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Effect of High-Pressure Treatment and a Bacteriocin-Producing Lactic Culture on the Odor and Aroma of Hispánico Cheese: Correlation of Volatile Compounds and Sensory Analysis

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The effect on the volatile compounds and on the odor and aroma of Hispánico cheese of a high-pressure (HP) treatment (400 MPa for 5 min at 10 °C, applied to 15-day-old cheeses), by itself or combined with the addition of a bacteriocin-producing (BP) culture to milk, was investigated. HP-treated cheeses showed higher levels of hexanal, 3-hydroxy-2-pentanone, 2-hydroxy-3-pentanone, and hexane and lower levels of ethanal, ethanol, 1-propanol, ethyl acetate, ethyl butanoate, ethyl hexanoate, 2-pentanone, and butanoic acid than untreated cheeses. HP cheeses received higher “milky” odor descriptor scores and lower scores for odor quality and intensity and for “buttery”, “yogurt-like”, and “caramel” odor descriptors. Addition of the BP culture enhanced the formation of three aldehydes, three alcohols, three ethyl esters, and three ketones but decreased the levels of seven ketones and butanoic acid. BP cheeses received higher scores for aroma intensity and for “yogurt-like” and “cheesy” aroma descriptors. Principal component analysis showed the correlation between diketones and aroma descriptors “caramel”, “buttery”, and “milky” and between 3-methylbutanal and the odor and aroma intensity scores and aroma descriptors “sheepy” and “meat broth”.

KEYWORDS: High pressure; bacteriocin; cheese; volatile compounds; odor; aroma

INTRODUCTION

Flavor is probably the most important characteristic of cheese quality. Low molecular weight compounds produced through glycolysis, proteolysis, lipolysis, and the secondary reactions that take place throughout cheese ripening are the most significant contributors to cheese flavor. Among them, more than 600 volatile compounds have been identified in different cheese varieties, and many have been related to particular odor and aroma notes (*1*, *2*). It is generally accepted that the volatile fraction gives an approximate objective image of the odor and aroma of cheeses. For this reason the headspace analysis can be useful to discriminate the effects of new technologies in cheese manufacture. However, the usually complex chromatographic profiles obtained make it necessary to use a multivariate statistical analysis, such as principal component analysis (PCA), to reduce the number of variables and to help find the best correlated chemical and sensory variables for the interpretation of results.

Lactic acid bacteria (LAB) are an important source of enzymes such as proteinases, peptidases, amino acid catabolic enzymes, and esterases, which transform milk constituents retained in the curd into low molecular weight compounds (*3*, *4*). Because most LAB enzymes are intracellular, cell lysis will favor the access of enzymes to their substrates and, presumably, will accelerate the development of cheese flavor (*5*).

Inoculation of milk with bacteriocin-producing (BP) adjunct cultures may enhance the lysis of LAB cells during cheese ripening. In cheese made from milk with *Enterococcus faecalis* INIA 4, a strain producing enterocin AS-48 added as an adjunct to the lactic starter, the formation of some volatile compounds and flavor intensity evolved more rapidly than in control cheese (*6*). The use of *Lactococcus lactis* subsp. *lactis* INIA 415, a strain producing nisin Z and lacticin 481, as BP culture enhanced the formation of some volatile compounds in Hispánico cheese (*7*).

Most applications of high-pressure (HP) treatment in cheese are related to the inactivation or reduction of pathogenic and spoilage microorganisms. When HP treatment was assayed for the acceleration of cheese ripening, an enhancement of proteolysis was observed (*8*−*11*), which was related to the higher cell-free aminopeptidase activities (*8*). As HP treatment increases cell membrane permeability (*12*−*14*), the release of intracellular peptidases to the medium is favored (*15*) and, therefore, proteolysis is accelerated.

Although the application of HP treatment to accelerated cheese ripening has been studied by different authors, few works have been published on its influence on the volatile compounds found in cheese. To our knowledge, it has been investigated only in Gouda cheese (*16*), Swiss cheese slurries (*17*), and

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Garrotxa cheese, a goat's milk variety (*18*). In the present work, the effect of HP treatment, by itself or combined with the addition of a BP adjunct culture to milk, on the volatile compounds, odor, and aroma of Hispánico cheese was investigated.

MATERIALS AND METHODS

Lactic Cultures and Cheese Manufacture. LAB strains used as lactic cultures were *L. lactis* subsp. *lactis* INIA 415, a producer of nisin Z and lacticin 481, as BP adjunct culture, *L. lactis* subsp. *lactis* INIA 415-2, a spontaneous bacteriocin-nonproducing mutant (BNP), as mesophilic starter culture, and commercial lactic culture TA052 (Rhodia, Iberia, Madrid, Spain) consisting of *Streptococcus thermophilus* strains of high aminopeptidase activity, as thermophilic starter culture.

Hispánico cheese was manufactured from a mixture of pasteurized cow's (80%) and ewe's (20%) milk in duplicate experiments, each consisting of two 100-L vats. Lactic cultures for vat 1 (BNP cheeses) were 1% BNP and 2% *S. thermophilus* culture and for vat 2 (BP cheeses) were 0.5% BP, 0.5% BNP, and 2% *S. thermophilus* culture. Six cheeses, ~2 kg in weight, were obtained from each vat. They were pressed overnight at 20 °C, salted for 24 h at 12 °C in a 160 g of NaCl/L brine, and ripened for 50 days at 12 °C. One cheese per treatment on days 15, 25, and 50 of ripening was used for volatile components and sensory analyses.

High-Pressure Treatments. Three cheeses from each vat (HP cheeses) were HP treated at 400 MPa for 5 min at 10 °C after 15 days of ripening, in a discontinuous isostatic press at NC Hyperbaric (Burgos, Spain). After HP treatment, cheeses followed ripening at 12 °C until day 50. The other three cheeses from each vat (NHP cheeses) were not HP treated.

Analysis of Volatile Compounds. Cheese pieces wrapped in aluminum foil were vacuum packed and frozen at −40 °C until analysis. Prior to volatile extraction, frozen pieces were thawed overnight at 4 °C. Duplicate 15 g cheese samples were homogenized in an analytical grinder (IKA, Labortechnik, Staufen, Germany), with 20 g of Na_2SO_4 and 25 μL of an aqueous solution containing 0.5 mg/mL cyclohexanone (Sigma-Aldrich Química, Alcobendas, Spain) as internal standard. An aliquot (2.25 g) of the mixture was subjected to dynamic headspace using helium (45 mL/min), in an automatic HP 7695 purge and trap apparatus (Hewlett-Packard, Palo Alto, CA), at 50 °C during 15 min, with 10 min of previous equilibrium. Volatile compounds were concentrated in a Tenax trap maintained at 30 °C and 6.5 psi back pressure, with a 1 min dry purge, and desorbed during 1 min at 230 °C directly into the injection port at 220 °C with a split ratio of 1:20, and 1.4 mL/min He flow (*15*).

Gas chromatography−mass spectrometry was carried out in a HP-6890 GC/HP-5973 MS apparatus equipped with a capillary column HP Innowax (60 m long; 0.25 mm o.d.; 0.5 μm film thickness). Chromatographic conditions were as follows: 12.5 min at 45 °C, raised at 4 °C/min to 114 °C, 6 min at 114 °C, raised at 7 °C/min to 143 °C and then at 15 °C/min to 240 °C, 4 min at 240 °C; He flow, 1 mL/min. Total analysis time was 55 min. Detection was performed with electron impact mass spectrometry operating in the scan mode, 2.6 scan/s, with 70 eV ionization energy, and source and quadrupole temperatures of 230 and 150 °C, respectively. Identification of most peaks was by comparison of retention times and ion spectra with those of authentic reference substances (Sigma-Aldrich). Twelve compounds were tentatively identified by spectra comparison with the Wiley 275 library (Wiley & Sons Inc., New York), usually with a quality level of >95%. Only compounds with abundance values >20000 in at least one cheese sample were further considered for quantification. For each compound, the peak areas of up to four characteristic ions were summed, and the result was divided by the sum of the peak areas of the characteristic ions of the internal standard. Levels of each volatile compound in the tables correspond to the so-obtained quotients multiplied by 10^3.

Sensory Evaluation. Eleven trained panelists evaluated the odor and aroma of cheeses after 15, 25, and 50 days of ripening for quality (overall acceptance) and intensity (overall intensity) of odor and aroma on a 0−10-point scale, using a horizontal line anchored in the middle and at both ends. Odor was defined as the olfactory sensation felt directly by the nose, and aroma as the olfactory sensation felt retronasally upon mastication. After removal of the rind, cheeses were cut in representative triangular slices (15−20 g). Slices of four cheeses per session, one HP cheese and one NHP cheese from each of the two vats manufactured on the same day, coded with random three-digit numbers, were presented to panelists in randomized order. The routine of sensory tasting was that the cheeses were first scored for odor by smelling and then introduced in the mouth and scored for the retronasal odorous sensation. Bread and water were provided to cleanse the mouth between cheeses.

A descriptive test was developed for Hispánico cheese based on the guidelines to the odor and aroma evaluation of hard and semihard cheeses given by Bérodier et al. (*19*). Panelists were asked to give scores for quality and intensity of odor and aroma on a 0−10-point scale. For both odor and aroma a series of attributes were scored on a 0−6-point scale, belonging to six families, namely, family "lactic", including the descriptors "milky", "buttery", "yogurt-like", and "cheesy"; the family "toasted", including the descriptor "caramel"; the family "animal", including the descriptors "sheepy" and "meat broth"; the family "fruity-flowery"; the family "vegetal"; and the family "others". Scores were very low for the last three families, which were not further considered.

Table 1. Volatile Compounds Identified in Hispánico Cheese Manufactured with a Bacteriocin-Producing Adjunct Culture and Treated by High Pressure

volatile compound	identification[a]	ions used for quantification
pentane	RCI	43, 42, 41, 57
hexane	RCI	57, 41, 43, 56
heptane	RCI	43, 71, 57, 41
ethanal	RCI	44, 43, 42, 41
octane	RCI	43, 85, 57, 71
2-methylpropanal	RCI	41, 72
acetone	RCI	43, 58
ethyl acetate	RCI	43, 70, 61, 45
2-butanone	RCI	43, 72
3-methylbutanal	RCI	44, 41, 43, 58
2-propanol	RCI	45, 43, 59
ethanol	RCI	45, 46, 43
2-pentanone	RCI	43, 86, 71, 41
2,3-butanedione	RCI	43, 86
ethyl butanoate	RCI	88
1-propanol	RCI	59
toluene	TI	91, 92, 43
2,3-pentanedione	TI	43, 57, 100
hexanal	RCI	44, 56
2-methyl-1-propanol	RCI	43, 41, 42
ethylbenzene	TI	91, 106
p-xylene	TI	91, 106
m-xylene	TI	91, 106
1-butanol	RCI	56, 41, 43
2-heptanone	RCI	43, 58, 71, 59
o-xylene	TI	91, 106
3-methyl-1-butanol	RCI	55, 70, 42, 41
ethyl hexanoate	RCI	88, 99, 43, 60
3-methyl-3-buten-1-ol	TI	68, 56, 41, 67
styrene	TI	104, 103, 78
3-hydroxy-2-butanone	RCI	45, 43, 88
3-methyl-2-buten-1-ol	TI	71, 41, 43, 53
3-hydroxy-2-pentanone	TI	59, 43, 41
2-hydroxy-3-pentanone	TI	45, 57, 102
2-nonanone	RCI	58, 43, 71, 59
nonanal	RCI	57, 41, 70, 98
2-ethyl-1-hexanol	RCI	57, 43, 55, 70
decanal	RCI	57, 70, 82, 112
benzaldehyde	RCI	105, 106
butanoic acid	RCI	60, 73
acetophenone	TI	105, 77, 120

[a] RCI, comparison of spectra and retention times with authentic reference compounds; TI, tentatively identified by spectra comparison using the Wiley 275 library.

Table 2. Aldehydes in Hispánico Cheese Manufactured with (BP) or without (BNP) a Bacteriocin-Producing Adjunct Culture, High-Pressure-Treated (HP) on Day 15 or Untreated (NHP)[a]

aldehyde	age (days)	cheese BNP-NHP	BNP-HP	BP-NHP	BP-HP
linear aldehydes					
ethanal	15	1.21 ± 0.33 b	0.85 ± 0.13 a	2.99 ± 0.19 d	2.20 ± 0.15 c
	25	1.27 ± 0.38 b	0.62 ± 0.08 a	2.50 ± 0.29 d	1.89 ± 0.17 c
	50	1.16 ± 0.28 b	0.59 ± 0.09 a	2.77 ± 0.13 d	1.77 ± 0.19 c
hexanal	15	0.73 ± 0.25 a	0.78 ± 0.18 a	0.66 ± 0.20 a	0.49 ± 0.09 a
	25	0.57 ± 0.14 a	1.10 ± 0.43 b	0.58 ± 0.14 a	0.51 ± 0.05 a
	50	0.54 ± 0.05 a	1.03 ± 0.07 b	0.68 ± 0.13 ab	1.05 ± 0.38 b
nonanal	15	0.32 ± 0.13 a	0.36 ± 0.22 a	0.28 ± 0.12 a	0.37 ± 0.15 a
	25	0.28 ± 0.10 a	0.27 ± 0.08 a	0.21 ± 0.14 a	0.22 ± 0.09 a
	50	0.22 ± 0.10 a	0.35 ± 0.12 a	0.40 ± 0.14 a	0.31 ± 0.11 a
decanal	15	0.27 ± 0.14 a	0.20 ± 0.07 a	0.21 ± 0.11 a	0.27 ± 0.08 a
	25	0.16 ± 0.05 a	0.20 ± 0.06 a	0.17 ± 0.09 a	0.18 ± 0.08 a
	50	0.18 ± 0.08 a	0.24 ± 0.08 a	0.23 ± 0.16 a	0.25 ± 0.09 a
branched-chain aldehydes					
2-methylpropanal	15	0.29 ± 0.03 a	0.29 ± 0.05 a	0.32 ± 0.02 ab	0.36 ± 0.03 b
	25	0.29 ± 0.07 a	0.25 ± 0.05 a	0.37 ± 0.03 a	0.35 ± 0.07 a
	50	0.30 ± 0.06 ab	0.26 ± 0.01 a	0.43 ± 0.06 b	0.36 ± 0.04 ab
3-methylbutanal	15	0.38 ± 0.03 a	0.34 ± 0.07 a	0.41 ± 0.11 a	0.41 ± 0.08 a
	25	0.36 ± 0.06 a	0.35 ± 0.02 a	0.49 ± 0.14 a	0.47 ± 0.11 a
	50	0.34 ± 0.08 a	0.42 ± 0.09 a	0.66 ± 0.15 b	0.85 ± 0.21 b

[a] Mean ± SD ($n = 4$) of duplicate determinations in two cheesemaking experiments, expressed as relative abundance to cyclohexanone. Means in the same row followed by different letters differ ($P < 0.05$).

Table 3. Ketones in Hispánico Cheese Manufactured with (BP) or without (BNP) a Bacteriocin-Producing Adjunct Culture, High-Pressure-Treated (HP) on Day 15 or Untreated (NHP)[a]

ketone	age (days)	cheese BNP-NHP	BNP-HP	BP-NHP	BP-HP
2-methylketones					
2-propanone	15	22.67 ± 1.39 b	21.65 ± 3.00 b	15.94 ± 0.74 a	15.49 ± 2.57 a
	25	19.68 ± 4.19 ab	21.60 ± 1.42 b	15.75 ± 1.46 ab	14.65 ± 2.46 a
	50	17.94 ± 4.58 a	18.81 ± 5.37 a	17.10 ± 2.38 a	15.49 ± 1.16 a
2-butanone	15	7.96 ± 0.45 b	7.45 ± 0.93 b	6.51 ± 0.26 a	6.31 ± 0.51 a
	25	8.03 ± 1.77 a	7.71 ± 1.00 a	6.67 ± 0.64 a	6.08 ± 0.91 a
	50	8.02 ± 1.66 a	7.97 ± 1.88 a	7.52 ± 0.90 a	6.91 ± 0.80 a
2-pentanone	15	0.72 ± 0.16 a	0.84 ± 0.09 a	1.68 ±0.14 b	1.63 ± 0.11 b
	25	1.14 ± 0.24 a	1.11 ± 0.19 a	2.04 ± 0.33 b	1.62 ± 0.23 ab
	50	1.90 ± 0.12 a	1.79 ± 0.12 a	2.70 ± 0.21 b	2.20 ± 0.31 ab
2-heptanone	15	2.37 ± 0.26 a	2.40 ± 0.20 a	4.34 ± 0.26 b	4.39 ± 0.09 b
	25	3.03 ± 0.52 a	2.97 ± 0.15 a	4.93 ± 0.40 b	4.39 ± 0.55 b
	50	4.12 ± 0.39 a	4.29 ± 0.41 a	6.08 ± 0.52 b	5.77 ± 0.21 b
2-nonanone	15	0.39 ± 0.15 a	0.33 ± 0.03 a	0.58 ± 0.02 b	0.60 ± 0.03 b
	25	0.35 ± 0.04 a	0.35 ± 0.03 a	0.61 ± 0.03 b	0.60 ± 0.10 b
	50	0.53 ± 0.09 a	0.52 ± 0.02 a	0.71 ± 0.06 b	0.67 ± 0.03 b
diketones					
2,3-butanedione	15	269.36 ± 30.79 c	224.03 ± 23.12 b	29.73 ± 6.11 a	12.90 ± 2.56 a
	25	245.32 ± 70.27 b	345.93 ± 85.94 b	36.67 ± 26.37 a	43.62 ± 12.77 a
	50	311.01 ± 24.88 b	350.20 ± 61.72 c	75.51 ± 6.66 a	74.68 ± 5.13 a
2,3-pentanedione	15	21.26 ± 2.40 c	14.58 ± 0.53 b	0.33 ± 0.02 a	0.22 ± 0.04 a
	25	12.94 ± 3.91 b	17.28 ± 4.45 b	0.32 ± 0.06 a	0.42 ± 0.12 a
	50	12.63 ± 3.02 b	17.32 ± 4.57 c	0.80 ± 0.14 a	0.81 ± 0.06 a
hydroxyketones					
3-hydroxy-2-butanone	15	23.44 ± 2.78 b	20.48 ± 2.30 b	3.05 ± 0.62 a	1.36 ± 0.45 a
	25	23.20 ± 4.51 b	31.16 ± 4.85 c	3.21 ± 3.18 a	4.29 ± 0.63 a
	50	31.35 ± 2.53 b	30.87 ± 6.53 b	6.51 ± 0.75 a	5.65 ± 1.22 a
3-hydroxy-2-pentanone	15	3.92 ± 0.39 b	4.69 ± 0.45 c	ND a	ND a
	25	4.24 ± 1.23 b	4.94 ± 0.58 b	ND a	ND a
	50	3.73 ± 0.85 b	4.48 ± 1.19 b	ND a	ND a
2-hydroxy-3-pentanone	15	2.51 ± 0.33 b	3.22 ± 0.45 c	ND a	ND a
	25	2.80 ± 0.90 b	3.18 ± 0.45 b	ND a	ND a
	50	2.39 ± 0.69 b	2.79 ± 0.94 c	ND a	ND a

[a] Mean ± SD ($n = 4$) of duplicate determinations in two cheesemaking experiments, expressed as relative abundance to cyclohexanone. Means in the same row followed by different letters differ ($P < 0.05$). ND, not detected.

Statistical Analysis. Statistics were performed by means of SPSS Win 8.0 program (SPSS Inc., Chicago, IL). Multifactor analysis of variance was carried out by using type of mesophilic starter, HP treatment, and cheese age as main effects. On the other hand, means for the four types of cheese (BNP-NHP, BNP-HP, BP-NHP, and BP-HP) at 15, 25, and 50 days were compared using Tukey's test. Principal component analysis (PCA) with Varimax rotation was carried out on highly correlated volatile compounds together with sensory attributes.

Table 4. Alcohols in Hispánico Cheese Manufactured with (BP) or without (BNP) a Bacteriocin-Producing Adjunct Culture, High-Pressure-Treated (HP) on Day 15 or Untreated (NHP)[a]

alcohol	age (days)	cheese			
		BNP-NHP	BNP-HP	BP-NHP	BP-HP
1- and 2-alkanols					
ethanol	15	79.2 ± 4.3 a	72.5 ± 12.0 a	696.6 ± 53.4 b	667.9 ± 102.9 b
	25	91.4 ± 16.0 a	69.4 ± 8.9 a	683.9 ± 78.3 b	601.7 ± 104.8 b
	50	118.0 ± 19.1 a	73.0 ± 11.0 a	753.1 ± 78.5 c	566.9 ± 38.2 b
1-propanol	15	0.68 ± 0.03 a	0.63 ± 0.10 a	1.52 ± 0.10 b	1.53 ± 0.09 b
	25	0.69 ± 0.09 a	0.64 ± 0.11 a	1.65 ± 0.20 b	1.40 ± 0.17 b
	50	0.87 ± 0.10 b	0.59 ± 0.11 a	1.71 ± 0.17 c	1.53 ± 0.11 c
1-butanol	15	7.87 ± 0.60 b	5.27 ± 1.26 a	8.27 ± 0.49 b	8.05 ± 1.26 b
	25	11.26 ± 4.31 a	9.72 ± 3.56 a	11.50 ± 3.04 a	9.91 ± 0.73 a
	50	10.90 ± 0.37 a	12.91 ± 2.66 b	11.41 ± 1.04 ab	12.15 ± 1.68 ab
2-propanol	15	1.02 ± 0.25 a	1.05 ± 0.38 a	0.95 ± 0.25 a	1.02 ± 0.26 a
	25	1.22 ± 0.29 a	1.17 ± 0.36 a	1.11 ± 0.21 a	4.31 ± 6.34 a
	50	5.70 ± 2.10 b	2.51 ± 0.70 a	1.33 ± 0.20 a	1.12 ± 0.08 a
branched-chain alcohols					
2-methyl-1-propanol	15	1.47 ± 0.26 b	1.33 ± 0.11 b	1.42 ± 0.22 b	0.81 ± 0.33 a
	25	1.18 ± 0.66 a	1.25 ± 0.33 a	1.44 ± 0.28 a	1.25 ± 0.08 a
	50	1.44 ± 0.21 a	1.27 ± 0.64 a	1.59 ± 0.31 a	1.36 ± 0.27 a
3-methyl-1-butanol	15	1.88 ± 0.24 ab	1.76 ± 0.11 a	2.13 ± 0.21 b	2.08 ± 0.16 b
	25	1.83 ± 0.46 a	1.89 ± 0.23 a	2.23 ± 0.40 a	1.94 ± 0.24 a
	50	2.00 ± 0.29 a	2.10 ± 0.32 a	2.36 ± 0.32 a	2.14 ± 0.26 a
3-methyl-3-buten-1-ol	15	1.43 ± 0.38 a	1.54 ± 0.11 a	3.15 ± 0.17 b	3.11 ± 0.10 b
	25	1.77 ± 0.29 a	1.89 ± 0.16 a	3.56 ± 0.27 b	3.24 ± 0.35 b
	50	2.47 ± 0.21 a	2.19 ± 0.48 a	4.35 ± 0.40 b	4.29 ± 0.17 b
3-methyl-2-buten-1-ol	15	1.11 ± 0.18 a	1.12 ± 0.09 a	3.90 ± 0.22 b	3.93 ± 0.16 b
	25	1.22 ± 0.26 a	1.18 ± 0.17 a	3.99 ± 0.19 b	3.63 ± 0.45 b
	50	1.44 ± 0.08 a	1.46 ± 0.11 a	4.82 ± 0.42 b	4.37 ± 0.22 b
2-ethyl-1-hexanol	15	0.78 ± 0.14 a	0.70 ± 0.19 a	0.64 ± 0.16 a	0.64 ± 0.18 a
	25	0.61 ± 0.22 a	0.58 ± 0.18 a	0.65 ± 0.22 a	0.56 ± 0.13 a
	50	0.62 ± 0.27 a	0.63 ± 0.26 a	0.62 ± 0.33 a	0.60 ± 0.25 a

[a] Mean ± SD ($n = 4$) of duplicate determinations in two cheesemaking experiments, expressed as relative abundance to cyclohexanone. Means in the same row followed by different letters differ ($P < 0.05$).

RESULTS

Table 1 lists the 41 volatile compounds identified in the headspace of Hispánico cheese together with the ions used for their quantification. Abundances (peak areas corrected by the internal standard) of 6 aldehydes, 10 ketones, 9 alcohols, 1 carboxylic acid, 3 esters, 4 alkanes, and 8 benzene compounds were determined in cheeses after 15, 25, and 50 days of ripening. The results will be ordered by chemical groups.

Aldehydes and Ketones. Among aldehydes, the ripening time was significant only for the abundance of 3-methylbutanal, which increased, and for that of ethanal, which decreased with age (**Table 2**). The addition of BP culture to milk caused a significant increase ($P < 0.05$) of the branched-chain aldehydes and ethanal, without a significant influence on the other linear aldehydes. The HP treatment produced a significant decrease of ethanal, whereas the higher molecular mass linear aldehydes tended to rise, this increase being significant only for hexanal in cheeses without BP addition.

In general, the abundance of 2-methylketones (except 2-propanone and 2-butanone), diketones, and 3-hydroxy-2-butanone increased ($P < 0.05$) with ripening time (**Table 3**). The addition of BP to milk produced a significant ($P < 0.05$) increase of 2-pentanone, 2-heptanone, and 2-nonanone and a decrease of 2-propanone, 2-butanone, diketones 2,3-butanedione (diacetyl) and 2,3-pentanedione, and their reduction products, 3-hydroxy-2-butanone (acetoin), 3-hydroxy-2-pentanone, and 2-hydroxy-3-pentanone with respect to BNP-NHP cheese. The HP treatment reduced the amounts of diketones on day 15, but afterward these compounds increased in cheeses without BP addition. Abundances of hydroxypentanones increased after HP treatment on day 15.

Alcohols, Carboxylic Acids, and Esters. The abundances of 1-butanol, and all of the saturated and unsaturated branched-chain alcohols except 2-ethyl-1-hexanol, increased significantly during ripening (**Table 4**). BP addition to milk produced a significant increase of ethanol, 1-propanol, 3-methyl-1-butanol, and the unsaturated alcohols. The HP treatment did not have a clear effect on alcohols, producing a slight decrease of ethanol, 1-propanol, and 2-methyl-1-propanol in some of the cheeses. Among carboxylic acids, only butanoic acid was observed in significant amounts, and higher levels were found in control cheeses not subjected to any treatment (**Table 5**). Ethyl esters significantly increased with age and with the addition of BP to milk and decreased with the HP treatment (**Table 5**).

Alkanes and Benzene Compounds. BP addition to milk lowered the abundances of hexane and heptane, whereas HP treatment of cheese raised that of hexane (data not shown). BP addition to milk resulted in significant ($P < 0.05$) styrene increases and toluene decreases in cheeses (data not shown). Levels of benzene compounds were not affected by the HP treatment.

Sensory Evaluation. Odor quality increased significantly ($P < 0.05$) with cheese age, aroma quality did not, and none of them were affected by BP addition to milk (**Table 6**). Both odor and aroma intensities increased significantly ($P < 0.05$) with cheese age. Aroma intensity was significantly ($P < 0.05$) higher in BP cheeses, with BP-NHP cheese showing the highest odor intensity (**Table 6**). HP treatment of cheeses had a detrimental ($P < 0.05$) effect on odor parameters, independent of the addition of BP as adjunct culture, and on aroma only when BP had been added to the milk. Odor and aroma intensities of pressurized cheeses especially decreased at 15 days, just after HP application.

Table 5. Carboxylic Acids and Esters in Hispánico Cheese Manufactured with (BP) or without (BNP) a Bacteriocin-Producing Adjunct Culture, High-Pressure-Treated (HP) on Day 15 or Untreated (NHP)[a]

compound	age (days)	cheese			
		BNP-NHP	BNP-HP	BP-NHP	BP-HP
carboxylic acids					
butanoic acid	15	0.49 ± 0.25 b	0.27 ± 0.09 ab	0.19 ± 0.02 b	0.12 ± 0.03 a
	25	0.22 ± 0.04 a	0.23 ± 0.02 a	0.30 ± 0.15 a	0.27 ± 0.12 a
	50	0.49 ± 0.11 b	0.30 ± 0.07 a	0.38 ± 0.16 a	0.32 ± 0.03 a
ethyl esters					
ethyl acetate	15	0.59 ± 0.05 a	0.49 ± 0.09 a	2.12 ± 0.19 b	2.42 ± 0.63 b
	25	0.75 ± 0.10 a	0.67 ± 0.18 a	2.11 ± 0.19 a	2.04 ± 0.42 a
	50	0.89 ± 0.25 a	1.21 ± 0.89 ab	3.60 ± 1.24 c	2.23 ± 0.60 b
ethyl butanoate	15	0.08 ± 0.01 a	0.10 ± 0.01 a	1.18 ± 0.23 c	0.51 ± 0.08 b
	25	0.14 ± 0.02 a	0.09 ± 0.01 a	0.84 ± 0.15 c	0.53 ± 0.08 b
	50	0.19 ± 0.02 a	0.12 ± 0.01 a	1.41 ± 0.12 c	0.81 ± 0.11 b
ethyl hexanoate	15	ND a	ND a	0.29 ± 0.04 c	0.21 ± 0.04 b
	25	ND a	ND a	0.36 ± 0.05 c	0.27 ± 0.04 b
	50	ND a	ND a	0.66 ± 0.11 c	0.45 ± 0.07 b

[a] Mean ± SD ($n = 4$) of duplicate determinations in two cheesemaking experiments, expressed as relative abundance to cyclohexanone. Means in the same row followed by different letters differ ($P < 0.05$). ND, not detected.

Table 6. Sensory Evaluation of Hispánico Cheese Manufactured with (BP) or without (BNP) a Bacteriocin-Producing Adjunct Culture, High-Pressure-Treated (HP) on Day 15 or Untreated (NHP)[a]

characteristic	age (days)	cheese			
		BNP-NHP	BNP-HP	BP-NHP	BP-HP
odor quality	15	5.41 ± 0.40 a	5.52 ± 0.03 a	5.92 ± 0.20 a	5.42 ± 0.10 a
	25	5.89 ± 0.27 a	5.85 ± 0.37 a	6.01 ± 0.11 a	5.52 ± 0.47 a
	50	6.23 ± 0.19 a	5.53 ± 0.28 a	6.26 ± 0.31 a	5.94 ± 0.05 a
odor intensity	15	4.14 ± 0.79 a	3.95 ± 0.30 a	4.87 ± 0.23 a	3.75 ± 0.61 a
	25	4.36 ± 0.22 a	4.60 ± 0.14 ab	5.49 ± 0.16 b	4.37 ± 0.47 a
	50	5.32 ± 0.32 a	5.05 ± 0.89 a	5.93 ± 0.24 a	5.19 ± 0.51 a
aroma quality	15	5.52 ± 0.44 a	5.41 ± 0.21 a	5.59 ± 0.15 a	5.42 ± 0.03 a
	25	5.67 ± 0.29 a	5.75 ± 0.05 a	5.41 ± 0.29 a	5.40 ± 0.44 a
	50	5.58 ± 0.40 a	5.49 ± 0.41 a	6.13 ± 0.18 a	5.75 ± 0.17 a
aroma intensity	15	4.60 ± 0.30 a	3.87 ± 0.33 a	5.03 ± 0.32 a	4.29 ± 0.91 a
	25	4.68 ± 0.37 a	4.63 ± 0.53 a	5.35 ± 0.26 a	5.25 ± 0.93 a
	50	5.14 ± 0.58 a	5.33 ± 0.61 a	6.10 ± 0.42 a	5.78 ± 0.67 a

[a] Mean ± SD ($n = 22$) of scores from 11 trained panelists in two cheesemaking experiments using a 10-point scale. Means in the same row followed by different letters differ ($P < 0.05$).

Cheese age significantly ($P < 0.05$) influenced the odor lactic family scores, which increased from 1.65 to 2.10, but did not influence the aroma lactic family scores (mean = 1.74). Among individual attributes, buttery odor scores decreased significantly ($P < 0.05$), from 2.21 to 1.78, with cheese age, but buttery aroma scores were not affected (mean = 1.76). Scores for sheepy and meat broth aromas increased significantly ($P < 0.05$) from levels below the perception threshold to 0.74 and 0.76, respectively. Scores for the yogurt-like attribute of both odor and aroma decreased significantly (from 1.49 to 0.80 and from 1.49 to 1.05, respectively).

The addition of BP to milk produced a significant increase ($P < 0.05$) in the yogurt-like (1.16 vs 1.48) and cheesy (1.35 vs 1.82) aroma scores and a significant decrease of aroma attributes milky (0.99 vs 0.70), buttery (2.04 vs 1.48), and caramel (0.93 vs 0.61).

The HP treatment increased scores for milky odor (1.01 vs 1.29) and aroma (0.75 vs 0.94) and lowered scores for yogurt-like odor (1.40 vs 1.01) and aroma (1.45 vs 1.19). It also lowered the odor attributes buttery (2.14 vs 1.83) and caramel (1.16 vs 0.92).

Principal Component Analysis. Figure 1 represents the loading plots of the variables in the plane defined by principal components 1 (PC1) and 2 (PC2), which explained 54.8 and 16.5% of the variance, respectively. 3-Methyl-2-buten-1-ol, ethanal, ethyl butanoate, ethyl hexanoate, 3-methyl-3-buten-1-ol, 2-methylpropanal, 2-pentanone, 2-heptanone, and the aroma descriptor yogurt-like correlated positively with PC1, whereas 2,3-butanedione, 2,3-pentanedione, 3-hydroxy-2-butanone, 3-hydroxy-2-pentanone, 2-hydroxy-3-pentanone, and the aroma descriptors buttery, milky, and caramel correlated negatively, occupying the left part of the plane. 3-Methylbutanal, odor and aroma intensity, odor and aroma quality, and aroma descriptors sheepy and meat broth correlated positively with PC2. A group of four compounds, 2-pentanone, 2-heptanone, 3-methyl-3-buten-1-ol, and ethyl hexanoate, showed correlation coefficients of >0.5 with both PCs.

Another group of variables occupies the upper right quarter of the plane. Some of them are positively correlated with PC1, namely, esters and unsaturated alcohols, and negatively correlated with diketones, whereas others, namely, 2-methylketones and 3-methylbutanal, are positioned in the central part of the quadrant, sharing positive correlation coefficients with the two PCs. The high correlation coefficients of sensory characteristics related to flavor intensity and quality with PC2 move them to the upper part of the plane. The yogurt-like aroma is positioned at the lower right quarter, closer than any other descriptor to the compound ethanal, but the correlation was low.

Figure 2 shows the loading plots of the factor scores for each cheese sample. One hundred percent of the cheeses were

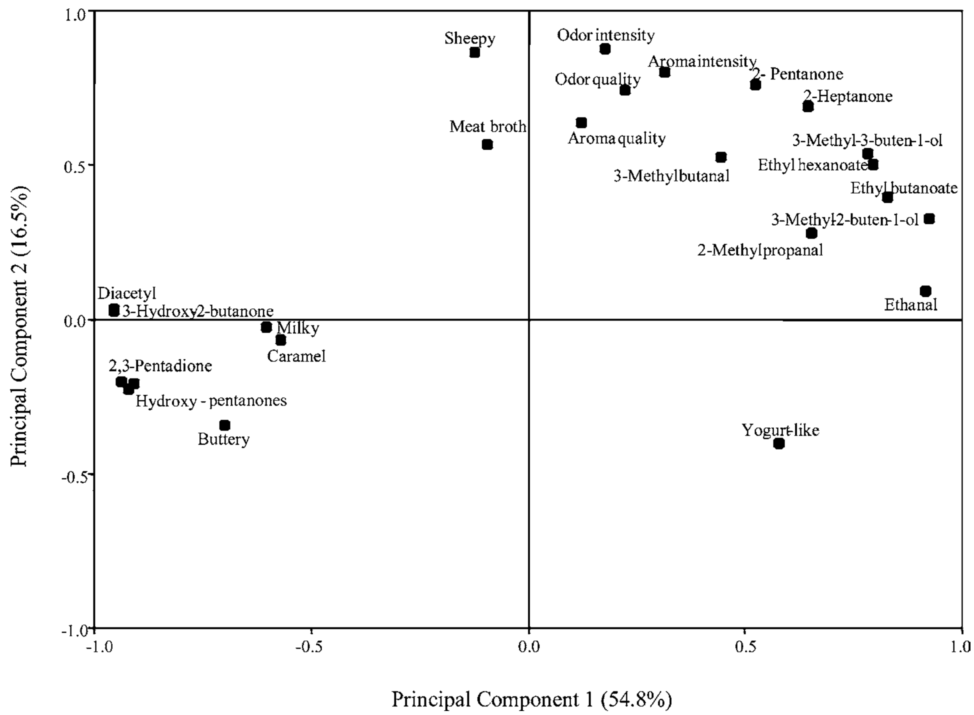

Figure 1. PCA showing the first two principal components of volatile compounds and sensory attributes.

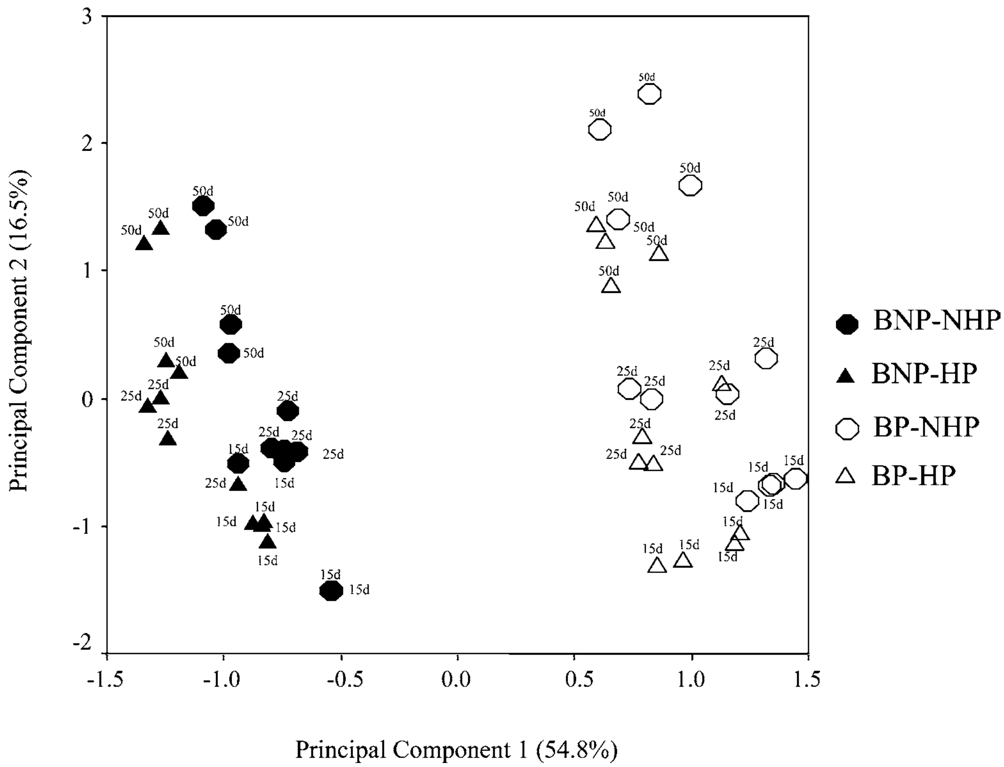

Figure 2. Distribution of cheeses at different ripening times (15, 25, and 50 days) using principal components 1 and 2. BP, cheese made with bacteriocin producer; BNP, cheese made without bacteriocin producer; HP, high-pressure-treated cheese; NHP, cheese not treated by high pressure.

separated on the basis of BP addition. However, HP treated and untreated cheeses were not satisfactorily differentiated.

DISCUSSION

PCA provides the basis to construct the discussion of the results. PC1 could be called "lactic and toasted" aromas, with their associated individual attributes milky, buttery, yogurt-like, and caramel, whereas PC2 would represent more of the animal aromas with the attributes sheepy and meat broth, together with the aroma and odor intensity and quality. As expected, diketones are positioned together with the aroma attributes buttery and milky, but also with the descriptor caramel. Diketones are key aroma components of many cheese varieties. Although diacetyl is derived from citrate metabolism, 2,3-pentanedione might be derived from the amino acid Ile (*20*). Presumably, the reduction of this substance would produce 3-hydroxy-2-pentanone and 2-hydroxy-3-pentanone. The adjunct *S. thermophilus* culture would likely be responsible for the high levels of diketones observed in this study, in agreement with Imhof et al. (*20*), and the cell lysis produced by the BP addition would most probably be responsible for the decrease in these compounds. These results are consistent with a previous study (*7*), and they suggest the necessity of the cellular integrity for these enzymatic processes. At the opposite side of PC1, ethanal and the yogurt-like descriptor show a certain correlation, the latter being clearly discriminated by panelists from the other attributes belonging to the lactic family. High levels of ethanal in BP-NHP cheeses were very probably derived from the amino acid Thr (*21*), which was also at higher levels in these cheeses (data not shown), in agreement with a previous study (*7*). On the other hand, the observed reduction of LAB viable counts caused by the HP treatment, of ∼90% on day 15 (data not shown), seemed to inhibit ethanal formation, independent of Thr concentration.

Cheeses richer in diacetyl had lower ethanal, ester, and alcohol contents according to the PCA. The lactic−toasted attributes and correlated compounds in PC1, whether with positive or negative coefficients, seem to be unlinked to the aroma or odor intensity or quality. In this cheese variety high amounts of diketones with their buttery−caramel flavor would be characteristic of young cheeses, whereas mature cheeses, possessing a more intense flavor, would be richer in the compounds positioned at the upper right side of the plane, such as 2-methylketones and 3-methylbutanal, which seem to be playing a role as aroma quality and intensity enhancers. The increments of these compounds, together with the decrease in the diketones produced by BP addition (**Tables 2** and **3**), seem to be the main reasons BP-NHP cheeses are scored higher than BNP-NHP cheeses for aroma intensity. These results are in agreement with the literature, as 3-methylbutanal has been repeatedly identified as a potent odorant in various cheese varieties (*1*). It is generally accepted that this compound originates from Leu by transamination or Strecker degradation (*22*), and, in fact, BP cheeses showed higher concentrations of Leu than BNP cheeses, a result already observed by Garde et al. (*7*). Formation of 3-methylbutanal could also be favored by an increase in Leu transamination activity due to cell lysis (*22*). A high level of 3-methylbutanal has been correlated to spicy chocolate-like flavors in Gouda-type cheese (*23*).

The descriptors meat broth and sheepy correlated with 2-methylketones and were considered to be quality and intensity enhancers. Although these substances are linked to free fatty acid metabolism by molds (*24*) and related to floral or moldy notes (*2*), an association of 2-heptanone with animal flavor notes has also been reported (*1*).

Unsaturated alcohols and esters shared a high positive correlation coefficient with PC1 and with ethanal, which is consistent with the origin of ethanol from ethanal and the subsequent formation of ethyl esters from ethanol and fatty acids. The fact that unsaturated alcohols were significantly more abundant in BP cheeses suggests that their production was enhanced by the lysis of LAB.

When cheese samples are given a value in PCs (**Figure 2**), the separation of cheeses with or without added BP is precise, not being so between HP treated or untreated cheeses. From a comparison of **Figures 1** and **2**, cheeses from milk with added BP, with lower abundances of diketones and higher abundances of esters, 2-methylketones, and ethanal, would shift to the right part. Only a low correlation was observed between the intensity of odor or aroma and ethyl hexanoate in the present study, despite the very low detection thresholds reported for esters (*1*). The higher abundance of esters in BP cheeses was consistent with their higher content of ethanol, which points to alcoholysis as the major formation mechanism (*25*).

Other alcohols, with nonsignificant correlations in the PCA, were also more abundant in BP cheeses. 1-Propanol and 3-methyl-1-butanol are reduction products of the corresponding aldehydes through the activity of LAB dehydrogenases or chemical reactions (*2*). Previous studies have reported higher levels of 3-methyl-1-butanol in Hispánico cheese made with BP adjunct cultures (*7*). This branched-chain alcohol has been associated either with a pleasant fresh aroma in Mozzarella cheese (*26*) or with unclean flavors in Cheddar cheese (*27*).

The influence of HP treatment on the sensory characteristics and on cheese volatiles was not as remarkable as that of the BP addition. The formation of a few volatile compounds, namely, hexanal, alkanes, hydroxyketones, and, in BNP cheese, diketones, was enhanced by the HP treatment, accompanied by higher scores for the milky descriptor. As a result, a displacement to the left of the HP-treated cheeses (within each group of cheeses in **Figure 2**) can be observed. Most compounds, such as ethanal, ethanol, other alcohols, ethyl esters, and butanoic acid, underwent a slight decrease, in agreement with the lower scores for most sensory characteristics. High pressure seems to have inactivated most of the enzymes as other authors have reported (*28*, *29*). However, the activity of those enzymes involved in citrate metabolism and in hexanal and alkane formation was apparently increased by HP treatment, probably because the access of intracellular enzymes to their substrates was favored by cell lysis or by an increase in bacterial membrane permeability. Hexanal originates from the β-oxidation of unsaturated fatty acids (*30*), and its increase might be related to the hexane increase. The few studies dealing with the influence of HP treatment on volatile constituents of cheese are very much in agreement with our results. Thus, butyric acid and acetoin were at lower concentrations in pressurized samples of Gouda cheese than in control cheese after ripening for 21 days (*16*). No effect on aldehyde, ketone, alkane, alcohol, or ester concentrations was observed in Swiss cheese slurries (*17*) or in goat's milk cheese (*18*) subjected to HP treatment. The lower levels of carboxylic acids reported for Swiss cheese slurries after HP treatment (*17*) and for caprine cheese treated at 400 MPa were assigned to the lower lactococci counts and the inactivation of lipases from the secondary microbiota by HP treatment (*18*).

The addition of a bacteriocin producer to milk significantly intensified cheese aroma, altering the balance of aroma compounds in the volatile fraction by enhancing the formation of some substances, mainly ethanol, branched-chain aldehydes, branched-chain alcohols (both saturated and unsaturated), ethyl esters, and 2-methylketones, and limiting the formation of others such as diketones or hydroxyketones. On the other hand, high-pressure treatment of the cheeses generally limited the formation of volatile compounds, producing a slight decrease of the odor quality and intensity scores of cheeses.

ABBREVIATIONS USED

LAB, lactic acid bacteria; PCA, principal component analysis; BP, made with bacteriocin producer; BNP, made without bacteriocin producer; HP, high-pressure-treated; NHP, not treated by high pressure; SD, standard deviation.

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