage, heated samples were also characterized by higher amounts of ketones originated by milk fat oxidation. The differences in the volatile profiles of HPH-treated and heated skim milk inoculated with *Leuconostoc* bacteria would probably be expressed as different off-flavor patterns in the final dairy product.

When *L. mesenteroides* subsp. *mesenteroides* D11 cells suspended in commercial skim milk were subjected to one single-pass HPH treatment at up to 400 MPa using the industrial-scale equipment, the reductions in viable cell counts were only more than 5 log when 300 and 400 MPa were applied. However, these treatments were ineffective in

completely destroying the bacteria, since remaining *Leuconostoc* cells were capable of growing rapidly after only 5 days of refrigerated storage at 8°C.

Many works have established that gram-positive bacteria are generally more resistant to HPH than gram-negative bacteria *(27, 28, 31)*, suggesting a correlation between cell wall structure and high pressure resistance. It was postulated that HPH kills vegetative bacteria suspended in a liquid especially through mechanical destruction of the cell integrity caused by hydrodynamic cavitation, impingement against static surfaces, high turbulence, and fluid shear *(31)*. In gram-positive bacteria, the robust peptidoglycan layer provides great structural strength which protects them from these phenomena. Other than the layer structure, some authors postulated that cell shape can be an additional factor contributing to susceptibility to high pressure treatments; smaller and/or spherically shaped microorganisms are expected to be more resistant than rod-shaped ones *(1, 25)*. Wuytack et al. *(31)* could not confirm this hypothesis, since the coccus *Leuconostoc dextranicum* strain tested was more sensitive to HPH than the rod *Lactobacillus plantarum*. In despite of this, the characteristics of *Leuconostoc* cells tested in our work could explain, at least partially, their high resistance to HPH treatments.

The *Leuconostoc* strains tested in this work were highly resistant to the inactivation treatments applied. Neither HPH nor heat treatment assured their total destruction, even if they were more sensitive to the thermal treatment. To enhance the inhibitory effect on *Leuconostoc* cells, HPH should be combined with a mild heat treatment which, besides efficient microbial inactivation, could allow maximal retention of the physicochemical properties of the product.

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REFERENCES

1. Arroyo, G., P. D. Sanz, and G. Prestamo. 1999. Response to high-pressure, low-temperature treatment in vegetables: determination of survival rates of microbial populations using flow cytometry and detection of peroxidase activity using confocal microscopy. *J. Appl. Microbiol.* 86:544–556.
2. Cardamone, L., A. Quiberoni, D. J. Mercanti, M. E. Fornasari, J. A. Reinheimer, and D. M. Guglielmotti. 2011. Adventitious dairy *Leuconostoc* strains with interesting technological and biological properties useful for adjunct starters. *Dairy Sci. Technol.* 91:457–470.
3. Chaves-López, C., R. Lanciotti, A. Serio, A. Paparella, E. Guerzoni, and G. Suzzi. 2009. Effect of high pressure homogenization applied individually or in combination with other mild physical or chemical stresses on *Bacillus cereus* and *Bacillus subtilis* spore viability. *Food Control* 20:691–695.
4. Diels, A. M. J., and C. W. Michiels. 2006. High-pressure homogenization as non-thermal technique for the inactivation of microorganisms. *Crit. Rev. Microbiol.* 32:201–216.
5. Fonberg-Broczek, M., B. Windyga, J. Szcsawinski, M. Szezawinka, D. Pietrzak, and G. Prestamo. 2005. High pressure processing for food safety. *Acta Bioquím. Pol.* 52:721–724.
6. Guerzoni, M. E., M. Ferruzzi, F. Gardini, and R. Lanciotti. 1999. Combined effects of ethanol, high homogenization pressure, and temperature on cell fatty acid composition in *Saccharomyces cerevisiae*. *Can. J. Microbiol.* 45:805–810.
7. Guerzoni, M. E., L. Vannini, C. Chaves López, R. Lanciotti, G. Suzzi, and A. Gianotti. 1999. Effect of high pressure homogenization on microbial and chemico-physical characteristics of goat cheeses. *J. Dairy Sci.* 82:851–862.
8. Hamasaki, Y., M. Ayaki, H. Fuchu, and M. Sugiyama. 2003. Behavior of psychrotrophic lactic acid bacteria isolated from spoiling cooked meat products. *Appl. Environ. Microbiol.* 69: 3668–3671.
9. Hemme, D., and C. Foucaud-Scheunemann. 2004. *Leuconostoc*, characteristics, use in dairy technology and prospects in functional foods. *Int. Dairy J.* 14:467–494.
10. Kheadr, E. E., J. F. Vachon, P. Paquin, and I. Fliss. 2002. Effect of dynamic high pressure on microbiological, rheological and microstructural quality of Cheddar cheese. *Int. Dairy J.* 12:435–446.
11. Lado, B. H., and A. E. Yousef. 2002. Alternative food-preservation technologies: efficacy and mechanisms. *Microbes Infect.* 4:433–440.
12. Lanciotti, R., N. Belletti, F. Patrignani, A. Gianotti, F. Gardini, and M. E. Guerzoni. 2003. Application of hexanal, (E)-2-hexenal, and hexyl acetate to improve the safety of fresh-sliced apples. *J. Agric. Food Chem.* 51:2958–2963.
13. Lanciotti, R., C. Chaves-López, F. Patrignani, A. Paparella, M. E. Guerzoni, A. Serio, and G. Guzzi. 2004. Effects of milk treatment with dynamic high pressure on microbial populations, and lipolytic and proteolytic profiles of Crescenza cheese. *Int. J. Dairy Technol.* 57:19–25.
14. Lanciotti, R., F. Patrignani, L. Iucci, P. Saracino, and M. E. Guerzoni. 2007. Potential of high pressure homogenization in the control and enhancement of proteolytic and fermentative activities of some *Lactobacillus* species. *Food Chem.* 102:542–550.
15. Lanciotti, R., L. Vannini, F. Patrignani, L. Iucci, M. Vallicelli, M. Ndagijimana, and M. E. Guerzoni. 2006. Effect of high pressure homogenisation of milk on cheese yield and microbiology, lipolysis and proteolysis during ripening of Caciotta cheese. *J. Dairy Res.* 73:216–226.
16. López-Pedemonte, T., W. J. Brine, A. X. Roig-Sagué, and B. Guarmis. 2006. Fate of *Staphylococcus aureus* in cheese treated by ultrahigh pressure homogenization and high hydrostatic pressure. *J. Dairy Sci.* 89:4536–4544.
17. Martley, F. G., and V. L. Crow. 1993. Interactions between non-starter microorganisms during cheese manufacture and ripening. *Int. Dairy J.* 3:461–483.
18. McSweeney, P. L. H., and M. J. Sousa. 2000. Biochemical pathways for the production of flavour compounds in cheeses during ripening: a review. *Lait* 80:293–324.
19. Patrignani, F., P. Burns, D. Serrazanetti, G. Vinderola, J. Reinheimer, R. Lanciotti, and M. E. Guerzoni. 2009. Suitability of high pressure-homogenized milk for the production of probiotic fermented milk containing *Lactobacillus paracasei* and *Lactobacillus acidophilus*. *J. Dairy Res.* 76:74–82.
20. Patrignani, F., L. Iucci, R. Lanciotti, M. Vallicelli, J. Maina Mathara, W. H. Holzapfel, and M. E. Guerzoni. 2007. Effect of high-pressure homogenization, nonfat milk solids, and milkfat on the technological performance of a functional strain for the production of probiotic fermented milks. *J. Dairy Sci.* 90:4513–4523.
21. Pereda, J., V. Ferragut, J. M. Quevedo, G. Guamis, and A. J. Trujillo. 2007. Effects of ultra-high pressure homogenization on microbial and physicochemical shelf life of milk. *J. Dairy Sci.* 90:1081–1093.
22. Quiberoni, A., D. Guglielmotti, and J. Reinheimer. 2008. New and classical spoilage bacteria causing widespread blowing in Argentinean soft and semihard cheeses. *Int. J. Dairy Technol.* 61: 358–363.
23. Rerkrai, S., I. J. Jeon, and R. Bassette. 1987. Effect of various direct ultrahigh temperature heat treatments on flavor of commercially prepared milks. *J. Dairy Sci.* 70:2046–2054.
24. Ross, A. I. V., M. W. Grifiths, G. S. Mittal, and H. C. Deeth. 2003. Combining nonthermal technologies to control foodborne microorganisms. Review. *Int. J. Food Microbiol.* 89:125–138.

25. Saboya, L. V., M.-B. Maillard, and S. Lortal. 2003. Efficient mechanical disruption of *Lactobacillus helveticus, Lactococcus lactis* and *Propionibacterium freudenreichii* by a new high-pressure homogenizer and recovery of intracellular aminotransferase activity. *J. Ind. Microbiol. Biotechnol.* 30:1–5.
26. Serra, M., A. J. Trujillo, J. Pereda, B. Guamis, and V. Ferragut. 2008. Quantification of lipolysis and lipid oxidation during cold storage of yogurts produced from milk treated by ultra-high pressure homogenization. *J. Food Eng.* 89:99–104.
27. Thiebaud, M., E. Dumay, L. Picart, J. P. Guiraud, and J. C. Cheftel. 2003. High-pressure homogenisation of raw bovine milk. Effects on fat globule size distribution and microbial inactivation. *Int. Dairy J.* 13:427–439.
28. Vachon, J. F., E. E. Kheadr, J. Giasson, P. Paquin, and I. Fliss. 2002. Inactivation of foodborne pathogens in milk using dynamic high pressure. *J. Food Prot.* 65:345–352.
29. Vannini, L., F. Patrignani, L. Iucci, M. Ndagijimana, M. Vallicelli, R. Lanciotti, and M. E. Guerzoni. 2008. Effect of a pre-treatment of milk with high pressure homogenization on yield as well as on microbiological, lipolytic and proteolytic patterns of ''Pecorino'' cheese. *Int. J. Food Microbiol.* 128:329–335.
30. Vedamuthu, E. R. 1994. The dairy *Leuconostoc*: use in dairy products. *J. Dairy Sci.* 77:2725–2737.
31. Wuytack, E. Y., A. M. J. Diels, and C. W. Michiels. 2002. Bacterial inactivation by high pressure homogenization and high hydrostatic pressure. *Int. J. Food Microbiol.* 77:205–212.