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Volatile Compounds in Hispánico Cheese Manufactured Using a Mesophilic Starter, a Thermophilic Starter, and Bacteriocin-Producing *Lactococcus lactis* Subsp. *lactis* INIA 415

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The effect of the addition of *Lactococcus lactis* subsp. *lactis* INIA 415, a strain harboring the structural genes of nisin Z and lacticin 481, on the formation of volatile compounds in Hispánico cheese manufactured with a mesophilic starter or with the mesophilic starter and a thermophilic starter was investigated. Addition of bacteriocin-producing *L. lactis* subsp. *lactis* INIA 415 to milk enhanced the formation of 2-methyl-propanal, 2-methylbutanal, 3-methylbutanal, 2-methyl-1-propanol, 3-methyl-1-butanol, 1-octanol, 2-butanone, and 2,3-butanedione. On the other hand, addition of thermophilic starter enhanced the formation of acetaldehyde, ethanol, 3-methyl-2-buten-1-ol, ethyl butanoate, ethyl hexanoate, 2-butanone, and 2,3-butanedione in Hispánico cheese. Stepwise discriminant analysis using the relative abundances of volatile compounds classified cheeses by type of starter, with function 1 related to thermophilic starter and function 2 to bacteriocin producer.

KEYWORDS: Volatile compounds; cheese; lactic acid bacteria; lysis; bacteriocin

INTRODUCTION

Cheese quality is determined by its flavor, rheological properties, and visual appearance (*1*). For this reason cheese flavor has been the subject of numerous scientific investigations. Originally it was thought that cheese flavor resulted from a single compound or class of compounds, but now it is generally accepted that the flavor of most cheese varieties is due to the correct balance and concentration of a wide range of taste and aromatic compounds, according to the component balance theory (*2*).

The flavor of matured cheese is the result of the interaction of milk enzymes, rennet enzymes, starter bacteria, and secondary microbiota (*3*). Flavor compounds are produced through glycolysis, lipolysis, and proteolysis, followed by the secondary reactions that take place throughout cheese ripening. All cheese varieties studied contain essentially the same flavor compounds, and their flavors differ owing to the absolute and relative concentrations of these compounds (*1*). Only lower molecular weight compounds contribute significantly to cheese flavor. An important group of low molecular weight molecules are the volatile compounds (*4*).

In a wide variety of cheeses lactic acid bacteria 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 flavor compounds and aroma precursors (*5*−*8*). Catabolism of free amino acids results in a number of volatile compounds, including ammonia, amines, aldehydes, phenols, indole, and alcohols, many of which contribute to cheese flavor (*9*), as well as the free fatty acids formed by the hydrolysis of triglycerides and the esters resulting from their reaction with alcohols. As most lactococcal enzymes are located in the interior of the cell, the lysis of starter bacteria will favor the access of enzymes to their substrates and may thus accelerate the development of cheese flavor (*10*, *11*).

Inoculation of milk with bacteriocin-producing adjunct cultures is a simple procedure to enhance the lysis of starter bacteria during ripening. Proteolysis was accelerated in cheese made with bacteriocin producers as adjuncts to the commercial starter (*10*−*12*), and in some cases flavor intensity evolved more rapidly (*11*, *12*). Formation of some volatile compounds in cheese was influenced by milk inoculation with *Enterococcus faecalis* INIA 4, a strain producing enterocin AS-48, with an increase in the levels of 3-methylbutanal, diacetyl, and acetoin (*12*).

As the dairy industry is reluctant to inoculate milk with enterococci, *Lactococcus lactis* subsp. *lactis* INIA 415, harboring the structural genes of nisin Z and lacticin 481 (*13*), was used as a bacteriocin-producing adjunct culture in the manufacture of Hispánico cheese, a semihard variety made from a mixture of cow's and ewe's milk. Changes in cheese proteolysis caused by the use of *L. lactis* subsp. *lactis* INIA 415 as adjunct culture

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were described in a previous work (*14*). Its effect on the formation of volatile compounds during cheese ripening is here reported.

MATERIALS AND METHODS

Lactic Cultures and Cheese Manufacture. Strains of lactic acid bacteria and cheese-making procedure were described in a previous work (*14*). Basically, *L. lactis* subsp. *lactis* INIA 437 and *L. lactis* subsp. *cremoris* INIA 450 were used as mesophilic starter, *Streptococcus thermophilus* INIA 463 and INIA 468 were used as thermophilic starter with high aminopeptidase activity, and *L. lactis* subsp. *lactis* INIA 415 was used as bacteriocin-producing adjunct culture.

Hispánico cheese was manufactured in duplicate experiments on different days from a mixture of pasteurized cow's (80%) and ewe's (20%) milk. Each experiment consisted of four 45-L vats. Milk in vat 1 was inoculated with 1% mesophilic starter, milk in vat 2 with 1% mesophilic starter plus 0.1% bacteriocin producer, milk in vat 3 with 1% mesophilic starter plus 1% thermophilic starter, and milk in vat 4 with 1% mesophilic starter plus 1% thermophilic starter plus 0.1% bacteriocin producer. Three cheeses, ∼2 kg in weight, were obtained from each vat. They were pressed for 18 h at 20 °C, salted in brine for 16 h at 12 °C, and ripened at 12 °C for 75 days.

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 kept overnight at 4 °C and then left to stabilize at room temperature. An automatic dynamic headspace apparatus (purge and trap, HP 7695, Hewlett-Packard, Palo Alto, CA) connected to a gas chromatograph−mass spectrometer (HP 6890-MSD HP 5973, Hewlett-Packard) was used for volatile compounds analysis. Duplicate 15 g cheese samples were homogenized in an analytical grinder (IKA, Labortechnik, Staufen, Germany), with 20 g of anhydrous Na_2SO_4 and 25 μL of an aqueous solution containing 0.5 mg/mL cyclohexanone (IS_1) and 0.5 mg/mL camphor (IS_2) (Sigma Chemical Co., St. Louis, MO) as internal standards. An aliquot (2 g) of this mixture was subjected to helium purge in a 25-mL glass sparger (Schmidlin Co., Neuheim, Switzerland). Volatile compounds were concentrated in a Tenax trap (Tekmar, Cincinnati, OH), kept at 30 °C. Operating conditions were as follows: line temperature, 200 °C; helium flow, 40 mL/min; sample temperature, 50 °C; equilibration time, 10 min; purge time, 20 min; dry purge time, 1 min; desorb temperature, 230 °C; desorb time, 1 min; split ratio, 1:20; injection port temperature, 220 °C.

Chromatography was carried out using an HP-Innowax column (60 m length × 0.25 mm i.d., 0.5-μm film thickness), with the following conditions: initial helium flow, 1.4 mL/min kept for 1.5 min; helium flow, 1 mL/min; initial temperature, 45 °C for 16 min; 4 °C/min up to 110 °C and kept for 10 min; 15 °C/min up to 220 °C and kept for 3 min. Mass detection was performed in the scan mode, from 33 to 220 amu at 2.23 scan/s, and ionization by EI at 70 eV. Data were collected with the HP ChemStation program, and volatile compounds were identified by comparison of spectra with the Wiley 275 library and by comparison of their retention times with authentic standards (Sigma Chemical Co.). Relative abundances were expressed as percentages of peak areas of the compounds on the cyclohexanone peak area.

Statistical Analysis. Analyses of variance with bacteriocin-producing adjunct culture addition, thermophilic starter addition, and cheese age as main effects were performed by means of the SPSS Win 5.4 program. Comparison of means between cheeses of the same age was carried out using Tukey's test (*15*).

Selected volatile compounds were used for canonical discriminant analysis to classify cheese by type of starter by means of the SPSS Win 5.4 program.

RESULTS AND DISCUSSION

Volatile Fraction. Forty-six compounds were identified in the volatile fraction of Hispánico cheese, including hydrocarbons, alcohols, ketones, aldehydes, esters, and sulfur compounds. The number was slightly higher than the 43 volatile compounds previously reported for Hispánico cheese (*12*). Eight volatile compounds were not significantly ($P > 0.05$) influenced by the addition of bacteriocin-producing adjunct culture, the addition of thermophilic starter, or cheese age, and their relative abundances were generally low (0.04−0.80). Overall mean values of relative abundances from all vats at 25, 50, and 75 days were 0.04 for pentane, 0.17 for hexane, 0.18 for heptane, 0.73 for octane, 0.80 for ethyl acetate, 0.13 for ethylbenzene, 0.06 for propanal, and 0.14 for α-pinene.

Thirteen volatile compounds were significantly ($P < 0.05$) influenced by cheese age but not by the addition of bacteriocin-producing adjunct culture or thermophilic starter. Their relative abundances (mean values from all vats) at 25 and 75 days, respectively, were 0.05 and 0.14 for methyl acetate, 0.02 and 0.03 for acetophenone, 0.40 and 0.28 for 2-nonanone, not detected and 0.02 for butanal, 0.05 and 0.08 for phenylacetaldehyde, 0.76 and 0.25 for heptanal, 0.04 and 0.05 for 1-pentanol, not detected and 0.05 for 1-hexanol, 1.00 and 1.62 for toluene, 0.09 and 0.14 for *p*-xylene, 0.08 and 0.13 for *m*-xylene, not detcted and 0.13 for *o*-xylene, and 0.03 and 0.04 for pyrrol.

The addition of bacteriocin-producing adjunct culture or thermophilic starter had a significant ($P < 0.05$) effect on the relative abundance of 25 volatile compounds (**Tables 1−4**). Fifteen of these compounds were also significantly ($P < 0.05$) influenced by cheese age: acetaldehyde, 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, nonanal, 2-propanol, 2-pentanol, 1-hexanol, ethyl butanoate, ethyl hexanoate, 2-butanone, 2-pentanone, 2,3-pentanedione, 2-hexanone, and 2-heptanone.

Aldehydes. The relative abundances of seven aldehydes were influenced by the addition of bacteriocin-producing adjunct culture or thermophilic starter (**Table 1**). Acetaldehyde is the most abundant volatile compound in yogurt and is also relevant to Domiati cheese flavor (*16*). In the present work it reached the highest level in cheese made with thermophilic starter and bacteriocin-producing adjunct culture after 25 days of ripening, decreasing thereafter. Acetaldehyde is produced during lactose metabolism by lactic acid bacteria (*17*), but it may also be derived from the breakdown of threonine (*18*, *19*). Cheese made with thermophilic starter and bacteriocin-producing adjunct culture showed the highest levels of threonine (*14*), and therefore the latter pathway may have been relevant for acetaldehyde formation in this cheese.

Levels of 2-methylpropanal, 2-methylbutanal, and 3-methylbutanal were generally higher in cheese made with mesophilic starter and bacteriocin producer and tended to increase during ripening. These aldehydes originate from Val, Ile, and Leu, respectively, by transamination or Strecker degradation (*20*), and are responsible for unclean and harsh flavors in Cheddar cheese (*21*). A high level of 3-methylbutanal, with spicy, malty, or cocoa-like flavor, was found in Gouda-type cheese made using *L. lactis* subsp. *lactis* S19 or wild lactococci of dairy and nondairy origin as starter cultures (*22*, *23*), in Proosdij cheese, in which it was responsible for a spicy chocolate-like flavor (*19*), and in Emmental cheese, where its level increased during ripening (*24*). High levels of 2-methylpropanal, 2-methylbutanal, and 3-methylbutanal were found in cheeses made with bacteriocin-producing adjunct culture (**Table 1**), which contained high amounts of the precursor free amino acids Val, Ile, and Leu (*14*). Higher levels of Leu and 3-methylbutanal were also found in cheese made with a commercial mesophilic starter and *E. faecalis* INIA 4 as bacteriocin-producing adjunct (*12*). In Saint-Paulin ultrafiltrate cheese, the addition of a crude broken suspension or cell-free extract of lactococci increased free amino acids content but did not change significantly the volatile compound profile (*25*). The mixed culture of wild *L. lactis*

Table 1. Aldehydes in Cheeses Manufactured with a Mesophilic Starter (MS), a Thermophilic Starter (TS), and Bacteriocin-Producing *L. lactis* Subsp. *lactis* INIA 415[a]

aldehyde	age (days)	1% MS		1% MS + 1% TS	
		0% INIA 415	0.1% INIA 415	0% INIA 415	0.1% INIA 415
acetaldehyde[b]	25	0.36 ± 0.16a	0.28 ± 0.04a	0.32 ± 0.07a	0.46 ± 0.08a
	50	0.25 ± 0.09ab	0.19 ± 0.04a	0.28 ± 0.03ab	0.36 ± 0.11b
	75	0.23 ± 0.08a	0.23 ± 0.06a	0.30 ± 0.05a	0.28 ± 0.05a
2-methylpropanal[b]	25	0.05 ± 0.02a	0.08 ± 0.02b	0.05 ± 0.01a	0.08 ± 0.01b
	50	0.06 ± 0.00a	0.05 ± 0.02a	0.08 ± 0.01a	0.08 ± 0.01a
	75	0.08 ± 0.01a	0.12 ± 0.02b	0.09 ± 0.01a	0.10 ± 0.01ab
2-methylbutanal[b]	25	0.07 ± 0.01a	0.11 ± 0.02bc	0.08 ± 0.02ab	0.12 ± 0.02c
	50	0.06 ± 0.01a	0.12 ± 0.04bc	0.08 ± 0.02ab	0.13 ± 0.02c
	75	0.09 ± 0.01a	0.18 ± 0.04b	0.11 ± 0.01a	0.13 ± 0.02ab
3-methylbutanal[b]	25	0.31 ± 0.05a	0.68 ± 0.16bc	0.40 ± 0.15ab	0.77 ± 0.16c
	50	0.45 ± 0.15a	1.17 ± 0.46b	0.73 ± 0.33ab	1.16 ± 0.16b
	75	0.73 ± 0.08a	1.77 ± 0.42b	1.08 ± 0.29a	1.19 ± 0.17a
hexanal	25	0.17 ± 0.05a	0.13 ± 0.04a	0.14 ± 0.01a	0.13 ± 0.04a
	50	0.15 ± 0.06a	0.14 ± 0.07a	0.13 ± 0.04a	0.15 ± 0.02a
	75	0.08 ± 0.02a	0.12 ± 0.02ab	0.12 ± 0.02ab	0.15 ± 0.03b
nonanal[b]	25	0.81 ± 0.45a	0.93 ± 0.79a	0.92 ± 0.26a	1.14 ± 0.44a
	50	0.42 ± 0.09a	0.72 ± 0.22ab	0.22 ± 0.23a	1.00 ± 0.17b
	75	0.70 ± 0.30a	0.53 ± 0.28a	0.63 ± 0.31a	0.34 ± 0.14a
decanal	25	0.04 ± 0.03a	0.03 ± 0.01a	0.04 ± 0.01a	0.03 ± 0.01a
	50	0.02 ± 0.00a	0.03 ± 0.02a	0.02 ± 0.01a	0.06 ± 0.02b
	75	0.04 ± 0.02a	0.03 ± 0.00a	0.03 ± 0.31a	0.01 ± 0.00a

[a] Mean ± SD of duplicate determinations in two cheese-making experiments, expressed as relative abundance to internal standard. Means not followed by the same letter in each row are significantly different ($P < 0.05$). [b] Significant ($P < 0.05$) effect of cheese age according to the analysis of variance.

subsp. *cremoris* B1157 and industrial *L. lactis* subsp. *cremoris* SK110 strains, but not the single cultures of individual strains, produced high levels of branched-chain aldehydes in milk, with a very strong chocolate-like flavor (*26*).

Hexanal, nonanal, and decanal originate from unsaturated fatty acids, with the formation of an intermediate hydroperoxide (*19*). The highest levels of hexanal were reached in cheeses made with bacteriocin-producing adjunct culture and thermophilic starter after 75 days of ripening. This volatile compound has been positively correlated with a caramel and creamy odor and a balanced flavor in a study carried out on 10 European cheese varieties (*27*). The highest levels of nonanal were detected in the same cheese as hexanal, after 25 days of ripening. Decanal was in all cheeses at low relative abundance during ripening.

Alcohols. The relative abundances of nine alcohols were influenced by the addition of the bacteriocin producer or the thermophilic starter (**Table 2**). Formation of alcohols from aldehydes through the activity of lactic acid bacteria dehydrogenases should be favored by the strong reducing conditions present in cheese (*18*, *19*). Levels of ethanol, the most abundant of volatile compounds, were higher in cheeses made with thermophilic starter. *S. thermophilus* produces high amounts of ethanol when compared to other homofermentative lactic acid bacteria (*28*). Ethanol was at slightly lower levels in cheeses made with bacteriocin-producing adjunct culture than in the respective cheeses made without bacteriocin-producing adjunct culture (**Table 2**). Besides lactate, the major product of pyruvate metabolism, lactococci produce ethanol, formate, and acetate when grown on galactose or on low concentrations of glucose or lactose (*29*), conditions similar to those present in cheese during ripening. Completion of metabolic pathways will be probably impaired in cells lysed or injured by bacteriocins. The release of intracellular contents of injured cells into the cheese matrix may contribute to an increase in the concentration of some intermediate metabolites.

Levels of 2-propanol and 2-pentanol were generally lower in cheeses made with the bacteriocin producer than in the respective cheeses made without it (**Table 2**). On the other hand, levels of 1-octanol tended to be higher in cheeses made with the bacteriocin-producing adjunct culture than in the respectives cheeses made without it. Higher levels of 1-hexanol, 3-methyl-2-buten-1-ol, and 3-methyl-3-buten-1-ol were occasionally found in cheeses made with thermophilic starter, whereas levels of 2-methyl-1-propanol and 3-methyl-1-butanol tended to be lower in cheeses made with thermophilic starter. 3-Methyl-1-butanol has a fruity and alcohol flavor note (*18*). This alcohol has been associated with unclean and harsh flavors in Cheddar cheese (*21*), but recently it has been positively correlated with a caramel odor (*27*). In cheeses made with bacteriocin-producing adjunct culture 2-methyl-1-propanol and 3-methyl-1-butanol were at higher levels than in the respective cheeses made without it.

Esters. The relative abundances of two esters were influenced by the addition of the bacteriocin producer or the thermophilic starter (**Table 3**). Esterification reactions occur between short- to medium-chain fatty acids and alcohols derived from lactose fermentation or from amino acids catabolism (*18*). Esters have been identified as major contributors to the flavor of Cheddar cheese (*30*), their role in cheese aroma being due to their high volatility at ambient temperatures (*31*) and the low perception threshold of these compounds (*18*). In our experiments, levels of ethyl butanoate and ethyl hexanoate were higher in cheeses made with thermophilic starter, which exhibited a higher concentration of ethanol. Ethyl butanoate has been positively correlated with a sweet odor (*27*). Ethyl hexanoate, with an aromatic note resembling pineapple or banana, has also been positively correlated with pungent odor and silage-like, salty, acidic, and peppery flavors (*27*).

Ketones. The relative abundances of seven ketones were influenced by the addition of the bacteriocin producer or the thermophilic starter (**Table 4**). Diacetyl (2,3-butanedione) originates from the unstable precursor α-acetolactate during citrate metabolism. It has buttery flavor notes. Diacetyl is the major aromatic compound in fermented milk and fresh cheese (*19*, *28*) and contributes to the flavor of Gouda-type cheese (*22*). In *L. lactis*, diacetyl is produced chemically by oxidative

Table 2. Alcohols in Cheeses Manufactured with a Mesophilic Starter (MS), a Thermophilic Starter (TS), and Bacteriocin-Producing *L. lactis* Subsp. *lactis* INIA 415[a]

alcohol	age (days)	1% MS		1% MS + 1% TS	
		0% INIA 415	0.1% INIA 415	0% INIA 415	0.1% INIA 415
ethanol	25	55.3 ± 11.2ab	44.7 ± 15.9a	95.0 ± 37.7b	78.0 ± 17.6ab
	50	57.3 ± 7.8b	31.3 ± 6.2a	80.0 ± 11.1c	50.3 ± 10.9ab
	75	43.7 ± 8.8a	38.7 ± 6.2a	78.0 ± 7.3b	83.0 ± 6.6b
2-propanol[b]	25	1.11 ± 0.48a	1.07 ± 0.11a	0.75 ± 0.08a	0.75 ± 0.19a
	50	1.87 ± 0.32b	1.51 ± 0.25ab	1.59 ± 0.72ab	0.86 ± 0.16a
	75	2.46 ± 0.15b	1.81 ± 0.69ab	1.63 ± 0.21a	1.34 ± 0.18a
2-methyl-1-propanol	25	0.33 ± 0.12a	0.44 ± 0.24a	0.28 ± 0.11a	0.34 ± 0.12a
	50	0.37 ± 0.03a	0.55 ± 0.10b	0.33 ± 0.06a	0.35 ± 0.06a
	75	0.38 ± 0.08a	0.49 ± 0.16a	0.32 ± 0.07a	0.46 ± 0.05a
2-pentanol[b]	25	0.78 ± 0.54a	1.02 ± 0.78a	0.88 ± 0.87a	0.64 ± 0.10a
	50	1.72 ± 0.76a	1.43 ± 1.17a	0.93 ± 0.90a	1.26 ± 0.03a
	75	4.00 ± 1.03b	2.95 ± 1.47ab	2.44 ± 1.66ab	1.06 ± 0.25a
3-methyl-1-butanol	25	4.97 ± 2.19a	7.31 ± 3.98a	3.65 ± 2.45a	3.51 ± 1.45a
	50	5.29 ± 0.80ab	7.65 ± 1.93b	3.89 ± 1.12a	4.89 ± 1.17ab
	75	6.40 ± 1.42a	7.80 ± 2.66a	4.44 ± 0.79a	5.44 ± 1.20a
3-methyl-2-buten-1-ol	25	0.55 ± 0.02a	0.62 ± 0.05ab	0.84 ± 0.07c	0.74 ± 0.09bc
	50	0.54 ± 0.03a	0.61 ± 0.04a	0.90 ± 0.06b	0.88 ± 0.03b
	75	0.59 ± 0.09a	0.61 ± 0.06a	0.92 ± 0.03c	0.78 ± 0.03b
3-methyl-3-buten-1-ol	25	0.89 ± 0.16a	1.01 ± 0.15a	1.05 ± 0.09a	0.89 ± 0.11a
	50	1.03 ± 0.11a	0.97 ± 0.12a	1.15 ± 0.19a	0.99 ± 0.07a
	75	1.01 ± 0.18ab	0.89 ± 0.06a	1.24 ± 0.17b	0.99 ± 0.05ab
1-hexanol[b]	25	ND	ND	ND	ND
	50	0.03 ± 0.00a	0.03 ± 0.00a	0.04 ± 0.00ab	0.06 ± 0.02b
	75	0.04 ± 0.01a	0.03 ± 0.00a	0.04 ± 0.00a	0.06 ± 0.04a
1-octanol	25	0.08 ± 0.03a	0.08 ± 0.06a	0.08 ± 0.03a	0.12 ± 0.03a
	50	0.06 ± 0.02a	0.14 ± 0.04b	0.07 ± 0.01a	0.13 ± 0.03b
	75	0.12 ± 0.02a	0.10 ± 0.04a	0.09 ± 0.01a	0.09 ± 0.03a

[a] Mean ± SD of duplicate determinations in two cheese-making experiments, expressed as relative abundance to internal standard. Means not followed by the same letter in each row are significantly different ($P < 0.05$). ND, below detection limit. [b] Significant ($P < 0.05$) effect of cheese age according to the analysis of variance.

Table 3. Esters in Cheeses Manufactured with a Mesophilic Starter (MS), a Thermophilic Starter (TS), and Bacteriocin-Producing *L. lactis* Subsp. *lactis* INIA 415[a]

ester	age (days)	1% MS		1% MS + 1% TS	
		0% INIA 415	0.1% INIA 415	0% INIA 415	0.1% INIA 415
ethyl butanoate[b]	25	0.05 ± 0.01a	0.05 ± 0.01a	0.13 ± 0.00c	0.09 ± 0.03b
	50	0.10 ± 0.02a	0.10 ± 0.01a	0.24 ± 0.02b	0.20 ± 0.05b
	75	0.15 ± 0.05a	0.14 ± 0.01a	0.37 ± 0.03b	0.33 ± 0.10b
ethyl hexanoate[b]	25	0.12 ± 0.02a	0.13 ± 0.01a	0.17 ± 0.02a	0.15 ± 0.06a
	50	0.14 ± 0.04a	0.16 ± 0.01a	0.26 ± 0.05b	0.27 ± 0.10b
	75	0.23 ± 0.08a	0.20 ± 0.03a	0.42 ± 0.06b	0.38 ± 0.15b

[a] Mean ± SD of duplicate determinations in two cheese-making experiments, expressed as relative abundance to internal standard. Means not followed by the same letter in each row are significantly different ($P < 0.05$). [b] Significant ($P < 0.05$) effect of cheese age according to the analysis of variance.

decarboxylation of the metabolic intermediate α-acetolactate (*32*). Levels of diacetyl were higher in cheeses made with thermophilic starter after 50 and 75 days of ripening (**Table 4**). Some *S. thermophilus* strains have been reported to produce high levels of diacetyl (*33*). In the present work, the highest level of diacetyl was recorded in cheese made with thermophilic starter and bacteriocin-producing adjunct culture (**Table 4**).

The highest level of 2,3-pentanedione was found after 25 days of ripening in cheeses made with thermophilic starter and bacteriocin producer. Imhof et al. (*33*) detected high levels of 2,3-pentanedione in milk inoculated with different *S. thermophilus* strains and suggested that 2,3-pentanedione might be formed from α-aceto-α-hydroxybutyrate, an intermediate of the isoleucine metabolism. On the other hand, 2-pentanone, 2-hexanone, and 2-heptanone tended to be at higher levels in cheeses made without thermophilic starter. Methyl ketones are produced by β-oxidation of free fatty acids, a pathway involving the release of fatty acids by lipolysis, their oxidation to β-ketoacids, and decarboxylation to methyl ketones with one fewer C atom (*9*). Ketones such as 2-pentanone and 2-hexanone have been

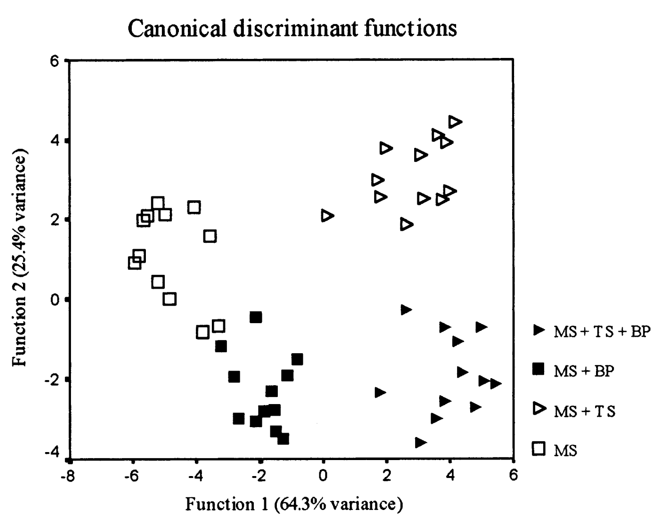

Figure 1. Distribution of cheeses using canonical discriminant functions 1 and 2 (MS, mesophilic starter; TS, thermophilic starter; BP, bacteriocin producer).

Table 4. Ketones in Cheeses Manufactured with a Mesophilic Starter (MS), a Thermophilic Starter (TS), and Bacteriocin-Producing *L. lactis* Subsp. *lactis* INIA 415[a]

ketone	age (days)	1% MS		1% MS + 1% TS	
		0% INIA 415	0.1% INIA 415	0% INIA 415	0.1% INIA 415
acetone	25	6.78 ± 1.17a	5.39 ± 0.21a	5.03 ± 0.83a	6.39 ± 1.08a
	50	7.09 ± 0.81a	7.72 ± 0.83a	4.93 ± 0.85a	5.74 ± 1.26a
	75	10.45 ± 1.74b	8.17 ± 1.21ab	6.66 ± 0.72a	7.57 ± 1.19ab
2-butanone[b]	25	0.65 ± 0.15a	0.66 ± 0.03a	0.77 ± 0.13a	1.08 ± 0.20b
	50	0.80 ± 0.09a	0.79 ± 0.11a	0.81 ± 0.15a	0.93 ± 0.16a
	75	0.89 ± 0.15a	1.04 ± 0.20a	0.94 ± 0.05a	1.17 ± 0.06a
2,3-butanedione	25	3.21 ± 2.08a	5.66 ± 3.46a	3.10 ± 2.59a	3.11 ± 0.44a
	50	1.75 ± 0.12a	3.82 ± 0.83ab	4.16 ± 1.40ab	5.84 ± 0.97b
	75	1.64 ± 0.29a	1.72 ± 0.18a	4.13 ± 1.03b	9.06 ± 1.27b
2-pentanone[b]	25	5.75 ± 3.75a	5.28 ± 2.33a	5.93 ± 4.56a	4.72 ± 0.74a
	50	21.10 ± 10.8a	18.11 ± 4.1a	10.20 ± 9.4a	4.41 ± 2.72a
	75	36.72 ± 13.2b	27.01 ± 2.4ab	18.32 ± 8.8ab	10.68 ± 3.1a
2,3-pentanedione[b]	25	0.29 ± 0.06a	0.33 ± 0.06ab	0.42 ± 0.08ab	0.50 ± 0.11b
	50	0.28 ± 0.01a	0.25 ± 0.01a	0.29 ± 0.03a	0.29 ± 0.10a
	75	0.37 ± 0.05a	0.36 ± 0.04a	0.36 ± 0.02a	0.30 ± 0.06a
2-hexanone[b]	25	0.05 ± 0.02a	0.08 ± 0.05a	0.06 ± 0.04a	0.03 ± 0.01a
	50	0.12 ± 0.05a	0.13 ± 0.08a	0.12 ± 0.04a	0.12 ± 0.02a
	75	0.33 ± 0.04b	0.27 ± 0.09b	0.20 ± 0.05ab	0.11 ± 0.06a
2-heptanone[b]	25	1.59 ± 0.31a	2.86 ± 1.05a	1.73 ± 0.33a	2.04 ± 0.53a
	50	4.67 ± 1.37a	6.21 ± 2.19a	3.48 ± 0.82a	3.20 ± 1.07a
	75	6.45 ± 0.72ab	6.98 ± 1.19b	2.99 ± 0.96ab	2.47 ± 0.45a

[a] Mean ± SD of duplicate determinations in two cheese-making experiments, expressed as relative abundance to internal standard. Means not followed by the same letter in each row are significantly different ($P < 0.05$). [b] Significant ($P < 0.05$) effect of cheese age according to the analysis of variance.

Table 5. Pooled Within-Groups Correlations between Discriminating Variables and Canonical Discriminant Functions

volatile compound	canonical discriminant functions	
	function 1	function 2
3-methyl-2-buten-1-ol	0.555[a]	0.346
acetone	0.296[a]	–0.039
heptanal	0.237[a]	0.080
ethyl butanoate	0.173[a]	0.035
2,3-pentanedione	–0.159[a]	0.092
2-pentanol	–0.126[a]	0.068
2-heptanone	–0.066[a]	0.007
2-hexanone	0.043[a]	0.023
2-methylbutanal	0.091	–0.359[a]
ethanol	0.226	0.295[a]
propanal	0.097	–0.252[a]
2-methyl-1-propanol	–0.053	–0.235[a]
3-methyl-3-buten-1-ol	0.066	0.227[a]
acetaldehyde	0.061	–0.220[a]
hexanal	–0.053	–0.219[a]
3-methylbutanal	0.076	–0.176[a]
2-methylpropanal	0.042	–0.151[a]
octane	0.037	–0.146[a]
2-pentanone	–0.058	0.109[a]

[a] Denotes largest absolute correlation between each variable and any discriminant function.

associated with fruity and floral flavor, and 2-heptanone was considered to be responsible for the characteristic aroma of Roquefort and Camembert cheeses (*18*). These three methyl ketones have been positively correlated with a moldy odor and a mushroom and moldy flavor (*27*).

Stepwise discriminant analysis using the relative abundances of volatile compounds classified correctly cheeses by type of starter in 97.9% of cases (**Figure 1**), with function 1 related to thermophilic starter and function 2 to bacteriocin producer. Canonical discriminant function 1 explained 64.3% of variance and separated the cheeses made with thermophilic starter from those made without it. The discriminant variables are summarized in **Table 5**. 3-Methyl-2-buten-1-ol, acetone, heptanal, ethyl butanoate, 2-hexanone, and cheeses made with thermophilic starter correlated positively with function 1, whereas 2,3-pentanedione, 2-pentanol, 2-heptanone, and cheeses made without thermophilic starter correlated negatively. Canonical discriminant function 2 explained 25.4% of variance and separated the cheeses made with bacteriocin producer from the cheeses made without it (**Figure 1**). Ethanol, 3-methyl-3-buten-1-ol, 2-pentanone, and cheeses made without bacteriocin producer correlated positively with function 2, whereas 2-methylbutanal, propanal, 2-methyl-1-propanol, acetaldehyde, hexanal, 3-methylbutanal, 2-methylpropanal, octane, and cheeses made with bacteriocin producer correlated negatively.

Conclusions. Many volatile compounds are responsible for cheese aroma and contribute to its overall flavor together with nonvolatile compounds such as acids, amino acids, and small peptides. In the present work, addition of bacteriocin-producing *L. lactis* subsp. *lactis* INIA 415 and addition of a thermophilic starter to milk in the manufacture of Hispánico cheese enhanced the formation of some volatiles considered to be impact aroma compounds such as acetaldehyde, 2-methyl-propanal, 2-methylbutanal, 3-methylbutanal, 2-methyl-1-propanol, ethyl butanoate, ethyl hexanoate, and 2,3-butanedione. The classification of cheeses manufactured using bacteriocin-producing adjunct and/or thermophilic starter was feasible by means of stepwise discriminant analysis of the relative abundances of volatile compounds (97.9% cases classified correctly). As reported previously (*14*), cheese made with bacteriocin-producing adjunct culture and thermophilic starter received the highest scores for flavor intensity and flavor quality. However, it is difficult to determine how the increase in the levels of volatile compounds could have enhanced flavor intensity and improved flavor quality. Further studies are necessary to establish the influence of volatile compounds on the sensory characteristics of Hispánico cheese.

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