3.4. Sensitivity of *Staphylococcus aureus* and *Lactobacillus helveticus* in Ovine Milk Subjected to High Hydrostatic Pressure. (Gervilla y col., 1999). *Journal of Dairy Science,* 82: 1099-1107.

Sensitivity of *Staphylococcus aureus* and *Lactobacillus helveticus* in Ovine Milk Subjected to High Hydrostatic Pressure

R. GERVILLA,[1] E. SENDRA, V. FERRAGUT, and B. GUAMIS
Unitat de Tecnologia dels Aliments (CeRTA), Facultat de Veterinària, Universitat Autònoma de Barcelona, 08193 Bellaterra (Barcelona), Spain

ABSTRACT

Ovine milk, standardized to 6% fat, was inoculated with *Staphylococcus aureus* CECT 534 and *Lactobacillus helveticus* CECT 414 at a concentration of 10^7 cfu/ml and treated by high hydrostatic pressure. Treatments consisted of combinations of pressure (200, 300, 400, 450, and 500 MPa), temperature (2, 10, 25, and 50°C), and time (5, 10, and 15 min). *Staphylococcus aureus* was highly resistant to pressure; only pressurizations at 50°C of 500 MPa for 15 min achieved reductions of ≥7.3 log units. For *L. helveticus*, the number of surviving cells was reduced considerably at pressures of 400 MPa or more (up to 4.5 log units at 50°C for 15 min), and pressure was more effective at low (2 and 10°C) and moderately high (50°C) temperatures than at room temperature (25°C). Both species showed first-order kinetics of destruction in the range 0 to 60 min. The *D* values for *S. aureus* were 20 min (2°C at 450 MPa) and 16.7 min (25°C at 450 MPa), and *D* values for *L. helveticus* were 7.1 min (2°C at 450 MPa) and 9.1 min (25°C at 450 MPa). *Lactobacillus helveticus* showed higher rates of survival of pressure than those reported in previous studies for other *Lactobacillus* spp.

(**Key words**: high hydrostatic pressure, *Staphylococcus aureus*, *Lactobacillus helveticus*, ovine milk)

Abbreviation key: **HPP** = high pressure processing, **MRS** = de Man-Rogosa-Sharpe, **PCA** = plate count agar.

INTRODUCTION

Nonthermal processing for food preservation is of great interest to researchers and industries (27). High pressure processing (**HPP**) is a physical process that can reduce microbial load (19, 38) with minimum alteration of food constituents (9, 10, 16, 32). The HPP is based on the application of two physical principles: Chatelier's principle and the principle of pressure transmission in a uniform and instantaneous manner. Also, HPP can be combined with other antimicrobial systems to obtain a synergistic effect. Vegetative cells in the growth phase are normally more sensitive to HPP than are cells in stationary or death phases (40); Gram-positive bacteria are more resistant than Gram-negative species, and bacterial spores are the most resistant forms.

The use of HPP (up to 1000 MPa) for food preservation was pioneered by Hite (17) and Hite et al. (18), but most of the studies are quite recent. Some authors have studied the effect of HPP on microorganisms in cultured and buffered media (35), meat (7), bovine UHT milk (34), ovine milk (12, 13), liquid cream (30), and other foods (5, 28). Ovine milk production is prevalent in Mediterranean countries, and this milk is mostly used for cheese making (90% of total production). The extensive popularity of cheeses from raw milk gives importance to the study of microbial inactivation in ovine milk under pressure.

The present study deals with pressure inactivation of two bacterial species: *Staphylococcus aureus* CECT 534 a pathogenic microorganism, and *Lactobacillus helveticus* CECT 414, a microorganism of technological interest in cheese production. Because of the presence of *S. aureus* and its enterotoxins, milk and dairy products have been involved in a number of food-poisoning outbreaks (3, 39). *Staphylococcus* spp. have been also reported to be the major cause of ovine mastitis (14). *Lactobacillus helveticus* can be used as a starter culture in the manufacture of a variety of fermented dairy products and as a nonstarter flavor enhancer that is capable of reducing bitterness and accelerating cheese flavor development (1, 2). Because of interest in other lactic bacterias (such as starters or starter adjuncts) in matured cheeses manufactured from milk treated by HPP, our study focused on the effect of the HPP on *L. helveticus*.

The main objective of this work was to study the effect of different pressures, times, and temperatures

Received June 19, 1998.
Accepted February 8, 1999.
[1]Author for correspondence.

on the destruction of *S. aureus* and *L. helveticus*, inoculated in ovine milk at about 10^7 cfu/ml. The *D* values (decimal reduction time, the time in minutes necessary to kill 90% of the microbial population at a certain temperature and pressure) were determined for both microorganisms to estimate the most effective application conditions of HPP technology.

MATERIALS AND METHODS

Bacterial Strain

Staphylococcus aureus CECT 534 and *L. helveticus* CECT 414 were obtained as freeze-dried cultures in thermosealed vials from the Spanish Type Culture Collection (University of Valencia, Valencia, Spain). The vials were maintained at 4°C until use. Bacteria were rehydrated in 3 ml of appropriate broth: *S. aureus* in tryptone-soy broth (Oxoid Ltd., Basingstoke, Hampshire, England) at 37°C for 24 h and *L. helveticus* in de Man-Rogosa-Sharpe (**MRS**) broth (Oxoid Ltd.) at 37°C for 24 h. Subsequently, 1 ml of each cultured broth was inoculated in 9 ml of the same broth and was incubated under the same conditions as rehydration. These broth cultures were used to inoculate tryptone-soy agar (Oxoid Ltd.) for *S. aureus* and MRS agar (Oxoid Ltd.) with *L. helveticus*, which were maintained at 4°C and transferred every 2 wk to provide stock cultures. For each experiment, a tube of stock media (tryptone-soy agar for *S. aureus* and MRS agar for *L. helveticus*) was inoculated with the appropriate bacteria in tryptone-soy broth and MRS broth and grown at 37°C for 24 h to achieve about 10^9 cfu/ml (stationary phase of growth).

Preparation and Inoculation of Milk Samples

Milk from Manchega ewes was obtained from the dairy farm of the Facultat de Veterinaria (Universitat Autònoma de Barcelona, Spain). Raw milk was collected from the first milking in the morning, centrifuged, and adjusted to 6% fat. Standardized milk was pasteurized at 75 ± 1°C for 1 min in a continuous tubular heat exchanger (Garvia S.A., Barcelona, Spain). Pasteurized milk was collected in 1-L sterile bottles, was adjusted to pH 6.7 (by adding 1*N* NaOH, or 1*N* HCl, or both), and was refrigerated at 4°C.

Separately, 10 ml of each broth culture (10^9 cfu/ml) were added to 1 L of pasteurized ovine milk to obtain approximately 10^7 cfu/ml. The milk samples were gently shaken by hand for 5 min, and then 30 ml of inoculated milk were pipetted into disinfected 30-ml polyester bottles. As much air as possible was expelled from the bottles, and caps were sealed with Teflon film.

Composition and Physicochemical Analyses of Milk

The total solids content was determined by drying at 102 ± 2°C in an oven until a constant weight was reached (21). Ash content was determined by gravimetric analysis after the sample had been calcinated in an oven at 550°C (20). Fat content was determined by the Gerber method (22). Total nitrogen was calculated using the digestion block method, a modification of the Kjeldahl method (23). The pH was measured by using a pH meter (micro-pH 2001; Crison Instruments S.A., Alella, Spain) (31).

HPP Treatments

Samples were pressurized by using discontinuous HPP equipment (ACB, Nantes, France) with a 2-L capacity pressure chamber. The time needed to achieve maximum pressure (500 MPa) was 2 min. The chamber and water (hydrostatic fluid medium) inside were cooled or heated to treatment temperature with a constant flow of ethyleneglycol and water (1:1) solution within the walls of the vessel. Samples were kept for 5 to 10 min at atmospheric pressure in the chamber until temperature equilibrium was established. The temperature of the samples was monitored by a thermocouple to determine the most extreme temperature conditions to which the samples were subjected.

Time, temperature, and pressure parameters were selected on the basis of previous, unpublished studies. The responses of *S. aureus* and *L. helveticus* to treatments at different conditions of pressure (200, 300, 400, 450, and 500 MPa), temperature (2, 10, 25, and 50°C), and time (5, 10, and 15 min) were studied. To evaluate the effect of temperature per se, inoculated samples of *S. aureus* and of *L. helveticus* were held at the most severe temperature-time conditions reached in the treatments (1°C for 65 min and 53°C for 20 min).

Kinetics of Destruction

To determine the kinetics of population reduction, assays were performed at 450 MPa and at 2 and 25°C over 60 min. Linear regression analyses of *S. aureus* and *L. helveticus* counts was computed for each temperature. An estimate of the *D* value was obtained by finding the absolute value of the inverse of the slope. Three replicates of each culture separately (inocu-

lated into ovine milk) were treated for each HPP combination.

Microbiological Assays

Samples were kept at 4°C prior to analysis (10 h approximately) to avoid postpressurization stress. Appropriate decimal dilutions in Ringer solution (9 ml) of each sample were made for microbial determinations.

To determine *S. aureus* counts, 1-ml volumes of sample or decimal dilutions were surface-plated in duplicate on Baird-Parker medium (Oxoid Ltd.) with egg yolk-tellurite emulsion (Oxoid Ltd.). The plates were incubated at 37°C and then were examined after 24 and 48 h of incubation. Representative numbers (5 to 10) of suspicious colonies (brown or black colonies with or without clear zones) were selected from Baird-Parker plates for identification by the tube coagulase test. At the same time, total counts were obtained with plate count agar (**PCA**) (Oxoid Ltd.) at 30°C for 48 h to determine any possible contamination of samples during manipulation, and differences in numbers between PCA and selective media were compared.

To determine *L. helveticus* counts, 1-ml volumes of sample or decimal dilutions were plated in duplicate on MRS agar (Oxoid Ltd.). To create microanaerobic conditions, a second layer of sterile MRS agar was added. The plates were incubated at 37°C for 72 h. Samples were plated in PCA as well.

Statistical Treatment of Data

Each experiment was run three times with duplicate analysis in each replicate. An ANOVA was performed using the GLM procedure of SAS (33). Duncan's new multiple range test and Student-Newman-Keuls test were used to obtain paired comparisons among sample means. Evaluations were based on a significance level of $P < 0.05$.

RESULTS

The mean (and SD) values for composition of ovine milk before standardization for fat content (wet weight basis) were DM, 18.73% (SD = 1.66%); fat, 7.61% (SD = 1.43%); protein, 5.78% (SD = 0.21%); and ash, 1.17% (SD = 0.11%); pH was 6.67 (SD = 0.08).

Bacterial inactivation increased linearly with pressure and exposure time; however, increased temperature in the pressurization treatments of milk did not lead to a linear response. The greatest reduction in counts was at 50°C for *S. aureus* (Figure 1) and at 2 and 50°C for *L. helveticus* (Figure 2).

Comparisons of significant differences between the HPP combinations illustrated in Figures 1 and 2 for *S. aureus* and for *L. helveticus* are shown in Table 1.

Inactivation of *S. aureus* CECT 534 increased with pressure ($500^a > 450^b > 400^c > 300^d > 200^e$ MPa) and exposure time ($15^a > 10^b > 5^c$ min), but temperature effect did not show a linear response ($50^a > 10^b > 25^c > 2^d$°C). Treatment variables just mentioned and subsequent comparisons without a common superscript differ ($P < 0.05$). Although some inactivation was observed for all treatments, only the treatment using 500 MPa at 50°C for 15 min reduced *S. aureus* to undetectable levels. From the analysis of the F values, pressure and temperature were the main factors, explaining 88% (at approximately equivalent levels) of the variability of the statistical model on destruction of *S. aureus* by HPP.

Lactobacillus helveticus CECT 414 showed higher sensitivity to HPP than did *S. aureus*. *Lactobacillus helveticus* counts were reduced to undetectable levels with 450 MPa at 50°C for 5 min. Inactivation rates increased linearly with pressure ($500^a > 450^b > 400^c > 300^d > 200^e$ MPa) and exposure time ($15^a > 10^b > 5^c$ min), but it is remarkable that temperature effect did not show a linear response ($50^a > 2^b > 25^c = 10^c$°C). No significant difference was found ($P > 0.05$) between treatments at 25 and 10°C. From the analysis of the F value, pressure and temperature explained 69 and 24%, respectively, of the variability in the statistical model.

Staphylococcus aureus showed less destruction at 500 MPa for 15 min at 2°C than at 10 and 25°C (Figure 3), whereas *L. helveticus* was more resistant at 10 and 25°C than at 2°C under the same conditions. Exposure time did not affect lethality of HPP as much as did pressure and temperature.

Kinetics of Destruction for *S. aureus* and *L. helveticus*

For *S. aureus*, the equations obtained by linear regression were as follows: at 2°C, $\log_{10}$ cfu/ml = 6.9 – 0.05 × t, where t is time of treatment ($r^2 = 0.955$); at 25°C, $\log_{10}$ cfu/ml = 6.9 – 0.06 × t ($r^2 = 0.963$). Similar to thermal treatments, HPP showed first-order kinetics for microbial destruction; D values for *S. aureus* treated at 450 MPa were 20 min at 2°C and 16.7 min at 25°C.

For *L. helveticus*, the equations obtained by linear regression were as follows: at 2°C, $\log_{10}$ cfu/ml = 6.5 – 0.14 × t ($r^2 = 0.989$); at 25°C, $\log_{10}$ cfu/ml = 6.7 – 0.11 × t ($r^2 = 0.996$). The D values obtained for *L. helveti-*

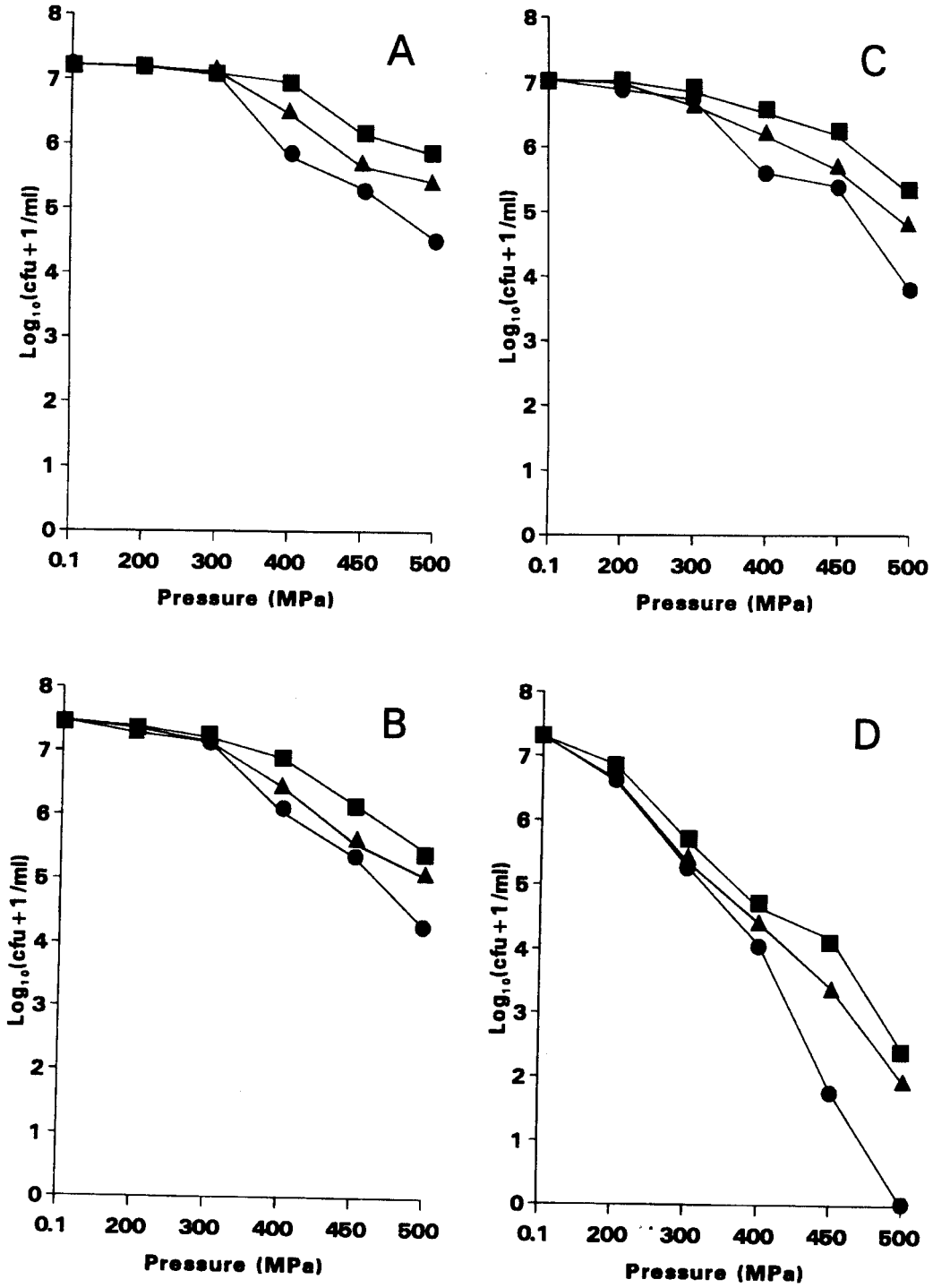

Figure 1. Effect of high hydrostatic pressure on *Staphylococcus aureus* CECT 534 in ovine milk. Pressure for 5 min (■), 10 min (▲), and 15 min (●). A: 2°C, B: 10°C, C: 25°C, and D: 50°C. Pressure at 0.1 MPa: logarithm of initial counts (control).

cus treated at 450 MPa were 7.1 min at 2°C and 9.1 min at 25°C.

To confirm total inactivation of microorganisms and to detect partially damaged microorganisms, 1 ml of sample from each treatment, where no microbial growth was detected, was directly plated on MRS (37°C for 72 h incubation), and another 1 ml of sample was incubated at 37°C for 72 h and afterward plated in MRS (37°C for 72 h of incubation). Both results from MRS plates were then compared. No

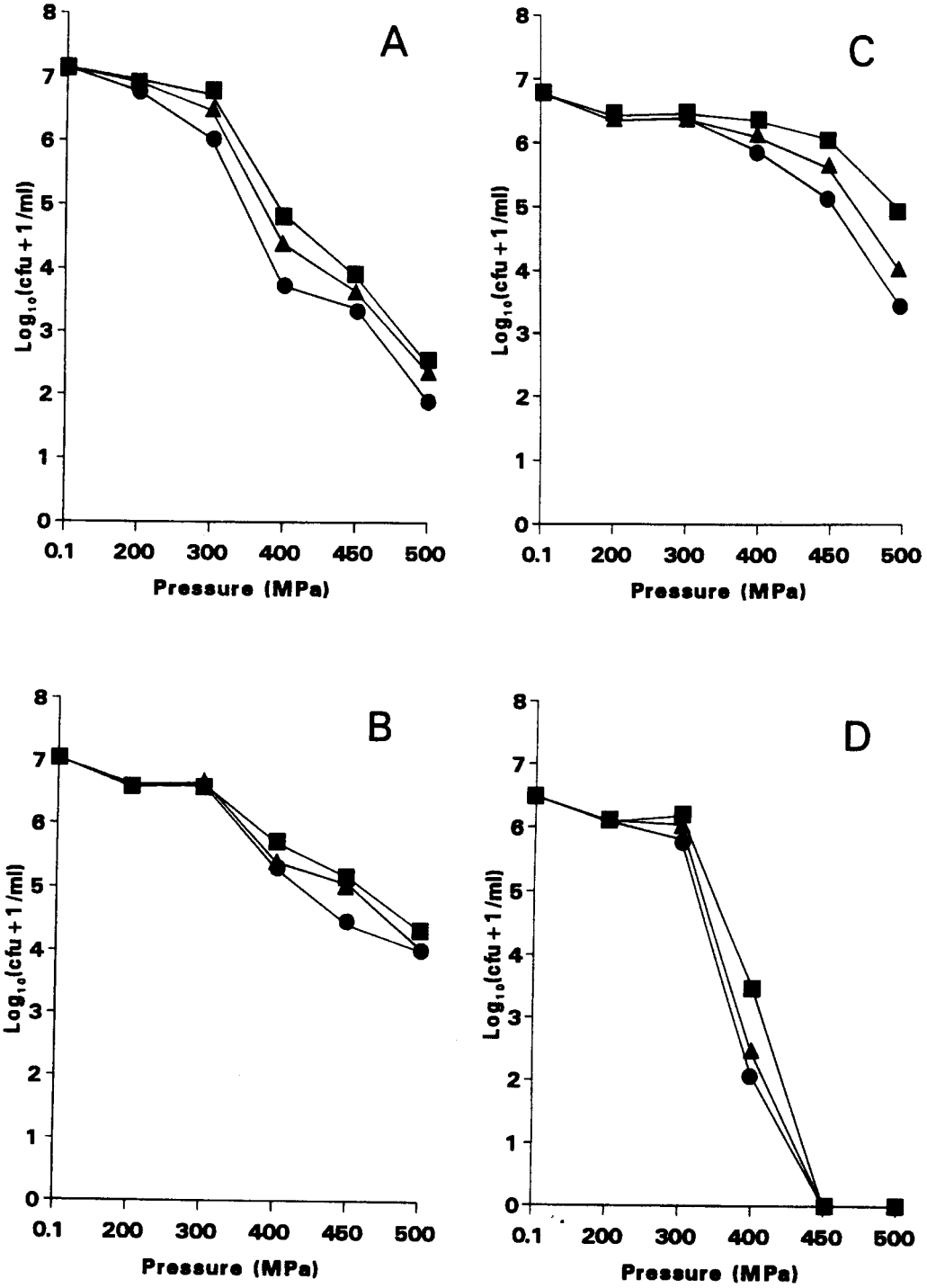

Figure 2. Effect of high hydrostatic pressure on *Lactobacillus helveticus* CECT 414 in ovine milk. Pressure for 5 min (■), 10 min (▲), and 15 min (●). A: 2°C, B: 10°C, C: 25°C, and D: 50°C. Pressure at 0.1 MPa: logarithm of initial counts (control).

microbial growth was detected in any sample and confirmed total inactivation and the absence of partially damaged microorganisms in samples in which no growth was detected (data not shown).

Counts obtained from inoculated samples that had been held at extreme time-temperature conditions, without pressure application, were not different from initial counts and indicated that temperature-time

TABLE 1. Significant differences between the high hydrostatic pressure combinations (at 5, 10, or 15 min) used to treat *Staphylococcus aureus* and *Lactobacillus helveticus* in ovine milk.

	2°C			10°C			25°C			50°C		
	5	10	15	5	10	15	5	10	15	5	10	15
S. aureus												
200[1]	a[2]	ab	ab	ab	abcd	abc	ab	ab	abcd	defg	ghi	hi
300	abc	ab	abcd	abcde	bcdef	bcdef	abcd	cdef	abcde	lmn	opq	pq
400	abcde	hi	kl	fgh	j	kl	fgh	ij	klm	s	t	u
450	j	lmn	opq	k	opq	q	ij	kl	mno	u	v	x
500	kl	nopq	s	pq	r	u	nop	r	u	w	x	y
L. helveticus												
200	a	ab	abc	abc	abc	abc	abc	c	c	abc	abc	abc
300	abc	c	de	abc	abc	abc	abc	bc	abc	abc	abc	c
400	i	k	m	eg	gh	fg	bc	d	ef	lm	p	pq
450	lm	m	n	h	gh	jk	d	gh	ij	s	s	s
500	qr	pq	r	jk	l	l	jk	o	pq	s	s	s

[1]Pressure (megaPascals).

[2]Each treatment combination is derived from three independent experiments and duplicate analyses (n = 6). Treatment combinations within each bacterial species without a common letter differ ($P < 0.05$).

combinations per se had no effect on microbial populations. Total counts obtained from PCA were never significantly different from those obtained with selective media.

DISCUSSION

Inactivation of *Staphylococcus* spp. by HPP has been studied by several groups under different conditions, and disparate responses to the treatment have been reported. Patterson et al. (29) studied the effect of HPP at 20°C on several microorganisms in different substrata or foods. *Staphylococcus aureus* NCTC 10652 was the most pressure-resistant of the pathogens tested. Pressures between 500 and 600 MPa were needed to obtain significant reductions in 10 m*M* PBS at pH 7.0, UHT milk, and raw poultry meat. We observed similar reductions of *S. aureus* CECT 534 in ovine milk at 400 MPa at 25°C and higher. All combinations evaluated with ovine milk samples were more effective than those reported for UHT milk (29). Earnshaw (11) studied the inactivation of *Staphylococcus carnosus* by HPP in nutrient broth. Reductions of 0, 0.5, 1.5, and 2.5 log units were reported at 300, 400, 500, and 600 MPa at 20°C for 30 min; higher reductions were obtained in our study with similar pressures. Takahashi (37) obtained reductions of 5 and ≥8 log units for *S. aureus* in 2 m*M* PBS at pH 7.0 treated at 300 and 400 MPa (20°C for 20 min). Lower bacterial reduction was obtained in our study with similar conditions (25°C for 15 min at 300 and 400 MPa), which gave reductions of 0.5 and 1.5 log units (Figure 1, c and d). In agreement with our results, Butz and Ludwig (4) reported reductions of 0.5 and 2 log units of *S. aureus* in sterile saline by HPP at 300 MPa and 40°C for 15 and 30 min. Carballo et al. (6) studied the effect of fat content on HPP inactivation of *S. aureus* and other microorgan-

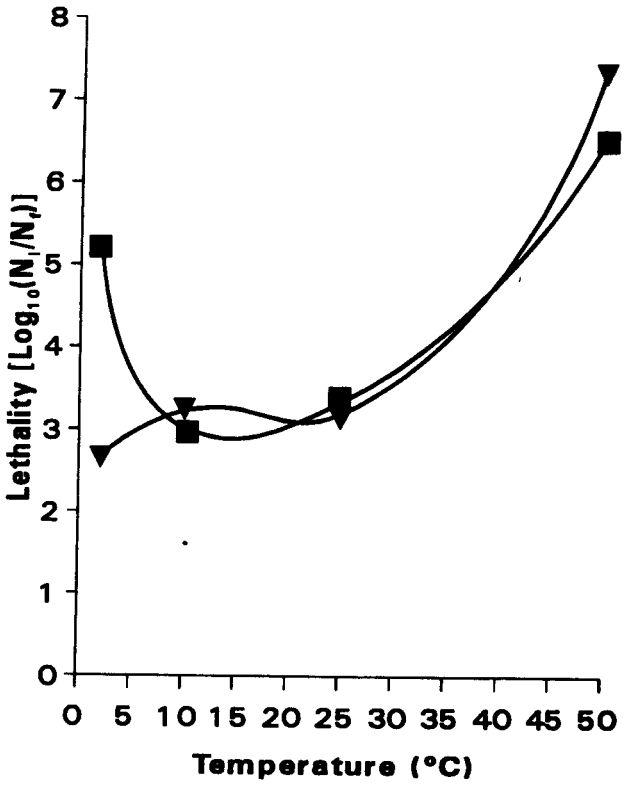

Figure 3. Effect of high hydrostatic pressure as a function of temperature at 500 (MPa) for 15 min on *Staphylococcus aureus* CECT 534 (▼) and *Lactobacillus helveticus* CECT 414 (■) in ovine milk. N_i = initial count; N_f = final count.

isms in beef patties at low temperature (5°C) with pressures of 100 and 300 MPa for 5 and 20 min. In high fat patties, 1 log reduction was achieved at 300 MPa for 20 min, and, in low fat samples, similar results were achieved in only 5 min.

All of the studies of HPP effects on *Staphylococcus* spp. agree that inactivation rates increase linearly with pressure and exposure time. The response of *S. aureus* to HPP depends on the temperature of the treatment as well, but *S. aureus* showed different behavior at low to ambient temperatures and at medium to high temperatures. In the first interval (low and ambient), *S. aureus* response to HPP was not affected by temperatures between –20 to 25°C; in the second interval, at 40 to 50°C and higher, *S. aureus* was much more sensitive to HPP as temperature increased (29, 37). Although no inactivation effect could be attributed to the temperature of the treatment per se between 1 to 50°C, temperatures over 40°C would seem to enhance the destructive effect of pressure on *S. aureus* (4).

Several factors can influence inactivation rates of *Staphylococcus* spp. under HPP: the medium in which the microorganism is pressurized and the species and strain (11, 29, 37) are probably the most influential of all factors. Those factors must be considered when making recommendations for improving the safety of pressure-treated foods. Low water activity, sodium chloride, sucrose, fat, and possibly other food constituents have been reported to provide protection against pressure (24, 26). It has also been shown that certain foods constituents, such as proteins, polysaccharides, certain organic acids, alcohols, lipids, and salts, can have a protective or synergistic effect on microbial inactivation (9). Presumably, *S. aureus* would be more resistant to HPP in foods than in physiological solutions or buffers. Comparisons of studies from different authors (4, 11, 29, 37) indicate that inactivation does not always follow the same pattern. It is also possible that other food constituents and changes (such as pH) in foods during pressurization would modulate inactivation of *S. aureus*. If inactivation in ovine milk is compared with UHT milk studies by Patterson (29), *S. aureus* is a microorganism of concern in foods treated by HPP because of high resistance to pressure.

Lactobacillus spp. are widely present in dairy products, and the response of lactobacilli to HPP has been studied for several species in different media. *Lactobacillus casei* was treated at 0 to 60°C and 0.1 to 400 MPa in 10 m*M* HEPES at pH 5.3 by Sonoike et al. (36), who observed that the contours of constant death rates of *L. casei* on the pressure-temperature plane were elliptical. These patterns were similar to those of free-energy differences for pressure-reversible denaturation of proteins. We agree with the observation that low (0 to 5°C) and moderately high temperatures (50 to 60°C) are very effective in the destruction of lactobacilli. *Lactobacillus delbrueckii* spp. *bulgaricus* in yogurt was studied by Kromkamp et al. (25). They observed microbial reductions between 4 and 5 log units at 200 and 300 MPa for 30 min at ambient temperature (20 to 25°C). Both studies showed higher inactivation rates than those observed in our study (Figure 2c). The factors could relate to species and media used in the studies (ovine milk at pH 6.7 and containing 6% fat vs. yogurt at pH 4.6 and no fat or 10 m*M* HEPES at pH 5.3). The response of *Lactobacillus* spp. to HPP in minced meat was studied by Carlez et al. (8), who showed that pressures under 400 MPa had little effect on their inactivation; at 400 MPa and higher, inactivation was extensive (reductions of 4 or >5 log units). In our experiments (Figure 2c), 500 MPa for 15 min was necessary to obtain reductions of about 3.5 log units. It seems that ovine milk may exercise a baroprotective effect on *Lactobacillus* spp., particularly on *L. helveticus*, and if ovine milk products are pressurized, *Lactobacillus* spp. will only be reduced slightly.

Both microorganisms studied in the present experiment are Gram-positive bacteria, and therefore, a certain amount of resistance to HPP was expected. Other factors are involved in its response to HPP, and the spherical morphology and cell-wall composition of *S. aureus* could enhance its resistance to pressure, but real causes of cellular damage are not well known. The kinetics of destruction of *L. helveticus* at constant HPP obtained at different temperatures followed the same pattern as that of protein denaturation. Some proteins are more susceptible to denaturation during pressurization at low temperatures (15), which suggests protein denaturation as a factor in the response of *L. helveticus* to HPP in our study.

To date, *S. aureus* is the most HPP-resistant vegetative microorganism studied in ovine milk of the microorganisms tested under the same conditions and pressurization medium (12, 13). *Pseudomonas fluorescens* is very sensitive, showing reductions of 7 log units when exposed to 400 MPa at 25°C for 5 min (13). *Escherichia coli* and *Listeria innocua* are less sensitive, reaching reductions of 6 log units above 450 MPa at 25°C for 10 min (12, 13). The microorganisms tested in this study (*S. aureus* and *L. helveticus*) required 500 MPa at 25°C for 5 to 15 min to obtain reductions of 2 to 3 log units.

From the critical review of the results reported by other researchers and our own, we can conclude that

the kinetics of destruction of most microorganisms at a given pressure are different depending on the temperature. Combinations of efficient temperatures (low or moderately high) and pressures are more effective than extending exposure time at lower pressures. The lethal effect at a given HPP on vegetative cells is strongly influenced by the composition of the media or food; the differences between the lethality of microorganisms subjected to HPP in buffer and real food systems need to be investigated. We believe that by investigating HPP in combination with other antimicrobial treatments (e.g., low pH, nisin, light pulses, and electric or magnetic fields) (27), a process can be developed that may be a good alternative to pasteurization. Studies to determine the effect of HPP on inactivation of other microorganisms in ovine milk and the effect of ovine milk constituents are being undertaken by our group.

ACKNOWLEDGMENTS

This study has been financially supported by the AIR-CT 92-0296 Project: High Hydrostatic Pressure Treatments: Its Impact on Spoilage Microorganisms, Biopolymer Activity, Functionality and Nutrient Composition of Food Systems. We thank the Spanish Type Culture Collection (CECT) for providing the strains.

REFERENCES

1 Bartels, H. J., M. E. Johnson, and N. F. Olson. 1987. Accelerated ripening of Gouda cheese. 1. Effect of heat shocked thermophilic lactobacilli and streptococci on proteolysis and flavour development. Milchwissenschaft 42:83–88.

2 Bartels, H. J., M. E. Johnson, and N. F. Olson. 1987. Accelerated ripening of Gouda cheese. 2. Effect of freeze-shocked *Lactobacillus helveticus* on proteolysis and flavour development. Milchwissenschaft 42:139–144.

3 Bryan, F. L. 1983. Epidemiology of milk-borne diseases. J. Food Prot. 46:637–649.

4 Butz, P., and H. Ludwig. 1986. Pressure inactivation of microorganisms at moderate temperatures. Physica 139 & 140B: 875–877.

5 Capellas, M., M. Mor-Mur, E. Sendra, R. Pla, and B. Guamis. 1996. Populations of aerobic mesophils and inoculated *E. coli* during storage of fresh goat's milk cheese treated with high pressure. J. Food Prot. 59:582–587.

6 Carballo, J., P. Fernández, A. V. Carrascosa, M. T. Solas, and F. Jiménez-Colmenero. 1997. Characteristics of low- and high-fat beef patties: effect of high hydrostatic pressure. J. Food Prot. 60:48–53.

7 Carlez, A., J. P. Rosec, N. Richard, and J. C. Cheftel. 1993. High pressure inactivation of *Citrobacter freundii*, *Pseudomonas fluorescens* and *Listeria innocua* in inoculated minced beef muscle. Lebensm. Wiss. Technol. 26:357–363.

8 Carlez, A., J. P. Rosec, N. Richard, and J. C. Cheftel. 1994. Bacterial growth during chilled storage of pressure-treatment minced meat. Lebensm. Wiss. Technol. 27:48–54.

9 Cheftel, J. C. 1991. Application des hautes pressions en technologie alimentaire. Ind. Aliment. Agric. 108:141–153.

10 Earnshaw, R. G. 1992. High pressure as a cell sensitiser: new opportunities to increase the efficacy of preservation processes. Pages 261–267 *in* High Pressure and Biotechnology. C. Balny, R. Hayahi, K. Heremans, and P. Masson, ed. Colloque INSERM/John Libbey Eurotext Ltd., London, England.

11 Earnshaw, R. G. 1995. High pressure microbial inactivation kinetics. Pages 37–46 *in* High Pressure Processing of Foods. D. A. Ledward, D. E. Johnston, R. G. Earnshaw, and A.P.M. Hasting, ed. Nottingham Univ. Press, Nottingham, England.

12 Gervilla, R., M. Capellas, V. Ferragut, and B. Guamis. 1997. Effect of high hydrostatic pressure on *Listeria innocua* 910 CECT inoculated into ewe's milk. J. Food Prot. 60:33–37.

13 Gervilla, R., X. Felipe, V. Ferragut, and B. Guamis. 1997. Effect of high hydrostatic pressure on *Escherichia coli* and *Pseudomonas fluorescens* strains in ovine milk. J. Dairy Sci. 80: 2297–2303.

14 Gutiérrez, L. M., M. L. García-López, A. Otero, M. C. García-Fernández, and B. Moreno. 1990. Incidence of staphylococci in ovine mastitic milk and antibiotic susceptibility of the strains. Milchwissenschaft 45:778–781.

15 Hawley, S. A. 1971. Reversible pressure-temperature denaturation. Biochemistry 10:2436–2442.

16 Hayashi, R. 1989. Applications of high pressure to food processing and preservation. Philosophy and development. Pages 815–829 *in* Engineering and Food. Vol. 2. W. E. Spiess and H. Schubert, ed. Elsevier Appl. Sci., London, England.

17 Hite, B. H. 1899. The effect of pressure in the preservation of milk. Bull. West Virginia Univ. Agric. Exp. Stn. 58:15–35.

18 Hite, B. H., N. J. Giddings, and C. E. Weakly. 1914. The effects of pressure on certain microorganisms encountered in the preservation of fruits and vegetables. Bull. West Virginia Univ. Agric. Exp. Stn. 146:1–67.

19 Hoover. D., C. Metrick, A. M. Papineau, D. Farkas, and D. Knorr. 1989. Biological effects of high hydrostatic pressure on food microorganisms. Food Technol. 43:99–107.

20 International Dairy Federation. 1964. Determination of the ash content of milk and processed cheese products. FIL-IDF Stand. 27. Int. Dairy Fed., Brussels, Belgium.

21 International Dairy Federation. 1987. Milk, cream and evaporated milk; total solids content. FIL-IDF Stand. 21B. Int. Dairy Fed., Brussels, Belgium.

22 International Dairy Federation. 1991. Milk and milk products—Fat content. General guidance on the use of butyrometric methods. FIL-IDF Stand. 52. Int. Dairy Fed., Brussels, Belgium.

23 International Dairy Federation. 1993. Milk-Nitrogen content. FIL-IDF Stand. 220B. Int. Dairy Fed., Brussels, Belgium.

24 Knorr, D., A. Bottcher, H. Dornenburg, M. Eshtiaghi, P. Oxen, A. Richwin, and I. Seyderhelm. 1992. High pressure effects on microorganisms, enzyme activity and food functionality. Pages 221–218 *in* High Pressure and Biotechnology. C. Balny, R. Hayahi, K. Heremans, and P. Masson, ed. Colloque INSERM/John Libbey Eurotext Ltd., London, England.

25 Kromkamp, J., R. M. Moreira, L.P.M. Langeveld, and P.J.J.M. van Mil. 1996. Microorganisms in milk and yoghurt: selective inactivation by high hydrostatic pressure. Pages 267–271 *in* Proc. Int. Dairy Fed. Symp. September 6–8, 1995. Heat treatments and alternative methods. Int. Dairy Fed., Brussels, Belgium.

26 Maggi, A., P. Rovere, S. Gola, and G. Dall'Agilo. 1994. Effect of high hydrostatic pressure on *Salmonella* and other strains of *Enterobacteriaceae* in culture broth at different pH and NaCl levels. Ind. Conserve. 68:232–235.

27 Mertens, B., and D. Knorr. 1992. Developments of nonthermal processes for food preservation. Food Technol. 46:124–132.

28 Ogawa, H., K. Fukuhisa, and H. Fukumoto. 1992. Effect of hydrostatic pressure on sterilization and preservation of citrus juice. Pages 269–278 *in* High Pressure and Biotechnology. C. Balny, R. Hayahi, K. Heremans, and P. Masson, ed. Colloque INSERM/John Libbey Eurotext Ltd., London, England.

29 Patterson, M. F., M. Quinn, R. Simpson, and A. Gilmour. 1995. Sensitivity of vegetative pathogens to high hydrostatic pressure treatment in phosphate-buffered saline and foods. J. Food Prot. 58:524–529.

30 Raffalli, J., J. P. Rosec, A. Carlez, E. Dumay, N. Richard, and J. C. Cheftel. 1994. Stress et inactivation par haute pression de *Listeria innocua* introduites dans une crème laitière. Sci. Aliment. 14:349–358.

31 Richardson, G. H., ed. 1985. Standard Methods for the Examination of Dairy Products. 15th ed. Am. Publ. Health Assoc., Inc., Washington, DC.

32 Rönner, U. 1994. Food preservation by ultra-high pressure. Pages 31–35 *in* Food Preservation by Combined Processes. L. Leistner and L.G.M. Gorris, ed. Eur. Comm. EUR 15776 EN, Final Report Flair Concerted Action No. 7, Subgroup B, The Netherlands.

33 SAS® User's Guide: Statistics, Version 5 Edition. 1985. SAS Inst., Inc., Cary, NC.

34 Styles, M., D. Hoover, and D. Farkas. 1991. Response of *Listeria monocytogenes* and *Vibrio parahaemolyticus* to high hydrostatic pressure. J. Food Sci. 56:1404–1407.

35 Shigehisa, T., T. Ohmori, A. Saito, S. Taji, and R. Hayashi. 1991. Effects of high hydrostatic pressure on characteristics of pork slurries and inactivation of microorganisms associated with meat and meat products. Int. J. Food Microbiol. 12: 207–216.

36 Sonoike, K., T. Setoyama, Y. Kuma, and S. Kobayashi. 1992. Effect of pressure and temperature on the death rates of *Lactobacillus casei* and *Escherichia coli*. Pages 297–301 *in* High Pressure and Biotechnology. C. Balny, R. Hayahi, K. Heremans, and P. Masson, ed. Colloque INSERM/John Libbey Eurotext Ltd., London, England.

37 Takahashi, K. 1992. Sterilization of microorganisms by hydrostatic pressure at low temperatures. Pages 303–307 *in* High Pressure and Biotechnology. C. Balny, R. Hayahi, K. Heremans, and P. Masson, ed. Colloque INSERM/John Libbey Eurotext Ltd., London, England.

38 Timson, W., and N. Short. 1965. Resistance of microorganisms to high pressure. Biotechnol. Bioeng. 7:139–159.

39 Wieneke, A. A., and R. J. Gilbert. 1987. Comparison of four methods for the detection of staphylococcal enterotoxin in foods from outbreaks of food poisoning. Int. J. Food Microbiol. 4: 135–143.

40 ZoBell, C. 1970. Pressure effects on morphology and life processes of bacteria. Pages 85–130 *in* High Pressure Effects on Cellular Processes. A. Zimmerman, ed. Acad. Press, New York, NY.