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Volatile Compounds Produced in Cheese by *Pseudomonas* Strains of Dairy Origin Belonging to Six Different Species

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Production of volatile compounds by seven *Pseudomonas* strains belonging to six different species, *Ps. brenneri*, *Ps. graminis*, *Ps. libanensis*, *Ps. lundensis*, *Ps. putida*, and *Ps. rhodesiae*, was investigated, with the aim of elucidating their possible contribution to the volatile profile of cheese. Laboratory-scale cheeses were made from pasteurized milk of low bacterial counts separately inoculated with $\sim 10^5$ colony-forming units/mL of each *Pseudomonas* strain and ripened for 12 days at 10 °C. A total of 122 volatile compounds were identified in cheeses by GC-MS of the dynamic headspace. The abundance of 62 compounds, belonging to eight chemical groups (aldehydes, ketones, acids, esters, alcohols, hydrocarbons, benzene compounds, and sulfur compounds) increased during ripening for at least one of the strains. Most groups of volatile compounds were more abundant in the outer part of cheeses than in the inner part, in agreement with the aerobic metabolism of the genus *Pseudomonas* and coinciding with the higher counts in the outer part. Production of volatile compounds in cheese by *Pseudomonas* was shown to be species-dependent.

KEYWORDS: Volatile compounds; cheese; *Pseudomonas*; psychrotrophs

INTRODUCTION

Psychrotrophic Gram-negative bacteria, mostly *Pseudomonas* spp., cause the spoilage of milk and dairy products (*1*). The main source of *Pseudomonas* and other psychrotrophic genera that become predominant during the refrigerated storage of raw milk is inadequately disinfected milking equipment (*2*). Common sources of postpasteurization contamination by *Pseudomonas* are improperly cleaned pasteurizers and filling machines (*3*). Within this genus, *Ps. fluorescens*, *Ps. fragi*, and *Ps. putida* are the three species of greatest concern (*4*, *5*).

Pseudomonas strains are responsible for textural changes in milk such as gelation and increased viscosity and for unclean and bitter flavors in cheese and other dairy products (*6*), due to the production of extracellular proteinases. On the other hand, rancid and fruity aromas are caused by extracellular lipases and esterases (*7*). Changes in milk flavor may be perceived when *Pseudomonas* counts exceed 5×10^6 colony-forming units (cfu)/mL (*2*). Defects in cheese flavor or texture have been reported for *Pseudomonas* counts in raw milk $>10^6$ cfu/mL (*8*−*10*).

Pseudomonas species produce different volatile compounds during growth in milk. Thus, *Ps. fragi* has been reported to produce high levels of ethyl esters of short-chain fatty acids, responsible for the fruity flavor defect (*11*). Five bacterial strains belonging to the species *Ps. fluorescens*, *Ps. fragi*, *Bacillus subtilis*, *Enterobacter aerogenes*, and *Lactococcus lactis* produced different volatile compound profiles when grown in milk (*12*). Sensory aroma characteristics of milk cultures of two strains of each of the species *Ps. fluorescens*, *Ps. fragi*, and *Ps. putida* were strain-dependent (*13*), as shown by descriptive aroma analysis.

It is generally assumed that raw milk cheeses possess flavor notes lacking in pasteurized milk cheeses (*14*), some of these flavor notes being traceable to the microbiota present in milk (*15*). The deleterious effects of *Pseudomonas* on milk coagulation characteristics and on proteolysis and lipolysis during cheese ripening are well-known (*2*, *6*, *7*). However, our knowledge of the effects of this genus on the volatile compound profile of cheese remains limited. The objective of the present work was to investigate the production of volatile compounds by strains of dairy origin belonging to different *Pseudomonas* species in laboratory-scale cheeses and to elucidate their possible contribution to the volatile profile of cheese.

MATERIALS AND METHODS

Strain Isolation and Identification. *Pseudomonas* strains were isolated from ten 1-day-old raw ewes' milk cheeses from five different dairies. Cheese homogenates (10% w/w) in 2% sodium citrate were serially diluted in 0.1% peptone solution and plated on PMK agar (Biolife, Milano, Italy). Ten colonies per plate were picked randomly and examined for Gram's stain, catalase test, and oxidase test. Five Gram-negative, catalase-positive, oxidase-positive rods from each of the 10 cheeses were grouped into different biochemical profiles with the aid of the API 20 NE system (bioMerieux, Marcy-l'Étoile, France).

One strain representative of each biochemical profile was selected and identified by 16S rRNA sequencing, as previously described (*16*).

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Lysates with lysozyme (1 mg/mL) and proteinase K (200 μg/mL) were serially diluted, and 1 μL of the 1:1000 dilution was used for PCR amplification. Partial sequencing (1013−1046 bp) of both strands of the amplified fragment was carried out, and the obtained sequence was compared by BLAST search analysis with sequences of Pseudomonadaceae collection strains in the NCBI database. Each strain was assigned to the species of the type strain with which it showed the highest degree of similarity after comparison. All of the strains were maintained at −80 °C in nutrient broth with 15% glycerol added.

Cheese Manufacture and Sampling. Cheeses were made at laboratory scale in duplicate experiments, carried out on different days. Each experiment consisted of eight 4-L vats of pasteurized (78 °C/15 s) cow's milk (total viable counts $<5 \times 10^2$ cfu/mL). Milk in seven vats was separately inoculated with each of seven *Pseudomonas* strains. Milk in the eighth vat was not inoculated with any strain, and 250 μg/mL amoxicillin and 62.5 μg/mL clavulanic acid were added to milk to prevent bacterial growth in cheese. The manufacturing procedure was that of Hispánico cheese, a semihard Spanish variety, except that glucono-δ-lactone was used instead of lactic starter cultures for milk and curd acidification to circumvent interferences by the metabolism of lactic acid bacteria. Milk at 31 °C, with 0.01% $CaCl_2$ and 1.0% glucono-δ-lactone (Chr. Hansen, Madrid, Spain) added, was inoculated with 2 mL of a fresh culture of the respective *Pseudomonas* strain in sterile milk, to yield $\sim10^5$ cfu/mL. After 20 min, 2.66 mL of a fresh 2% dilution of Maxiren 150 rennet (Gist Brocades, Delft, The Netherlands) was added, and milk was held at 31 °C for 40 min to coagulate. The curd was cut to rice grain size, heated to 38 °C, held for 15 min at this temperature to favor whey expulsion, and transferred into molds. Two cheeses, ∼250 g in weight, were obtained per vat. They were pressed overnight at 20 °C and 0.7 kg/cm^2 pressure. The next morning cheeses were salted for 1 h in a 15% NaCl solution at 20 °C and left to ripen at 10 °C.

Curds were sampled after 2 h in the press and cheeses (outer part, 5 mm thick; inner part, the rest of the cheese) after 6 or 12 days of ripening. Samples of 2 h curds from milk not inoculated with *Pseudomonas* strains were used to determine the initial levels of volatile compounds in each experiment. Samples for volatile compound analysis were wrapped in aluminum foil, vacuum packed, and kept at −40 °C.

Microbiological Analysis and pH. Curd and cheese samples were homogenized (10%, w/w) in a sterile 2% sodium citrate solution using a homogenizer (IUL, Barcelona, Spain), and decimal dilutions were prepared in sterile 0.1% peptone solution. Viable counts were determined on PMK duplicate plates incubated at 30 °C for 24 h. Dilutions (0.1 mL) were also spread plated on plate count agar (PCA, Oxoid) to check for homogeneity of colonies and the absence of contaminants. VRBGA (VRBA, Oxoid with 1% glucose added) was used to check for the absence of Enterobacteriaceae and MRS (Biolife), pH 5.7, to check for the absence of lactic acid bacteria.

Cheese pH was measured in duplicate using a penetration electrode (Xerolyt 52-32; Crison, Barcelona, Spain).

Volatile Compound Analysis. Duplicate cheese samples (10 g) were homogenized in an analytical blender with 20 g of anhydrous Na_2SO_4 and 30 μL of an internal standard aqueous solution containing 0.65 mg/mL cyclohexanone (Sigma-Aldrich Química, Alcobendas, Spain). 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.

Gas chromatography−mass spectrometry was carried out in an 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, 4 °C/min to 114 °C, 6 min at 114 °C, 7 °C/min to 143 °C, 15 °C/min to 240 °C, 4 min at 240 °C; He flow, 1 mL/min. Total analysis time was 51 min. Detection was performed with the mass spectrometer 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. Peak identification was by comparison of retention times and ion spectra from real standards (Sigma-Aldrich Química, Madrid, Spain) and/or spectra from the Wiley 275 library (Wiley, New York). Only compounds with abundance values >20 000 in at least one cheese sample were further considered and semiquantified. 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.

Statistical Analysis. One-way analysis of variance was carried out by means of SPSS Win 8.0 program on pH values, on log counts, and on the abundances of 63 individual volatile compounds with *Pseudomonas* species as the main effect, outer 6-day-old, outer 12-dat-old, and inner 12-dat-old cheese samples being separately treated. One-way analysis of variance was also performed on the sums of eight chemical groups of volatile compounds (aldehydes, ketones, acids, esters, alcohols, hydrocarbons, benzene compounds, and sulfur compounds), with cheese sample (outer 6-day-old, outer 12-day-old, inner 12-daya-old) as the main effect. Comparison of means was carried out by Tukey's test. A stepwise discriminant analysis was performed to classify the six studied *Pseudomonas* species by their ability to produce volatile compounds, using Wilk's lambda as the statistical criterion for the selection of variables and considering separately the samples from the inner and outer parts of cheeses.

RESULTS AND DISCUSSION

***Pseudomonas* Strains.** *Pseudomonas* is the psychrotrophic genus of greatest concern with respect to the spoilage of milk and dairy products (*4*, *5*). Within this genus, *Ps. putida*, *Ps. fluorescens*, and *Ps. fragi* have been reported as the most abundant species in raw and processed milk (*16*). In the present work, 50 *Pseudomonas* isolates were obtained from 1-day-old raw milk cheeses and grouped into 12 different biochemical profiles with the aid of the API 20 NE system. The characterization of 12 strains, 1 per biochemical profile, by comparison of their 16S rRNA sequences with those of collection strains gave the following results: 5 strains (representing 21 of the 50 isolates) were ascribed to the species *Ps. fragi*, 2 strains (representing 4 isolates) to *Ps. libanensis*, 1 strain (representing 16 isolates) to *Ps. graminis*, 1 strain (representing 3 isolates) to *Ps. brenneri*, 1 strain (representing 3 isolates) to *Ps. putida*, 1 strain (representing 2 isolates) to *Ps. rhodesiae*, and 1 strain (representing 1 isolate) to *Ps. lundensis.* Production of volatile compounds by the 5 *Ps. fragi* strains was studied and reported elsewhere (*17*), and references to this study will be made throughout the text. The coincidence of the nucleotide sequences of the remaining 7 *Pseudomonas* strains with the nucleotide sequence of the closest type strain ranged from 98.6% for *Ps. putida* 32 to 100% for *Ps. brenneri* 46. These strains were used in cheesemaking experiments.

Bacterial Counts and Cheese pH. The mean count of the seven *Pseudomonas* strains in inoculated milk was 4.76 log cfu/mL (**Table 1**), a number frequently reached by *Pseudomonas* populations in milk for cheese production (*2*, *8*). Counts of *Pseudomonas* strains increased on average by 1.96 log units from inoculated milk to curds after 2 h in the press, because of cell retention in the curds during whey drainage and bacterial growth in milk and curds during cheese manufacture. All seven *Pseudomonas* strains were able to grow in the outer part of cheeses during the first 6 days, despite the early decline in pH caused by glucono-δ-lactone, and in cheeses made from milk inoculated with *Ps. graminis* and *Ps. libanensis* growth continued from day 6 to day 12. In the inner part of cheeses, a decrease in counts from curds to day 6 was observed for *Ps. graminis* and *Ps. brenneri*, but afterward *Ps. graminis* was able to grow from day 6 to day 12. Populations of the rest of the

Table 1. Microbial Counts and pH Values of Curds and Cheeses Made with Seven *Pseudomonas* Strains[a]

	Ps. brenneri	*Ps. graminis*	*Ps. libanensis*	*Ps. lundensis*	*Ps. putida*	*Ps. rhodesiae*
log cfu/g						
inoculated milk	4.89a	4.51a	4.90a	4.53a	4.84a	4.75a
2 h curds	6.51ab	5.94a	7.02b	6.73b	6.73b	7.12b
6-day-old outer sample	8.92b	8.16×bb	9.20b	9.03b	8.83b	9.39b
6-day-old inner sample	4.97a	4.22a	6.95bc	7.47c	6.23b	7.33c
12-day-old outer sample	8.88a	9.12a	9.83c	9.48b	9.13a	9.84c
12-day-old inner sample	5.06a	6.94bc	7.18bc	7.48c	6.80b	7.01bc
pH values						
2 h curds	5.41a	5.41a	5.22a	5.33a	5.33a	5.33a
6-day-old outer sample	5.76a	5.63a	5.51a	5.45a	5.55a	5.66a
6-day-old inner sample	5.40a	5.42a	5.39a	5.44a	5.45a	5.41a
12-day-old outer sample	6.14a	5.72a	6.17a	5.45a	5.51a	5.97a
12-day-old inner sample	5.45a	5.51a	5.51a	5.40a	5.40a	5.53a

[a] Mean values followed by the same letter are not significantly different ($P > 0.05$). One strain per species except *Ps. libanensis*, with two strains.

Table 2. Mean Levels[a] of the Main Groups of Volatile Compounds in 2 h Curds and in Cheeses Made from Milk Inoculated Separately with *Ps. brenneri*, *Ps. graminis*, *Ps. libanensis*, *Ps. lundensis*, *Ps. putida*, and *Ps. rhodesiae* Strains[b]

group of compounds	2 h curds	6-day-old cheeses, outer samples	12-day-old cheeses, outer samples	12-day-old cheeses, inner samples
aldehydes ($n = 3$)	1.54a	7.10a	24.61b	3.17a
ketones ($n = 7$)	5.14a	64.96a	171.59b	78.49ab
acids ($n = 4$)	3.36a	13.75a	38.66a	1.38a
esters ($n = 10$)	0.17a	6.07a	37.65b	0.93a
alcohols ($n = 15$)	46.28a	170.16ab	414.85c	203.92b
hydrocarbons ($n = 7$)	8.28a	85.77b	217.19c	18.77a
benzene compounds ($n = 6$)	19.38a	23.52a	26.77a	17.80a
sulfur compounds ($n = 10$)	4.82a	19.25a	65.20b	34.21ab

[a] Sums of areas of volatile compounds corrected by the internal standard. [b] Means in the same row followed by the same letter are not significantly different ($P > 0.05$). One strain per species except *Ps. libanensis*, with two strains.

strains did not change significantly in the inner part of cheeses during ripening. The higher counts recorded for the seven *Pseudomonas* strains in the outer part of cheeses than in the inner part were in agreement with the aerobic metabolism of this genus. No contamination of cheeses by lactic acid bacteria or Enterobacteriaceae at levels capable of influencing the volatile profile of cheeses was detected. Counts in the inner part of 12-day-old cheeses were 1−3 log units lower than those of *Ps. fragi* (*17*), whereas counts in the outer part of cheeses were <1 log unit lower. The survival and growth of *Pseudomonas* strains during the manufacture and ripening of Manchego cheese, also with pH values close to 5, have been reported (*10*).

A low pH, with 5.32 as mean value (**Table 1**), was recorded for curds after 2 h in the press, because of a rapid acidification of the substrate caused by the hydrolysis of glucono-δ-lactone. Slight changes in pH value were recorded in the inner part of cheeses during ripening. However, mean pH value in the outer part of cheeses after 6 days of ripening was 0.26 unit higher than the pH of 2 h curds and still increased 0.29 unit from day 6 to day 12. No significant differences in pH values were recorded between cheeses made with the seven *Pseudomonas* strains throughout ripening.

Volatile Compounds. More than 600 volatile compounds have been identified so far in different cheese varieties (*18*, *19*). In the present work, 122 volatile compounds were detected by gas chromatography−mass spectrometry analysis of the volatile fraction of cheeses made from milk inoculated with *Pseudomonas* strains, 99 of which were already present in 2 h curds made from milk not inoculated with *Pseudomonas* (data not reported). Forty of the 122 volatile compounds detected in the present work were among the 76 compounds found in cold-stored raw milk (*20*). Approximately 90 compounds, 26 of which were odor-active, have been reported for the volatile fraction of *Ps. fragi* milk cultures (*21*). In cheeses made by Morales et al. (*17*) from milk inoculated with five *Ps. fragi* strains using identical manufacturing procedures, 131 volatile compounds were detected by means of the same analytical methods.

Twenty-five of the 122 volatile compounds had abundance values under 20 000 in all samples. Twelve compounds decreased during cheese ripening, and 23 compounds did not vary significantly during ripening in any of the cheeses. Sixty-two volatile compounds that, according to the analysis of variance, increased significantly with cheese age for at least one of seven *Pseudomonas* strains were further considered **(Tables 2−7)**. They were grouped into 7 ketones, 3 aldehydes, 4 acids, 10 esters, 15 alcohols, 7 hydrocarbons, 6 benzene compounds, and 10 sulfur compounds (**Table 2**). Compared with a previous study with *Ps. fragi* strains (*17*), esters were found at lower levels in cheeses made with strains of other *Pseudomonas* species. Volatile profiles of cheeses made from milk inoculated with Enterobacteriaceae were richer in ketones and alcohols (*22*), and those of cheeses made with *L. lactis* strains were richer in branched-chain alcohols and ketones (*23*).

Higher levels of aldehydes, esters, alcohols, and hydrocarbons were detected in the outer part of cheeses than in the inner part, in agreement with the higher counts and the aerobic metabolism of the genus *Pseudomonas*. Inner samples of 6-day-old cheeses were not analyzed for volatile compounds after consideration of the low *Pseudomonas* counts in those samples (**Table 1**). Only the levels of total hydrocarbons increased significantly from 2 h curds to outer samples of 6-day-old cheeses (**Table 2**), but all groups of volatile compounds except total acids and total benzene compounds increased significantly in the outer part of cheeses from day 6 to day 12.

Table 3. Mean Levels[a] of Aldehydes and Ketones in 12-Day-Old Cheeses (Outer and Inner Samples) Made from Milk Inoculated Separately with Seven *Pseudomonas* Strains and in Noninoculated 2 h Curds[b]

compound	ID[c]	sample	*Ps. brenneri*	*Ps. graminis*	*Ps. libanensis*	*Ps. lundensis*	*Ps. putida*	*Ps. rhodesiae*	2 h curds
2-methyl-1-propanal	ST	outer	2.84a	0.12a	7.95b	0.06a	2.12a	10.77b	0.86
		inner	0.42a	1.32a	5.10a	0.49a	0.95a	1.39a	
2-methyl-1-butanal	ST	outer	3.13b	0.00a	3.11b	0.00a	0.00a	10.26c	0.26
		inner	0.00a	0.00a	0.00a	0.00a	0.00a	0.29a	
3-methyl-1- butanal	ST	outer	11.32a	0.00a	24.14b	0.00a	0.14a	61.10c	0.41
		inner	0.69a	0.00a	1.82ab	0.00a	0.00a	2.77b	
total aldehydes		outer	17.30b	0.12a	35.20c	0.06a	2.26ab	82.13d	1.54
		inner	1.11a	1.32a	6.92a	0.49a	0.95a	4.45a	
2-pentanone	ST	outer	17.21ab	2.99a	13.08ab	245.83c	12.91ab	57.50b	1.06
		inner	17.16ab	3.25a	6.71ab	216.67c	6.00ab	49.65b	
2-heptanone	ST	outer	46.08b	7.34a	27.02ab	268.52d	23.23ab	224.63c	3.15
		inner	6.09a	5.91a	5.31a	88.20b	8.87a	18.27a	
2-octanone	ST	outer	0.15a	0.00a	0.00a	9.68c	0.00a	1.71b	0.03
		inner	0.00a	0.02a	0.00a	1.54b	0.00a	0.00a	
2-nonanone	ST	outer	11.24b	2.76a	6.59ab	63.18d	5.21a	27.34c	0.68
		inner	1.37a	1.50a	1.10a	4.23b	1.70a	1.90a	
2-undecanone	ST	outer	1.97b	0.50a	0.96a	0.76a	0.77a	3.12c	0.20
		inner	0.42bc	0.51c	0.17ab	0.00a	0.00a	0.00a	
3-methyl-2-butanone	MS	outer	0.00a	0.00a	0.23a	1.79b	0.00a	1.82b	0.00
		inner	0.00a	0.00a	0.48a	2.48b	0.00a	5.51c	
3-methyl-2-pentanone	MS	outer	4.77ab	0.00a	11.67ab	13.45bc	0.00a	25.52c	0.01
		inner	4.85a	0.00a	13.82a	10.17a	0.00a	37.97b	
total ketones		outer	81.41b	13.58a	59.55ab	603.22d	42.13ab	341.65c	5.14
		inner	29.88a	11.18a	27.59a	323.28c	16.57a	113.31b	

[a] Areas of volatile compounds corrected by the internal standard. [b] Means in the same row followed by the same letter are not significantly different ($P > 0.05$). One strain per species except *Ps. libanensis*, with two strains. [c] Compound identification: ST, authentic standard injection; MS, tentatively identified by spectra comparison using Wiley 275 Library.

Aldehydes and Ketones. Production of aldehydes and ketones in cheeses manufactured from milk inoculated with the seven *Pseudomonas* strains is shown in **Table 3**. *Ps. rhodesiae* produced the highest levels of all three aldehydes quantified, which were as a whole 18.5-fold higher in the outer part of 12-day-old cheeses than in the inner part and 3.2-fold higher than in the outer part of 6-day-old cheese made with this strain. The three branched-chain aldehydes, 2-methylpropanal, 2-methylbutanal, and 3-methylbutanal, found in the present work were produced only by *Ps. rhodesiae*, *Ps. libanensis*, and *Ps. brenneri* strains, generally at low levels. The most probable origin of these aldehydes are transamination reactions of Val, Ile, and Leu, respectively (*24*), and are considered to be potent odorants in some cheeses (*18*). *Ps. fragi* strains did not produce detectable levels of aldehydes in cheese (*17*).

Ketones are a group of volatiles in which many odor-active compounds have been identified (*18*). Strains belonging to *Ps. rhodesiae* and *Ps. lundensis* produced seven different ketones, with 2-pentanone and 2-heptanone as the predominant ones (**Table 3**). *Ps. lundensis* was the strongest producer of ketones, with levels in the outer and inner parts significantly higher than for the strains ascribed to the other species of *Pseudomonas*. All of the species studied produced a higher number of ketones and higher ketone levels than *Ps. fragi* strains, which produced only 2-pentanone and acetoxypropanone, with a mean total abundance of ∼3, as reported in a previous study (*17*). The total amount of ketones in the outer part of 12-day-old cheese made with *Ps. lundensis* was 1.9-fold higher than the amount found in the inner part and 3.3-fold higher than that found in the outer part of 6-day-old cheese made with this strain. Higher amounts of branched-chain ketones were produced in the inner part of cheese than in the outer part by *Ps. rhodesiae*. There were no significant differences in total aldehyde and total ketone production between the two strains of *Ps. libanensis*.

Acids and Esters. Levels of acids and esters in cheeses made from milk inoculated with the different *Pseudomonas* strains are shown in **Table 4**. No significant differences in levels of acids or esters were recorded between the two *Ps. libanensis* strains. *Ps. rhodesiae* produced high amounts of butanoic and hexanoic acids and the highest levels of total acids. The total level of acids in the outer part of 12-day-old cheese made with *Ps. rhodesiae* was 32-fold higher than the level found in the inner part and 3.1-fold higher than that found in the outer part of 6-day-old cheese made with this strain, values which could be explained by a higher esterase or lipase activity in the outer part of cheese. Butanoic acid was also produced by *Ps. brenneri*, *Ps. putida*, and *Ps. lundensis*, but no production of this acid was detected in cheeses made from milk inoculated with the other two species, in coincidence with the results obtained for *Ps. fragi* (*17*). Enterobacteriaceae strains produced very low amounts of volatile carboxylic acids in laboratory-scale cheeses (*22*), and the production of acids by *L. lactis* strains was not detectable (*23*).

Ps. rhodesiae was by far the strongest ester-producing species, both in number (10 different esters) and in levels (**Table 4**). This species exhibited high levels of methyl butanoate and hexanoate, ethyl butanoate, hexanoate, and octanoate, and isoamyl butanoate, although not reaching the abundance values reported for some of the *Ps. fragi* strains, which ranged between 700 and 1000 (*17*). The total amount of esters in the outer part of 12-day-old cheeses made with *Ps. rhodesiae* was 106-fold higher than in the inner part and 5.8-fold higher than in the outer part of 6-day-old cheese made with this strain, values that can be related to the higher levels of acids in the outer part of 12-day-old cheeses. *Ps. libanensis* and *Ps. brenneri* strains only produced significant amounts of ethyl butanoate and ethyl hexanoate, and only *Ps. putida* produced a significant amount of ethyl butanoate. Many of the esters found in our cheeses were present in cold-stored milk (*20*) and in cheeses made from refrigerated raw ewe's milk (*10*, *25*, *26*) and have been identified as odor-active compounds in different cheese varieties (*18*). Ester production by *Pseudomonas* in milk has been associated

Table 4. Mean Levels[a] of Acids and Esters in 12-Day-Old Cheeses (Outer and Inner Samples) Made from Milk Inoculated Separately with Seven *Pseudomonas* Strains and in Noninoculated 2 h Curds[b]

compound	ID[c]	sample	*Ps. brenneri*	*Ps. graminis*	*Ps. libanensis*	*Ps. lundensis*	*Ps. putida*	*Ps. rhodesiae*	2 h curds
butanoic acid	ST	outer	6.24a	0.00a	1.01a	14.19a	7.65a	188.22b	2.43
		inner	1.20ab	0.00a	0.11a	0.00a	0.59a	5.73b	
pentanoic acid	ST	outer	0.00a	0.00a	0.00a	0.00a	0.00a	1.50b	0.00
		inner	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	
hexanoic acid	ST	outer	1.73a	0.00a	0.18a	3.93a	1.46a	41.06b	0.93
		inner	0.45a	0.00a	0.00a	0.00a	0.00a	1.46a	
3-methyl-1-butanoic acid	ST	outer	0.00a	0.00a	0.00a	0.00a	0.00a	2.22b	0.00
		inner	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	
total acids		outer	7.97a	0.00a	1.19a	18.12a	9.11a	233.00b	3.36
		inner	1.65ab	0.00a	0.11ab	0.00a	0.59ab	7.19b	
methyl butanoate	ST	outer	0.56a	0.00a	0.63a	0.00a	0.00a	20.33b	0.06
		inner	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	
methyl hexanoate	ST	outer	0.94a	0.00a	0.70a	0.00a	0.00a	12.19b	0.03
		inner	0.03a	0.07a	0.00a	0.00a	0.00a	0.00a	
methyl octanoate	MS	outer	0.41a	0.00a	0.12a	0.00a	0.00a	6.97b	0.00
		inner	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	
ethyl butanoate	ST	outer	5.27ab	0.22a	10.10b	0.49a	6.35ab	66.70c	0.08
		inner	0.38ab	0.18a	1.19cd	0.91bc	0.70abc	1.86d	
ethyl hexanoate	ST	outer	6.48b	0.00a	8.11b	0.00a	0.11a	30.27c	0.00
		inner	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	
ethyl octanoate	ST	outer	1.54a	0.00a	0.65a	0.00a	0.11a	13.96b	0.00
		inner	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	
isobutyl butanoate	ST	outer	0.00a	0.00a	0.06a	0.00a	0.00a	5.01b	0.00
		inner	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	
butyl butanoate	ST	outer	0.00a	0.00a	0.00a	0.00a	0.00a	1.30b	0.00
		inner	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	
isoamyl butanoate	MS	outer	0.54a	0.00a	1.17a	0.00a	0.00a	37.73b	0.00
		inner	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	
isoamyl hexanoate	MS	outer	0.00a	0.00a	0.00a	0.00a	0.00a	2.97b	0.00
		inner	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	
total esters		outer	15.74a	0.22a	21.53a	0.49a	6.57a	197.43b	0.17
		inner	0.41a	0.25a	1.19bc	0.91ab	0.70ab	1.86c	

[a] Areas of volatile compounds corrected by the internal standard. [b] Means in the same row followed by the same letter are not significantly different ($P > 0.05$). One strain per species except *Ps. libanensis*, with two strains. [c] Compound identification: ST, authentic standard injection; MS, tentatively identified by spectra comparison using Wiley 275 Library.

with the appearance of fruity aromas (*15*, *21*, *28*, *29*), and *Ps. fragi* strains have been previously reported as strong ester producers (*13*, *21*, *29*). Our results agree partially with those reported for the headspace of milk inoculated with *Pseudomonas* strains held for 3 days at 7 °C (*20*), with ethyl acetate, butanoate, and hexanoate as the three major ethyl esters, and methyl acetate as the only non-ethyl ester.

Alcohols. Major alcohols in our cheeses (**Table 5**) were 2-propanol, mainly produced by *Ps. graminis*, 2-pentanol, mainly produced by *Ps. lundensis*, 3-methylbutanol, mainly produced by *Ps. libanensis* and *Ps.* rhodesiae, and 2-ethylhexanol, mainly produced by *Ps. brenneri*, *Ps. libanensis*, and *Ps. rhodesiae*. Higher amounts and variety of alcohols have been found in the present study than in cheeses inoculated with *Ps. fragi* strains, where 2-propanol, ethanol, and 3-methylbutanol were the most abundant alcohols (*17*). Ethanol, 2-propanol, 1-propanol, and 3-methylbutanol were the predominant alcohols in the headspace of refrigerated milk (*20*). Other dairy microorganisms also produce alcohols, with ethanol, 3-methylbutanol, and 2-methylpropanol as the predominant alcohols in cheeses inoculated with Enterobacteriaceae (*22*) or *L. lactis* strains (*23*). Considerable levels of 2-propanol were already present in 2 h curds, and the highest level of this alcohol, with an abundance of 138 (data not shown), was found in the outer part of 6-day-old cheese made with *Ps. lundensis*. Significant increases in 2-propanol content were recorded during ripening of cheeses made with *Ps. graminis* and *Ps. lundensis*, with slightly higher levels in the inner part than in the outer part. Total alcohols in the outer part of 12-day-old cheeses made from milk inoculated with *Ps. rhodesiae*, the strongest alcohol producer, were 1.6-fold higher than in the inner part and 2.4-fold higher than in the outer part of 6-day-old cheese made with this strain. No significant differences in the production of total alcohols between the two *Ps. libanensis* strains were recorded.

Levels of alcohols in our cheeses were not related to the levels of the respective esters. Thus, no significant production of ethanol was observed from 2 h curds to 6-day-old or 12-day-old cheeses (data not shown), whereas three ethyl esters were present at higher levels in cheeses than in 2 h curds.

Hydrocarbons. Hydrocarbons are common components of the volatile fraction of cheeses (*31*). Higher levels of hydrocarbons (**Table 6**) were observed in cheeses made with the *Pseudomonas* species studied in the present work, with the only exception of *Ps. graminis*, than in cheeses made with *Ps. fragi*, which showed a mean abundance of 73 in the outer part after 12 days of ripening (*17*). Undecene was the most abundant hydrocarbon produced in cheese by *Pseudomonas* strains, mostly in those cheeses made with *Ps. libanensis*, *Ps. rhodesiae*, *Ps. lundensis*, and *Ps. brenneri*. This particular hydrocarbon was produced abundantly in laboratory-scale cheeses (abundance = 223) by only one of five *Ps. fragi* strains (*17*), and its production by *Ps. fluorescens* and *Ps. putida* on solid culture media had been previously reported (*32*). Other microorganisms of dairy origin, such as Enterobacteriaceae or *L. lactis*, did not show any ability to produce undecene (*22*, *23*). Higher levels of cyclohexane in the inner than in the outer part of 12-day-old cheeses made with *Ps. rhodesiae*, *Ps. putida*, and *Ps. lundensis* were recorded. There were significant differences in the levels

Table 5. Mean Levels[a] of Alcohols in 12-Day-Old Cheeses (Outer and Inner Samples) Made from Milk Inoculated Separately with Seven *Pseudomonas* Strains and in Noninoculated 2 h Curds[b]

compound	ID[c]	sample	*Ps. brenneri*	*Ps. graminis*	*Ps. libanensis*	*Ps. lundensis*	*Ps. putida*	*Ps. rhodesiae*	2 h curds
2-propanol	ST	outer	48.06ab	111.32c	25.97a	68.77b	21.22a	31.56a	35.10
		inner	31.34a	132.12c	26.55a	79.10b	19.48a	21.73a	
2-pentanol	ST	outer	37.56a	0.91a	3.42a	214.57b	0.00a	71.81a	0.00
		inner	5.22a	0.00a	0.27a	164.68b	0.00a	7.79a	
2-heptanol	ST	outer	10.66ab	0.21a	0.80a	25.02c	0.00a	12.65b	0.04
		inner	0.60a	0.24a	0.04a	3.33b	0.00a	0.33a	
1-propanol	ST	outer	3.65d	0.00a	2.15c	0.79ab	0.71ab	1.59bc	0.61
		inner	1.22b	0.33a	0.76ab	0.61ab	0.51ab	1.07ab	
1-pentanol	ST	outer	0.00a	0.34a	0.52a	4.11c	3.43c	1.73b	1.80
		inner	1.01a	0.92a	1.65ab	3.81c	3.52c	3.10bc	
1-hexanol	ST	outer	1.14ab	0.00a	0.39a	1.94b	0.89ab	0.39a	0.04
		inner	0.34ab	0.03a	0.35ab	1.09bc	0.63ab	1.63c	
1-octanol	ST	outer	0.02a	0.08a	0.20a	0.37a	0.34a	0.29a	0.13
		inner	0.00a	0.02a	0.20a	0.37a	0.38a	0.84b	
2-methyl-2-propanol	ST	outer	1.31ab	1.21a	2.33abc	4.21c	4.12bc	3.85abc	1.54
		inner	1.33a	0.96a	3.13ab	5.15b	4.43b	4.64b	
2-methyl-1-propanol	ST	outer	5.86a	1.96a	21.77b	7.27a	3.24a	43.79c	1.06
		inner	4.71ab	1.05a	9.08b	5.65ab	3.05a	31.51c	
3-methyl-1-butanol	ST	outer	75.60ab	1.59a	264.30b	3.16a	6.78a	308.82b	0.29
		inner	52.28a	2.79a	82.07a	1.90a	2.68a	248.15b	
3-methyl-2-pentanol	MS	outer	3.57a	0.00a	2.84a	0.00a	0.00a	12.59b	0.04
		inner	0.54a	0.00a	0.03a	0.00a	0.00a	0.60a	
3-methyl-3-buten-1-ol	MS	outer	5.30bc	0.00a	8.17c	6.66bc	4.30b	6.56bc	0.00
		inner	7.07cd	0.00a	7.99d	4.94ab	3.27b	12.36e	
3-methyl-2-buten-1-ol	MS	outer	2.44a	0.00a	5.66b	1.22a	1.03a	1.94a	0.00
		inner	3.54b	0.00a	6.48c	1.15a	0.86a	6.50c	
2-ethyl-1-hexanol	ST	outer	288.49b	7.33a	254.50ab	8.00a	15.06a	192.39ab	3.56
		inner	62.47b	1.72a	45.81ab	4.00a	7.95a	82.45b	
cyclohexanol	MS	outer	1.82bc	3.02c	2.62c	0.22ab	0.00a	1.78bc	0.06
		inner	0.21a	0.32a	0.39a	0.00a	0.00a	0.24a	
total alcohols		outer	485.50bc	127.98ab	595.63c	346.31abc	61.12a	691.76c	46.28
		inner	171.88bc	140.51ab	184.78bc	275.79c	46.75a	422.92d	

[a] Areas of volatile compounds corrected by the internal standard. [b] Means in the same row followed by the same letter are not significantly different ($P > 0.05$). One strain per species except *Ps. libanensis*, with two strains. [c] Compound identification: ST, authentic standard injection; MS, tentatively identified by spectra comparison using Wiley 275 Library.

of total hydrocarbons produced by the two strains belonging to *Ps. libanensis*, which was the strongest producing species. On average, the two *Ps. libanensis* strains produced in the outer part of 12-day-old cheeses levels of total hydrocarbons 24-fold higher than in the inner part and 2.3-fold higher than in the outer part of 6-day-old cheeses. Hydrocarbons are not supposed to be key odorants in cheese (*18*).

Benzene Compounds. A significant production of some benzene compounds was observed for some of the assayed *Pseudomonas* species (**Table 6**). However, because these compounds were already present in curds, and we are dealing with ubiquitous substances, a contamination from air, water, or tools cannot be dismissed. Levels of benzene compounds were generally higher in the outer than in the inner part of 12-day-old cheeses, which would be in agreement with *Pseudomonas* aerobic metabolism. *Ps. putida* seemed to be the strongest producer of benzene compounds. There were significant differences between the two *Ps. libanensis* strains for total benzene compounds in the inner and outer parts of 12-day-old cheeses. The benzene compounds identified in this study have not been reported as odor-active compounds in cheeses (*18*).

Sulfur Compounds. A great diversity of sulfur compounds, most of which were not found in control curds, were produced in cheeses made with the seven *Pseudomonas* strains investigated in the present work (**Table 7**). *Ps. libanensis*, *Ps. brenneri*, and *Ps. rhodesiae* were the strongest producers, the levels reached by these species being higher than in cheeses made with *Ps. fragi* strains, in which abundances were below 20 (*17*). Dimethyl sulfide, the major sulfur compound found in the present work, was present at high levels in the headspace of refrigerated milk (*20*) and also in cheeses made from milk inoculated with Enterobacteriaceae strains from dairy origin (*22*). *Ps. libanensis* was the species producing the highest levels of total sulfur compounds. There were significant differences in total sulfur compounds between the two *Ps. libanensis* strains for the inner and outer parts of 12-day-old cheeses. Mean levels of total sulfur compounds were 1.7-fold higher in the outer part of 12-day-old cheeses made with the two *Ps. libanensis* strains than in the inner part and 4.8-fold higher than in the outer part of the respective 6-day-old cheeses.

Discriminant Analysis. The complexity of the volatile profiles obtained makes it necessary to apply a multivariate statistical analysis, such as discriminant analysis, to reduce the amount of variables and to help interpreting the results. T**able 8** lists the standardized discriminant function coefficients separately calculated for the inner and outer parts of the cheeses, with *Pseudomonas* species as the grouping variable. One hundred percent of the experimental cheeses were correctly classified for the *Pseudomonas* species. Volatile compounds used in the classification of the inner and outer samples were not coincident. For the outer part of the cheeses function 1 explained 52.3% of the variance. The production of high amounts of esters by *Ps. rhodesiae* determined the position of the cheese samples at the right extreme of the plane, whereas the active production of branched-chain alcohols by *Ps. libanensis* moved these samples to the central upper part (**Figure 1A**). Function 2 explained 32.1% of the variance, and together with function 1 determined the positions of *Ps. lundensis*, *Ps.*

Table 6. Mean Levels[a] of Hydrocarbons and Benzene Compounds in 12-Day-Old Cheeses (Outer and Inner Samples) Made from Milk Inoculated Separately with Seven *Pseudomonas* Strains and in Noninoculated 2 h Curds[b]

compound	ID[c]	sample	*Ps. brenneri*	*Ps. graminis*	*Ps. libanensis*	*Ps. lundensis*	*Ps.putida*	*Ps. rhodesiae*	2 h curds
1-pentene	MS	outer	17.03b	0.00a	0.00a	0.09a	0.00a	0.12a	0.79
		inner	0.00a	1.11a	0.11a	0.09a	0.29a	0.17a	
1-heptene	MS	outer	16.33b	0.19a	0.23a	0.38a	0.28a	0.64a	2.35
		inner	0.00a	1.07a	0.00a	0.24a	0.74a	0.64a	
octane	ST	outer	5.55a	4.82a	5.66a	4.02a	5.68a	10.90b	4.78
		inner	2.57a	3.24ab	4.43ab	4.73ab	6.37bc	9.16c	
1-nonene	MS	outer	9.14c	0.00a	4.63abc	8.36c	2.01ab	6.15bc	0.26
		inner	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	
1-undecene	MS	outer	154.41bc	0.23a	287.88d	231.70cd	72.37ab	246.39cd	0.00
		inner	1.80a	0.00a	4.28a	4.27a	0.69a	1.00a	
cyclohexane	MS	outer	4.90a	0.91a	14.84a	13.79a	10.68a	15.73a	0.10
		inner	2.34a	1.47a	4.71ab	16.79ab	26.04b	19.54ab	
cycloundecene	MS	outer	8.52b	0.00a	11.04b	5.70ab	4.09ab	10.68b	0.00
		inner	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	
total hydrocarbons		outer	215.89bc	6.15a	324.28c	264.04c	95.11ab	290.60c	8.28
		inner	6.71a	6.89a	13.52ab	26.12ab	34.14b	30.52ab	
ethylbenzene	MS	outer	1.28a	1.23a	3.66ab	5.15bc	6.82c	5.91bc	3.49
		inner	0.38a	1.33a	3.54a	3.20a	4.18a	2.54a	
o-xylene	MS	outer	3.03a	2.90a	8.87ab	13.98cd	18.42d	15.63cd	9.25
		inner	1.06a	3.60a	10.38a	8.97a	11.50a	7.25a	
p-xylene	MS	outer	0.71a	0.72a	2.21ab	3.37bc	4.68c	3.88bc	1.93
		inner	0.21a	0.71a	1.96a	2.14a	2.69a	1.94a	
styrene	MS	outer	3.95b	2.19a	2.73ab	2.35a	3.11ab	3.14ab	0.65
		inner	0.43ab	1.00b	0.16a	0.36ab	0.77ab	0.59ab	
trimethylbenzene	MS	outer	0.61a	0.78a	2.52ab	4.65bc	5.28c	4.71bc	2.66
		inner	0.26a	0.57a	6.88a	2.81a	3.44a	2.71a	
o-dichlorobenzene	MS	outer	1.93a	2.63a	3.82a	5.63a	5.54a	5.56a	1.39
		inner	0.61a	0.96a	0.56a	3.69a	4.12a	3.62a	
total benzene compounds		outer	11.52a	10.45a	23.81ab	35.06ab	43.79b	38.83b	19.38
		inner	2.96a	8.16ab	23.48ab	21.16ab	26.63b	18.66ab	

[a] Areas of volatile compounds corrected by the internal standard. [b] Means in the same row followed by the same letter are not significantly different ($P > 0.05$). One strain per species except *Ps. libanensis*, with two strains. [c] Compound identification: ST, authentic standard injection; MS, tentatively identified by spectra comparison using Wiley 275 Library.

Table 7. Mean Levels[a] of Sulfur Compounds in 12-Day-Old Cheeses (Outer and Inner Samples) Made from Milk Inoculated Separately with Seven *Pseudomonas* Strains and in Noninoculated 2 h Curds[b]

compound	ID[c]	sample	*Ps. brenneri*	*Ps. graminis*	*Ps. libanensis*	*Ps. lundensis*	*Ps. putida*	*Ps. rhodesiae*	2 h curds
carbon disulfide	MS	outer	7.76a	16.55b	5.58a	4.08a	3.38a	4.80a	2.72
		inner	6.47a	7.13a	7.26a	3.33a	2.93a	4.52a	
dimethyl sulfide	MS	outer	1.85a	4.09a	12.02a	6.75a	2.64a	4.75a	1.70
		inner	2.71ab	1.38a	8.64b	3.73ab	1.07a	2.94ab	
dimethyl disulfide	ST	outer	44.69b	2.61a	42.64b	1.05a	2.39a	19.90ab	0.40
		inner	12.73bc	1.49ab	21.29c	0.72a	1.68ab	8.86ab	
dimethyl trisulfide	MS	outer	3.98a	0.00a	3.91a	0.00a	0.00a	4.87a	0.00
		inner	0.00a	0.00a	0.36a	0.00a	0.00a	0.00a	
methanethiol	MS	outer	0.00a	0.00a	10.26b	0.00a	0.00a	1.97ab	0.00
		inner	0.00a	0.00a	4.55b	0.00a	0.00a	0.00a	
methyl thiol acetate	MS	outer	15.65ab	0.00a	32.98b	0.00a	0.00a	13.23ab	0.00
		inner	12.17ab	0.00a	26.72b	0.00a	0.00a	8.07ab	
methyl thiopropionate	MS	outer	5.33a	0.00a	8.64a	0.00a	0.00a	9.84a	0.00
		inner	0.40a	0.00a	2.01a	0.00a	0.00a	1.37a	
methyl thiobutanoate	MS	outer	2.71b	0.00a	1.20ab	0.00a	0.00a	0.69a	0.00
		inner	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	
methyl 3-methylbutane-thioate	MS	outer	1.81a	0.00a	2.98a	0.00a	0.00a	0.25a	0.00
		inner	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	
methyl thiocyanate	MS	outer	0.00a	1.57a	7.32b	1.68a	7.33b	3.17a	0.00
		inner	0.32a	0.78ab	4.50b	2.07ab	1.42ab	0.55a	
total sulfur compunds		outer	83.79ab	24.81ab	127.53b	13.56a	15.73ab	63.48ab	4.82
		inner	34.79ab	10.77a	75.32b	9.85a	7.10a	26.32ab	

[a] Areas of volatile compounds corrected by the internal standard. [b] Means in the same row followed by the same letter are not significantly different ($P > 0.05$). One strain per species except *Ps. libanensis*, with two strains. [c] Compound identification: ST, authentic standard injection; MS, tentatively identified by spectra comparison using Wiley 275 Library.

brenneri, *Ps. graminis*, and *Ps. putida*, the three latter species being close to each other. The higher production of 1-pentanol and 2-methyl ketones and the low production of esters and branched-chain alcohols by *Ps. lundensis* determined the position of these cheeses at the left lower extreme of the plane.

For the inner part of the cheeses the classification was also precise (**Figure 1B**). Function 1 explained as much as 82.3% of the variance and shifted *Ps. lundensis* cheeses to the left and *Ps. libanensis* cheeses to the right side of the plane due to the active production of methyl ketones and methanethiol, respec-

Table 8. Standardized Discriminant Function Coefficients for the Production of Volatile Compounds in Cheeses, with *Pseudomonas* Species as the Grouping Variable

	function	
	1	2
outer part		
variance (%)	52.3	32.1
methyl hexanoate	5.70	–1.85
3-methyl-2-pentanol	–4.46	3.18
3-methyl-2-buten-1-ol	3.67	1.48
3-methylbutanol	2.29	1.90
ethyl butanoate	1.50	–0.76
1-heptene	–1.00	0.04
1-pentanol	–0.70	–0.66
2-propanol	–0.50	–0.18
2-nonanone	–0.52	–0.84
2-pentanone	0.00	–0.15
inner part		
variance (%)	82.3	12.6
2-pentanone	–11.33	7.85
methyl thiol acetate	7.95	0.66
3-methyl-2-buten-1-ol	7.91	3.69
2-pentanol	8.35	–0.74
2-methylbutanal	3.01	1.06
methanethiol	2.91	1.00
dimethyl trisulfide	–2.72	–1.32
2-methylpropanol	–1.65	–1.18
1-pentanol	1.36	0.50
3-methyl-2-butanone	–0.29	–3.86
2-heptanol	2.94	–3.78
2-methyl-2-propanol	–0.91	–1.56
2-propanol	–0.30	–0.71

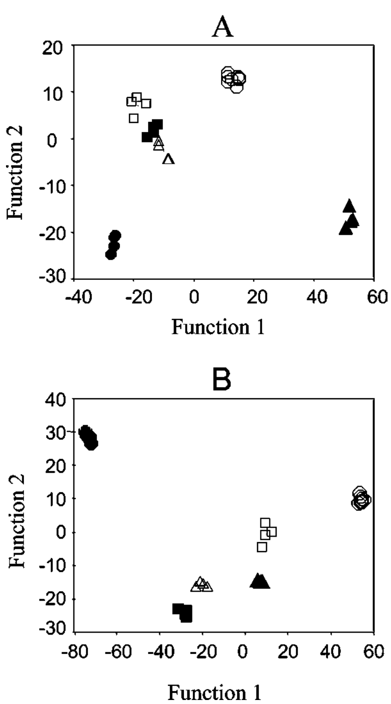

Figure 1. Outer (**A**) and inner (**B**) parts of cheeses, respectively, plotted as distribution using the two canonical discriminant functions. Cheeses were made from milk inoculated with *Ps. brenneri* 46 (□), *Ps. graminis* 1 (■), *Ps. libanensis* 23 and 35 (○), *Ps. lundensis* 48 (●), *Ps. putida* 32 (△), and *Ps. rhodesiae* 24 (▲) strains.

tively, in those cheeses. Function 2, explaining 12.6% of the variance, determined the separation of *Ps. brenneri* from *Ps. rhodesiae* samples and of *Ps. graminis* from *Ps. putida* samples.

Discriminant analysis can help in the imaging of the volatile profile produced by strains belonging to different *Pseudomonas* species. As the volatile patterns of the six studied species were very different from each other, it was possible to classify 100% of the experimental cheeses by only two discriminant functions, which were combinations of a low number of volatile compounds. *Ps. rhodesiae* and *Ps. lundensis* were active producers of volatile compounds of different nature, whereas *Ps. libanensis* was a strong producer of sulfur compounds, and this made them easily classifiable by the discriminant functions, separating them from the other three species, which were much less active in the production of volatile compounds.

It may be concluded from the results obtained in the present work that strains belonging to different species of the genus *Pseudomonas* are capable of survival, growth, and production of a large variety of volatile compounds during cheese ripening and that these abilities are species-dependent. Therefore, the presence of *Pseudomonas* in milk might affect negatively the sensory characteristics of cheese, even though most groups of volatile compounds are found at higher levels in the outer part than in the edible inner part of cheeses. Lowering of *Pseudomonas* counts in raw milk, particularly if there is no heat treatment prior to cheese manufacture, is crucial to prevent the appearance of undesirable off-flavors during ripening.

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