Kluyveromyces lactis but Not *Pichia fermentans* Used as Adjunct Culture Modifies the Olfactory Profiles of Cantalet Cheese

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ABSTRACT

Yeasts are commonly detected in cheese. Two yeast species, *Kluyveromyces lactis* and *Pichia fermentans*, were isolated at high populations from raw-milk Cantal cheese, a French Protected Denomination of Origin hard cheese. To investigate the interest of these 2 species as adjunct cultures to promote flavor development of Cantalet cheese, they were added at 10^5 cfu/mL to microfiltered milk. The global microbiological, biochemical, and flavor changes induced by the presence of the yeasts in cheese were determined. Adjunct yeasts were present at 10^6 cfu/g in curd, declined to 10^4 to 10^5 cfu/g in cheese, and did not influence gross composition, content of free amino acids, or content of free fatty acids. By using 8-way gas chromatography-olfactometry in parallel with gas chromatography-mass spectrometry, 30 odorous compounds of Cantalet cheese were identified. The olfactory profiles of *K. lactis* cheeses contained significantly greater levels of 8 odorous compounds (ethanol, ethyl hexanoate, 4 aldehydes, and 2 branched-chain acids) compared with the control and *P. fermentans* cheeses. Sensory analysis of cheeses flavor discriminated *K. lactis* cheeses on only 2 attributes (acetaldehyde and alcohol odors). This study shows that yeast contribution is species-specific and that *K. lactis*, at a population of 10^6 viable cells/g, can influence Cantalet cheese flavor.

Key words: *Kluyveromyces lactis*, Cantalet cheese, adjunct culture, odorous compound

INTRODUCTION

Yeasts are frequently detected within the microflora of various cheese types (Fleet, 1990), which can be attributed to their ability to grow at low temperatures, to assimilate and ferment lactose, assimilate organic acids such as succinic, lactic, and citric acids, and to resist high salt concentrations and cleaner products and sanitizers

(Viljoen, 2001). Yeasts are common inhabitants of the surface of smear-ripened cheeses, where they grow during the initial stages of ripening, deacidifying the cheese surface, which allows the subsequent development of surface bacteria (Lenoir, 1984). As a result, they are used as adjunct cultures mainly in surface-ripened cheeses (Chamba and Irlinger, 2004). Yeasts can also be detected at levels as high as 10^7 to 10^8 cfu/g in the interior of cheese, where many species have been identified, the most commonly found being *Debaryomyces hansenii*, *Kluyveromyces lactis*, *Kluyveromyces marxianus*, *Pichia membranaefaciens*, *Pichia fermentans*, *Saccharomyces cerevisiae*, *Yarrowia lipolytica*, and *Geotrichum candidum* (Fleet, 1990; Chamba and Irlinger, 2004).

In raw-milk Cantal cheese, a French Protected Denomination of Origin hard cheese that is manufactured in a similar way to Cheddar cheese, the population of yeasts enumerated reached $9 \log_{10}$ cfu/g of curd (I. De Freitas and N. Pinon; unpublished data). *Kluyveromyces lactis* and *P. fermentans* were the 2 main species identified, accounting for 80 and 10% of the isolates identified, respectively (I. De Freitas and N. Pinon; unpublished data). Earlier studies showed that most of the lactose-fermenting yeasts isolated from Cantal cheese were identified as *K. lactis* (formerly *Saccharomyces lactis* and *Torulopsis sphaerica*), and the non lactose-fermenting yeast species being identified as *Pichia* spp. and *Rhodotorula* spp. (Millet et al., 1974).

Yeasts can play a role in the ripening process, either directly or via their interactions with the other cheese microorganisms. The most studied species are *D. hansenii*, *K. lactis*, *Y. lipolytica*, *S. cerevisiae*, and *G. candidum*. Yeasts can either positively contribute to the formation of cheese flavor, or be responsible for yeasty flavors and other off-flavors (Jakobsen and Narvhus, 1996). Other defects caused by the presence of spoilage yeasts are gas production, discoloration, and changes of texture. *Kluyveromyces lactis* is known for its ability to assimilate lactose and its enzymatic potential such as aminopeptidase activity (Lenoir, 1984). The interactions between bacteria and yeasts may result in the stimulation or the inhibition of the growth of one or both popula-

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tions (Viljoen, 2006). Yeasts, however, are considered to stimulate the growth of lactic acid bacteria, via the synthesis of growth factors such as vitamins (Addis et al., 2001; Viljoen, 2006).

Yeasts are able to produce a variety of volatile odorous compounds in dairy products such as esters (mainly ethyl acetate), volatile organic sulfur compounds derived from the conversion of Met, various compounds of the catabolism of aromatic and branched-chain amino acids, and methyl-ketones (Spinnler et al., 2001; Arfi et al., 2004). As a result, yeasts have the potential to contribute to the formation of cheese flavor (Lenoir, 1984). The potential of *Pichia* spp. to produce volatiles in cheese has not been studied.

The present work aimed to determine the interest of 2 yeasts, *K. lactis* and *P. fermentans*, isolated from the dominant yeast population of a typical raw milk Cantal cheese, as adjunct cultures to promote the formation of flavor of Cantalet cheese. We characterized the global microbiological, biochemical, and sensory changes induced by the presence of yeasts in microfiltered milk Cantalet cheese, with a particular focus on the formation of odorous compounds, determined by using a recently developed multi gas chromatography-olfactometry (GC-O) device (Berdaqué et al., 2007).

MATERIALS AND METHODS

Cheese Manufacture and Sampling

Cantalet cheese is a small Cantal cheese, with a weight of 8 to 10 kg and a cylindrical shape (20 to 22 cm in diameter $\times$ approximately 20 cm high). The manufacturing process is close to that of Cantal cheese (De Freitas et al., 2007). Six Cantalet cheeses, 2 experimental and 1 control repeated twice on 2 consecutive days, were manufactured from microfiltered skim milk and pasteurized cream. Skimmed milk (~350 L) and pasteurized (80°C for 20 s) cream (~40 L) were refrigerated and carried at 4°C to the manufacture plant. Skimmed milk was then heated at 40°C and microfiltered by microfiltration. The pilot installation Bactocatch type (MFS1, Alfa Laval, Les-Clayes-sous-Bois, France) was equipped Sterilox ceramic membrane with a porosity gradient of mean diameter 1.4 μm (1P1940, length 1.02 m, filtration area of 0.24 m^2 , Société des Céramiques Techniques, Tarbes, France). Pasteurized cream was added to the microfiltration permeate to adjust the fat content to 3.3%, constituting cheese milk. The milk was then divided into 3 vats of 100 L each and heated to 31°C. Lactic starters and yeast cultures were then added. Calf rennet (33 mL/100 L of milk) was added 10 min later. Coagulation took around 25 min, and the curd was cut for 10 min. The mixture of curd and whey was then directed toward a pressing vat in which whey drainage and press-

ing took place at room temperature. The curd was turned and pressed 6 times in pressure cycles with an increase from 1.8×10^5 to 6.0×10^5 Pa for 60 min. Then, the pressed curd was matured overnight at 17°C to a pH of about 5.1 to 5.2. The matured curd was then ground and mixed with 2.0% of dry salt. One hour later, the salted curd was molded and pressed for 48 h (pressure was increased from 0.9×10^5 to 5.0×10^5 Pa) at 16°C. After removal from the molds, the cheeses were ripened under controlled conditions (10°C and 95% relative humidity).

The control cheeses were made by adding only commercial lactic starters (F-DVS DCC-260 and CHN19, Chr. Hansen, St-Germain Les Arpajon, France), to obtain a global population of 10^5 cfu/mL in cheese milk. Starters contained lactococci, leuconostocs, *Streptococcus thermophilus*, *Lactobacillus helveticus*, and *Lactobacillus casei*. The 2 experimental cheeses were made by adding the same lactic starters and the yeasts *K. lactis* F41 no. 1 (**KI**) or *P. fermentans* F31 no.2 (**PI**) (2 strains isolated from raw milk Cantal cheese, from LFO, Villefranche de Lauragais, France) inoculated at 10^5 cfu/mL in cheese milk. For each cheese trial, 5 samples were analyzed: microfiltered milk and cream mixture (**T₀**), curd after maturation (**T_{14h}**), cheese after demolding (**T_{3d}**), and cheeses after 45 and 95 d of ripening. Samples were stored at -20°C before analyses, except those for volatile compound and FFA analyses, for which samples were kept at -80°C. Preliminary results obtained by enumerating yeasts in the same Cantalet cheese sample either freshly sampled or after being stored for 15 d at -20°C showed that counts did not differ by more than 0.5 log₁₀ cfu/g.

Microbiological Analyses

Microbial Counts. Cheese samples were prepared as previously described (De Freitas et al., 2005). Mesophilic aerobic microflora were enumerated on PCA (Biokar Diagnostics, Beauvais, France), lactococci on M17 agar (Difco, BD Diagnostics, Pont de Claix, France) incubated for 7 d at 15°C, thermophilic streptococci on M17 agar (Difco) incubated for 1 d at 43°C, thermophilic lactobacilli on de Man, Rogosa, and Sharpe, pH 5.4, incubated for 2 d at 43°C under anaerobiosis, and facultative heterofermentative lactobacilli on FH agar, incubated for 3 d at 37°C under anaerobiosis, as previously described (De Freitas et al., 2005). The use of M17 at 15°C for lactococci enumeration was adapted from the IDF Standard to improve the selective recovery of lactococci from mixed lactic acid bacteria populations, because it is known that M17 is poorly selective. Yeasts were enumerated on oxytetracycline glucose agar (Biokar Diagnostics) after incubation for 5 d at 24°C. Mean values resulting from duplicate plates are given on the log₁₀ basis.

Compositional Analyses

Sampling. For all analyses, an inner cheese sample was grated, thoroughly mixed, and used directly for pH, TS content, and fat determinations. pH was measured by using a pH meter (HI 9025, Hanna Instruments, Vila do Conde, Portugal); TS was determined in triplicate by drying cheese samples (2 g) mixed with sand at 102°C for 7 h (FIL-IDF, 1987b); fat was determined in duplicate by the gravimetric method (FIL-IDF, 1987a). Thirty grams of grated cheese was homogenized with 60 g of distilled water by mixing in an ice bath for 2×1 min at 20,500 rpm using an Ultraturrax blender (Janke & Kunkel, Staufen, Germany). The homogenates were frozen at -20°C before analysis of acidity, ethanol, and lactose. Lactose contents were determined by an enzymatic method through the use of Boehringer kits (Ref. 10176303, R-Biopharm, Darmstadt, Germany).

Free AA. Individual free AA (FAA) were determined in duplicate from a homogenate of 5 g of grated cheese with 10 g of lithium citrate buffer, 0.5 M, pH 7.0. An aliquot of the homogenate was deproteinized by addition of a 10% sulfosalicylic acid solution (1:4, wt/wt), incubated for 1 h at 4°C, and centrifuged at $1,000 \times g$ at 4°C for 15 min. The supernatant was filtered on a 0.2- μ m pore diameter membrane, and diluted (1:4, vol/vol) with lithium citrate buffer, 0.2 M, pH 2.2. Amino acids were analyzed using an amino acid analyzer (AlphaPlus series 2, Pharmacia, Uppsala, Sweden).

Individual FFA. Free fatty acid contents were determined at 95 d of ripening after extraction according to the method of De Jong and Badings (1990). Two independent extractions of cheese lipids were performed using aminopropyl solid-phase extraction columns (500 mg/3 mL; Phenomenex, Torrance, CA) and a vacuum manifold (Macherey Nagel, 52313 Duren, Germany). Individual FFA (from C2:0 to C18:3) were quantified by GC using a Varian 3800 gas chromatograph with flame-ionization detection, a Varian 1079 universal capillary injector, a Varian 8410 liquid auto sampler, and Varian Star operating software (Varian Analytical Instruments, Harbor City, CA). A fused-silica semicapillary column BP21 (SGE Europe, Courtaboeuf, France; 25 m $\times$ 0.53 mm $\times$ 0.5 μ m film thickness) was used. Direct cold on-column injection took place at 65°C, the oven temperature increased from 65°C to 240°C at a rate of 10°C/min, and was then held at 240°C for 20 min. The flame-ionization detection temperature was 250°C. The flow rate of the carrier gas (hydrogen) was 9.7 mL/min at 65°C.

Quantification of Volatile Compounds. Twenty-one neutral volatile compounds were preselected on the basis of 1) previous analyses of volatile compounds in Cantal cheese (De Freitas et al., 2007) and 2) volatile compounds that were reported to be produced in cheese

by yeasts, such as products from the catabolism of methionine and of branched-chain AA, ethanol, and ethyl esters (Spinnler et al., 2001; Arfi et al., 2004; Leclercq-Perlat et al., 2004). Selected compounds included 6 alcohols (ethanol, propanol, 2-butanol, 2-methyl-1-propanol, 3-methyl-butanol, and 2-methyl-butanol), 7 ketones (2,3-butanedione, 2-butanone, 2-pentanone, 2,3-pentanedione, 3-methyl-2-pentanone, 2-heptanone, and 2-nonanone), 5 esters (ethyl acetate, ethyl butanoate, ethyl hexanoate, ethyl octanoate, and 3-methylbutyl acetate), 2 aldehydes (ethanal and 3-methyl-butanal), and 1 sulfur compound (dimethyldisulfide). These selected neutral volatile compounds were extracted, identified, and quantified using a first dynamic headspace (DH)-GC-MS method. The DH-GC-MS equipment comprised a Tekmar 3000 headspace device (Tekmar Inc., Cincinnati, OH) coupled to a GC HP5890A (Agilent Technologies, Massy, France) and MS HP 7972A (Agilent Technologies). A 20-g sample was homogenized with 80 g of refrigerated solution of boiled distilled water by mixing for 2 min at 20,500 rpm using an Ultraturrax blender. A 7-g sample of this cheese homogenate (± 0.05 g) was used for each headspace GC-MS analysis, performed in duplicate. Samples were purged with ultrapure helium gas (35 mL/min) at 65°C for 15 min to isolate headspace volatiles, which were adsorbed on a Vocab 3000 trap (Supelco, Bellefonte, PA). The trapped compounds were thermally desorbed at 250°C over 4 min and cryofocused at -100°C before being injected by heating at 270°C. They were separated on an Agilent Technologies HP5 capillary column (60 m $\times$ 0.32 mm $\times$ 1.0 μ m film thickness) as previously described (Thierry et al., 2005b). Peaks were quantified by the areas of the total ion current (TIC). To avoid the approximation related to the commonly used internal standard calibration, volatiles were quantified (ng/g) using an external calibration method based on the addition of standards to the cheese homogenate (Thierry et al., 2005b). Volatiles were identified by comparison of mass spectra and retention times with those of authentic standards purchased from Sigma-Aldrich (St. Quentin Fallavier, France). Ethanol was detected by DH-GC-MS, but it was quantified by an enzymatic method through the use of Boehringer kits (Ref. 10176290).

Determination of Odorous Compounds

Extraction of the Volatile Compounds. For 8-way GC-olfactometry (8W-GC-O) analyses, volatile compounds were extracted using a second DH-GC method. This DH-GC method was used in parallel to identify the candidate chemical entities to account for the odorous zones previously detected by 8W-GC-O, by using an independent single GC-O/MS set-up. A 3-g aliquot of grated

cheese was placed on glass wool at the bottom of a Pyrex extractor (M3, Maillères, Aubière, France) of a Tekmar headspace device (Tekmar Inc.). The extractor was maintained at ambient temperature and purged for 60 min with a helium stream at a flow rate of 60 mL/min (Air Liquide, He/U purity: 99.995%). The trap (Tenax TA 60/80, Supelco) was operated at 30°C. The dry purge step was set at 3 min. The volatile components were then desorbed from the trap at 180°C for 10 min using helium (Air Liquide, He/N55 purity: 99.9995%) and cryofocused at -150°C before splitless injection at 220°C.

8W-GC-O. The volatile components were separated on an RTX-5 capillary column (60 m $\times$ 0.53 mm $\times$ 1.5 μ m film thickness, Restek, Evry, France). The chromatograph oven temperature was programmed as follows: 5 min isothermal at 40°C, 4°C/min increase to 205°C, and 5 min isothermal at 205°C. The duration of the GC-O analyses was 2,100 s; the helium flow rate was 8 mL/min. The 8W-GC-O device previously described in detail was designed to distribute the volatile components separated by chromatography to 8 sniffing ports synchronously and evenly (Berdagué et al., 2007). Thus, 8 sniffers can detect the substances eluted in a single chromatographic separation simultaneously and under identical conditions, thereby improving the quality of the data obtained. The compounds separated by GC (Hewlett Packard 4890D, Agilent Technologies) were transferred via a transfer line heated at 210°C from the end of the capillary column to the effluent divider of the 8W-GC-O device. The total olfactory signal (**TOS**), corresponding to the overall response of the sniffer panel, was expressed by the sum of the intensities of each individual aromagram from sniffer 1 to sniffer 8 against time. To study the information in the vocabulary items, the VIDEO-Sniff (Berdagué and Tournayre, 2004) method was used, which breaks up the TOS into olfactory classes. The deconvolution of the olfactory signal to classes facilitates a consensual description of the odor of the olfactory peaks.

Identification of the Odorous Compounds. To identify the candidate chemical entities to account for odorous zones, an independent single DH-GC-O/MS set-up composed of a chromatograph (GC 6890, Agilent Technologies), a mass detector (MSD 5973 Inert, Agilent Technologies), and a homemade sniffing port was used. The split between sniffing port and MS was 1:1. The purge and trap and the chromatographic conditions were the same as those used for 8W-GC-O, except for the capillary column used (RTX-5, 60 m $\times$ 0.32 mm $\times$ 1.0 μ m film thickness, Restek) and the helium flow rate (2 mL/min). The volatile components were ionized by electronic impact at 70 eV. Ions were scanned over the range 18 < *m/z* < 220 amu and identified using databases of spectra (NIST/EPA/NIH 2005 V2.0d <http://www.nist.gov/srd/>

nist1a.htm; Masslib 1999, MSP Köfel, Koeniz, Switzerland), relative retention indices, and odors (Flavor-Base 2004, Leffingwell and Associates, Canton, GA; Flavornet 2006, www.flavornet.org; LRI & Odor Database 2006, www.odour.org.uk).

The computer coupling between the 8W-GC-O set-up and the independent GC-O/MS system made it possible to obtain an alignment that was sufficiently precise for candidate structures to be matched with olfactory peaks. This coupling was obtained by importing the aromagram of the TOS (in the *netCDF* exchange format) into the mass spectrometry software MSDChem Agilent D.01.02 (Agilent Technologies), to place the TOS in parallel with the TIC or with specific ions. Before export, the 8W-GC-O aromagram retention time was resynchronized with the independent GC-O/MS aromagram/chromatogram retention time using olfactory standards (occurring naturally in the mixture or added specifically). To localize the standards accurately by mass spectrometry, a search based on specific ions was necessary, because of the frequent occurrence of coelutions. The best alignment was calculated using a third-order polynomial regression model between the start of the odor-active peaks and the start of the mass spectrometry peaks.

Flavor Assessment

Descriptive Evaluation by Trained Panel. Sensory evaluations of ripened cheeses were performed by Les Maisons du Goût (Bourg-en-Bresse, France) as previously described (Thierry et al., 2005a). A panel of 18 highly experienced trained assessors, selected for their ability to memorize various odor and flavor attributes, participated in the sensory analysis. The cheeses were evaluated in duplicate by each assessor, at an interval of 2 d. On the day of assessment, the core cheese samples were cut into 30-g sticks, placed in disposable flasks, and allowed to warm to 15°C for 2 h before evaluation. Cheese odor (nasal perception) and flavor (defined as the global perception of aroma and taste when samples are put in the mouth) intensities were rated from 0 (none) to 10 (intense) by each panelist. Four attributes of taste (salty, sweet, acidic, and bitter) and 53 attributes of odor (nasal perception) and aroma (retronasal perception) were used to describe Cantalet cheeses. For each of these attributes, the assessors indicated whether the corresponding odor, aroma, and taste were perceived or not, allowing a frequency of perception (number of "presence" responses out of the total number of responses; i.e., 18 assessors $\times$ 2 replicates = 36) to be calculated and expressed as a percentage. The numbers of attributes cited to describe odor and flavor were also determined. This method of sensory evaluation was devised to show a maximum of odor and aroma attributes, whereas classi-

cal sensory evaluation by scaling the intensity of odor/aroma attributes considers only a limited number of attributes. This method proved useful to compare the impact of different starters or to show the sensory particularities of Protected Denomination of Origin cheeses (J. F. Clément, Les Maisons du Goût, Bourg-en-Bresse, France; personal communication). Data were collected and analyzed using FIZZ software (Biosystèmes, Couternon, France).

Statistical Analyses

Averaged concentration data of duplicate analyses of each compound were used for statistical analysis. One-way ANOVA were performed by using the GLM procedure of Statgraphics Plus (Statistical Graphic Corp., Englewood Cliffs, NJ) to determine the effect of the addition of yeasts on the concentration data of each compound. The Fisher's least significant difference (LSD) test was used to determine which means were significantly different at a 95% confidence level.

In the same way, the Fisher test was used to determine which of the olfactory zones of the 3 TOS corresponding to the 3 cheese treatments were significantly influenced by the addition of yeasts. An ANOVA was performed in each of the 2,100 scans (S_i) of the aromagrams within this model: $S_i = \mu + \text{yeast effect}_p + \text{judge effect}_j + \epsilon_{i,p,j}$ (with μ = average effect, $p = 3$, $j = 8$, and $n = 2$). The 2,100 ANOVA thus calculated made it possible to build an "ANOVAgram" to identify the zones of aromagrams that were most influenced by the treatment (here the addition of adjunct yeasts).

Sensory data were analyzed by using FIZZ software (Biosystèmes). The ability of odor and flavor intensity scores and richness to discriminate between cheeses was investigated using 1-way ANOVA. The Newman Keuls test was used to determine which means were significantly different at a 95% confidence level. Only odor/aroma attributes having a perception frequency over 10% for at least 1 cheese, were subjected to statistical analyses. Perception frequencies lower than 10% (less than 4 presence responses out of 36) can be considered as the noise of the method. The statistical significance of differences between the frequencies of this attribute in the 3 cheeses was investigated using a χ^2 test, by using STATITCF version 5 (ITCF, Paris, France).

RESULTS

Lactic Starters and Yeast Adjuncts

Figure 1 shows the changes occurring in the starter and adjunct populations. Total mesophilic bacteria counts were 2.9 and 2.7 $\log_{10}$ cfu/mL in pasteurized creams, and 1.1 and 1.4 $\log_{10}$ cfu/mL in microfiltered

milks on the 2 d of manufacture, respectively. Yeasts were not detected in microfiltered milks or in pasteurized creams. As expected, after addition of the starter and adjuncts, the population of yeasts was 5.11 ± 0.09 and $5.07 \pm 0.03 \log_{10}$ cfu/mL in Kl and Pf cheese milks, respectively, whereas yeasts were not detected in the control cheese milks. Yeasts counts were stable during manufacture (5.87 ± 0.04 and 5.80 ± 0.07 after maturation at T_{14h} , and 5.99 ± 0.19 and $5.94 \pm 0.08 \log_{10}$ cfu/g at T_{3d} in Kl and Pf cheeses, respectively). During the ripening period, yeast populations decreased to 4.1 and 4.7 $\log_{10}$ cfu/g in Kl and Pf cheeses at 45 d, respectively, and then increased to 5.4 and 5.0 $\log_{10}$ cfu/g at 95 d. All the colonies of yeasts on agar plates prepared from Kl and Pf cheese samples had the same morphotype, showed the same morphology under microscopic observation, and corresponded to those of *K. lactis* and *P. fermentans*, respectively. The dominant populations of yeasts in Kl and Pf cheeses thus represented the added yeast species throughout the ripening period. In contrast, colonies of different sizes, colors, and aspects were observed on the oxytetracycline glucose agar plates made from the control cheeses. These contaminating yeasts probably originated from the plant environment, and increased in cheese during acidification to reach 2.7 $\log_{10}$ cfu/g at T_{3d} . Thereafter, yeast counts of the control cheeses decreased to 1.8 $\log_{10}$ cfu/g at 45 d. Some significant differences of the populations of lactococci and thermophilic lactobacilli ($P = 0.02$ and $P = 0.01$, respectively) in the 3 cheeses were observed at 45 d of ripening (Figure 1). Hence, the cultivable populations of lactococci decreased by 81% in the control cheeses during the 45-d ripening period, vs. 16 and 57% decreases in Kl and Pf cheeses, respectively.

Compositional Attributes

The pH of matured curd (T_{14h}) was 5.16 ± 0.09 , 5.18 ± 0.01 , and 5.12 ± 0.04 in the control, Kl, and Pf cheeses, respectively, showing that the addition of yeasts did not modify the rate of acidification. In agreement, the addition of yeasts did not influence the concentrations of residual lactose at 3 d, which were similar to that found in 3-d-old Cantal cheese (De Freitas et al., 2007). Lactose concentrations were thus not further determined during the ripening. There were no significant differences of gross composition between the control and the 2 experimental Cantalet cheeses (Table 1), demonstrating that the addition of yeasts did not modify the characteristics of the cheese. All cheeses showed the same changes over the ripening: increase in TS and fat contents due to loss of moisture, and increase in pH, acidity, and FAA concentrations due to proteolysis (Table 1). Five individual FAA (Pro, Arg, Orn, Phe, and Leu) showed slight but significant differences of concentrations between the

Figure 1. Changes in the microbial populations of lactic starters and yeast adjuncts throughout the ripening in 3 Cantalet cheeses manufactured without (control) and with addition of yeasts (*Kluyveromyces lactis* and *Pichia fermentans*) as adjunct cultures.

control and the experimental cheeses at 45 d of ripening only. The control and Kl cheeses had 11 to 16% greater concentrations of Pro, Phe, and Leu than did Pf cheeses ($P < 0.05$). Arginine was at 3- to 5-fold greater concentrations in the control cheeses than in Kl and Pf cheeses ($P < 0.01$), and Orn was at a 14% greater concentration in Kl cheeses than in the other cheeses ($P = 0.02$; data not shown). The Cantalet cheese composition values were in the range of those of Cantal cheese, as expected (De Freitas et al., 2005).

Volatile Composition

Levels of FFA derived from lipolysis (C4:0 to C18:3) did not differ significantly in the control and the experimental cheeses (mean value 1.78 ± 0.07 g/kg at 95 d of ripening) and represented 0.6% of the fat content (Table 2). The 3 Cantalet cheeses also contained VFA derived from carbohydrate (acetic acid) and branched-chain AA (methylpropanoic and methylbutanoic acids) catabolism. Methyl-branched acid concentrations were about 2-fold

Table 1. Gross composition and free amino acids (FAA) for 3 Cantalet cheeses manufactured without (control) and with addition of yeasts (*Kluyveromyces lactis*, *Pichia fermentans*) as adjunct cultures in matured curd (3 d) and during ripening (45 and 95 d)

Item ¹	Cheese age, d	Control	<i>K. lactis</i>	<i>P. fermentans</i>	<i>P</i> -value ²
pH	3	5.16 $\pm$ 0.09	5.18 $\pm$ 0.01	5.12 $\pm$ 0.04	NS
	95	5.42 $\pm$ 0.12	5.47 $\pm$ 0.04	5.44 $\pm$ 0.08	NS
Acidity, Dornic °	3	115 $\pm$ 7	110 $\pm$ 0	113 $\pm$ 4	NS
	95	208 $\pm$ 25	180 $\pm$ 22	215 $\pm$ 15	NS
TS, g/kg	3	523.1 $\pm$ 13.9	524.0 $\pm$ 7.5	524.4 $\pm$ 5.8	NS
	95	613.9 $\pm$ 5.8	611.2 $\pm$ 9.9	618.8 $\pm$ 1.7	NS
Fat, g/kg	3	265.5 $\pm$ 3.5	269.0 $\pm$ 8.5	264.0 $\pm$ 5.7	NS
	95	303.8 $\pm$ 8.8	296.3 $\pm$ 0.6	296.3 $\pm$ 8.8	NS
Fat/TS, %	3	50.8 $\pm$ 0.8	51.3 $\pm$ 0.9	50.4 $\pm$ 0.5	NS
	95	49.5 $\pm$ 1.0	48.5 $\pm$ 1.0	47.9 $\pm$ 1.3	NS
Lactose, g/kg	3	4.7 $\pm$ 1.2	4.8 $\pm$ 2.7	4.8 $\pm$ 1.6	NS
FAA, g/kg	45	5.94 $\pm$ 0.18	5.84 $\pm$ 0.28	5.28 $\pm$ 0.38	NS
	95	14.39 $\pm$ 0.55	17.46 $\pm$ 1.55	15.86 $\pm$ 0.63	NS

¹Values are means $\pm$ standard deviation of 4 values (2 Cantalet cheese trials $\times$ duplicate analyses).

²Results of the ANOVA: probability of *F*-test: NS: $P > 0.05$.

Table 2. Concentrations of selected volatile compounds in Cantalet cheeses manufactured without (control) and with addition of yeasts (*Kluyveromyces lactis*, *Pichia fermentans*) as adjunct cultures in matured curd (3 d) and during ripening

Compound ¹	Cheese age, d	Control	<i>K. lactis</i>	<i>P. fermentans</i>	P-value
Neutral volatiles ²					
Ethanal, AU	3	ND ³	ND	ND	— ⁴
	45	ND ^a	51 ± 49 ^a	ND ^a	NS
	95	ND ^a	61 ± 30 ^b	ND ^a	NS
Ethanol, µg/g	3	26 ± 1 ^a	207 ± 35 ^b	29 ± 1 ^a	**
	45	47 ± 2 ^a	355 ± 41 ^b	48 ± 5 ^a	**
	95	29 ± 3 ^a	86 ± 97 ^a	19 ± 2 ^a	NS
Ethyl acetate, ng/g	3	8 ± 1 ^a	1,101 ± 299 ^b	9 ± 2 ^a	*
	45	66 ± 37 ^a	2,928 ± 228 ^b	145 ± 67 ^a	***
	95	147 ± 3 ^a	4,448 ± 278 ^b	332 ± 406 ^a	***
2-Methylpropanol, ng/g	3	ND ^a	101 ± 62 ^a	ND ^a	NS
	45	ND ^a	996 ± 348 ^b	ND ^a	*
	95	42 ± 59 ^a	911 ± 16 ^b	ND ^a	*
3-Methylbutanal, ng/g	3	ND ^a	193 ± 17 ^b	ND ^a	***
	45	7 ± 0.2 ^a	171 ± 53	9 ± 1 ^a	*
	95	62 ± 23 ^a	183 ± 147 ^a	35 ± 17 ^a	NS
3-Methylbutanol, ng/g	3	ND ^a	318 ± 135 ^b	ND ^a	*
	45	70 ± 10 ^a	2,762 ± 781 ^b	36 ± 8 ^a	*
	95	446 ± 50	3,283 ± 199 ^b	143 ± 115 ^a	***
2-Methylbutanol, ng/g	3	ND	ND	ND	—
	45	ND	ND	ND	—
	95	46 ± 65 ^a	432 ± 87 ^b	ND ^a	*
Ethyl butanoate, ng/g	3	ND	16 ± 1 ^b	ND	***
	45	8 ± 3 ^a	83 ± 0.4 ^b	12 ± 4 ^a	***
	95	19 ± 10 ^a	124 ± 12 ^b	16 ± 2 ^a	**
3-Methylbutyl acetate, ng/g	3	ND	ND	ND	—
	45	ND ^a	18 ± 2 ^b	ND ^a	***
	95	ND ^a	150 ± 13 ^b	ND ^a	***
Ethyl hexanoate, ng/g	3	ND	ND	ND	—
	45	6 ± 8 ^a	121 ± 25 ^b	27 ± 2 ^a	**
	95	45 ± 25 ^a	182 ± 39 ^b	36 ± 5 ^a	*
VFA, µg/g					
Acetic acid, µg/g	95	1,262 ± 78 ^a	1,438 ± 3 ^a	1,025 ± 166 ^a	NS
Methylpropanoic acid, µg/g	95	4.8 ± 3.8 ^a	10.6 ± 5.4 ^a	9.4 ± 2.9 ^a	NS
Methylbutanoic acids, µg/g	95	6.5 ± 6.4	15.2 ± 9.3 ^a	13.2 ± 6.1 ^a	NS
Butanoic acid, µg/g	95	63.4 ± 15.4 ^a	88.4 ± 18.6 ^a	78.2 ± 6.5 ^a	NS
Hexanoic acid, µg/g	95	18.1 ± 1.1 ^a	18.2 ± 1.5 ^a	19.8 ± 2.6 ^a	NS
FFA (sum C4:0 to C18:3), µg/g	95	1,755 ± 19 ^a	1,775 ± 150 ^a	1,803 ± 6 ^a	NS

^{a,b}Means within a row with different superscripts differ ($\alpha < 0.05$).

¹Values are means of 4 values (2 Cantalet cheese trials × duplicate analyses).

²Neutral volatile compounds were identified by comparison of mass spectra and retention times with those of authentic standards, and quantified by total ion current. Compounds were quantified by a regression curve obtained using authentic standards, excepted for 2-methyl-propanol and 2-methylbutanol, quantified using the regression curve of 3-methylbutanol, and 3-methylbutyl acetate, quantified using the regression curve of ethyl hexanoate, and are expressed in nanograms per gram, except for ethanal, which was expressed in arbitrary units (AU). Ethanol was determined by enzymatic method.

³ND = not detected (zero value was used for ANOVA).

⁴ANOVA not performed.

⁵Sum of 2-methylbutanoic and 3-methylbutanoic acids.

*** $P \leq 0.001$; ** $0.001 < P \leq 0.01$; * $0.01 < P \leq 0.05$; NS = $P > 0.05$.

greater in both experimental cheeses but these differences were not statistically significant (Table 2).

Selected volatile compounds (see Materials and Methods) were quantified in Cantalet cheeses by DH-GC-MS. Ten of the 21 selected compounds (ethanol, ethanal, 3 ethyl esters, and 5 branched-chain compounds; Table 2) were present at significantly greater concentrations in the Kl cheeses at 95 d compared with the control and

Pf cheeses. Ethanol was produced early during manufacture, whereas most of the other compounds increased in concentrations throughout ripening. Esters showed the greatest change in concentration.

Odorous Compounds Analysis

Thirty-three odors were perceived by using 8W-GC-O. Half of the odors perceived (16 of 33) could be classified

Table 3. List of the odorous compounds identified by gas chromatography-olfactometry/mass spectrometry (GC-O/MS) of Cantalet cheese at 95 d of ripening

Peak no. ¹	Chemical name	Retention index ²	Reliability of ID ³	Odor ⁴
1	Ethanol	477	Very high	Alcohol
2	2-Methylpropanal	551	Very high	Butter
3	1-Propanol	559	Very high	Empyreumatical
4	2,3-Butanedione	589	Very high	Butter
5	Acetic acid	610	Very high	Vinegar
6	2-Methylpropanol	632	Very high	Acid – alcohol
7	3-Methylbutanal	652	Very high	Empyreumatical (various attributes)
8	Pentanal	696	Very high	Fruity
9	2-Ethylfuran	701	Very high	Cream
10	3-Hydroxy-2-butanone	712	Very high	Butter – yogurt
11	2-Methylpropanoic acid	738	Very high	Feet – vomit
12	2-Pentenal	756	Very high	Fruity
13	1-Hexen-3-one	776	High	Plastic
14	Butanoic acid	791	Very high	Vomit – feet – cheesy
15	Hexanal	803	Very high	Green apple
16	3-Methylbutanoic acid	838	Very high	Feet – cheesy
17	Coeluted esters	851	Tentative	Fruity
18	2-Hexenal	854	High	Fruity
19	1-Hepten-3-ol	881	Very high	Sulfur – blue cheese
20	Heptanal	903	Very high	Fruity
21	Methional	907	Very high	Cooked potato
22	1-Octen-3-one	979	Very high	Mushroom
23	1-Octen-3-ol	980	Very high	Mushroom
24	Methionol	980	High	Potato
25	3-Octanone	986	High	Moss – undergrowth
26	Ethyl hexanoate	998	Very high	Fruity (strawberry aroma)
27	Octanal	1004	Very high	Citrus fruit – fruity
28	2-Ethyl-3-methoxypyrazine	1062	High	Empyreumatical – caramel – burnt
29	1-Nonen-3-ol	1079	High	Mushroom
30	Nonanal	1105	Very high	Stuffy/rancid
31	Phenylethanol	1123	Very high	Floral
32	Unidentified	1190	Tentative	Empyreumatical – caramel – toasted bread
33	Unidentified pyrazine	1222	Tentative	Empyreumatical – peanut – roasted – cheesy

¹Peak numbers as in Figure 2.²Retention indices calculated for the DB5 capillary column of the GC-O/MS system.³The reliability of identification or structural proposal is indicated as follows: Very high = mass spectrum, retention index, and odor identical to those of authentic standards; High = mass spectrum, retention index, and odor in agreement with the corresponding literature data; Tentative = tentative identification by mass spectra.⁴Main odors given by the sniffers.

in “fruity-floral” and “lactic-cheesy” odor families (9 and 7 occurrences, respectively). In total, 30 of 33 odorous compounds were identified with a high reliability, and their odor attributes, as described in literature and databases, were in agreement with 8W-GC-O data (Table 3). The total olfactory signal (TOS, sum of the 16 individual aromagrams) of the control and the 2 experimental cheeses were compared to investigate the effect of yeast adjuncts on the olfactory characteristics of Cantalet cheese (Figure 2). The Fisher test values revealed that 12 zones of the TOS differed significantly ($P < 0.01$) between the 3 cheeses. In most cases, the Kl cheeses showed a more intense olfactory signal than the other cheeses (Figure 2). Ten of these 12 olfactory signals were related to 3 acids, 4 aldehydes, 2 alcohols, and 1 ester (Table 3). Regarding the 2 remaining olfactory zones, the fruity odor (peak 17) likely corresponded to one or

several (coeluted) esters, and the empyreumatical odor (peak 32), described as caramel/toasted bread/roasted, could not be identified. To further confirm the link between the olfactory peaks and the 10 identified volatile compounds, peak areas of the corresponding DH-GC-MS chromatograms were compared for the 3 cheeses (Table 4). Eight of the 10 volatile compounds were effectively detected at greater concentrations in the Kl cheeses than in the other cheeses (Table 4), as expected from the analysis of olfactometry data (Figure 2).

Sensory Analyses

Cheeses were first evaluated by 2 industrial graders experienced in Cantal/Cantale cheese tasting, who considered that all the Cantale cheeses had typical Cantal characteristics. Cantale cheeses were also evaluated by

Figure 2. Total olfactory signals obtained by using 8-way GC-olfactometry in 3 Cantale cheeses without (control) and with addition of yeasts (*Kluyveromyces lactis* and *Pichia fermentans*) as adjunct cultures, at 95 d of ripening, and “ANOVagram” showing the results of the ANOVA.

the trained assessors of Les Maisons du Goût to be further characterized. The average intensities of odor and flavor did not significantly vary between the 3 cheeses (Table 5), but more attributes were used to describe the odor of Kl cheeses ($P = 0.060$), and the flavor of both Kl and Pf cheeses ($P = 0.053$; Table 5). Twenty-five and 26 out of the 53 odor and aroma attributes used to de-

scribe the flavor of Cantale cheeses were cited at a frequency >10% (shown in Figure 3). The attributes used frequently to describe the odor of cheeses were “butter”, “boiled milk”, “whey”, “lemon”, and “fresh milk”, which represented more than 30% of the total citations for at least 1 trial. The attributes used frequently to describe the taste and aroma of cheeses were “acidic”, “boiled

Table 4. Odorous compounds in the GC-MS profiles corresponding to the olfactory zones that differed significantly between 3 Cantale cheeses manufactured without (control) and with addition of yeasts *Kluyveromyces lactis* (Kl) and *Pichia fermentans* (Pf) adjunct cultures, at 95 d of ripening

Peak no. ¹	Compound ²	Cheese		
		Control	Kl	Pf
1	Ethanol (31)	2.0	13.9	1.8
11	2-Methylpropanoic acid (73)	Tr ³	0.7	Tr
12	2-Pentenal (55)	0.4	2.3	0.7
14	Butanoic acid (60)	3.9	4.6	3.0
15	Hexanal (ion 44)	15.1	69.3	26.2
16	3-Methylbutanoic acid (60/87)	Tr	Tr	Tr
18	2-Hexenal (TIC)	5.2	19.8	8.4
23	1-Octen-3-ol (57)	0.2	1.1	0.3
26	Ethyl hexanoate (88)	0.6	3.7	0.4
27	Octanal (41)	1.5	1.8	1.8

¹Peak numbers as in Figure 2.

²Volatiles were quantified by total ion current (TIC) or selected fragments (m/z , in parentheses), as indicated. Values are expressed in arbitrary units.

³Tr (trace): area <0.1.

Table 5. Results of ANOVA on the intensity of odor and flavor and of the numbers of attributes cited to describe odor and flavor of 3 Cantalet cheeses manufactured without (control) and with addition of the yeasts *Kluyveromyces lactis* (Kl) and *Pichia fermentans* (Pf) as adjunct cultures, evaluated after 95 d of ripening

		Control	Kl	Pf	P-value ¹
Intensity ²	Odor	4.2 ^a	4.1 ^a	4.1 ^a	NS
	Flavor	4.4 ^a	4.5 ^a	4.4 ^a	NS
Number of attributes cited ³	Odor	5.9 ^a	6.2 ^b	5.8 ^a	0.060
	Flavor	7.9 ^a	8.2 ^b	8.3 ^b	0.053

^{a,b}Values in the same row with the same letter were not significantly different according to the Newman Keuls multiple-comparison test ($\alpha < 0.05$).

¹Probability of *F*-test; NS = *P* > 0.10.

²Intensity scored on a 10 point-scale

³The number of attributes cited theoretically varied from 0 (no attribute cited) to 53 and 57, for odor and flavor, respectively (all the attributes cited). Values are means of 36 assessments (18 assessors, duplicate evaluations).

milk”, “salty”, “butter”, “bitter”, “whey” and “lemon”, which represented more than 38% of the total citations. Cheeses significantly differed in the frequency of only few attributes (Figure 3). The control cheeses were characterized by “apricot/peach” (*P* = 0.02) odor and “hazel-

nut” odor (*P* = 0.02) and aroma (*P* = 0.08). The Kl cheeses were characterized by “acetaldehyde” and “alcohol” odors (*P* < 0.001). At lower levels of significance, Kl cheeses differed from the other cheeses by “yeast” (*P* = 0.12) odor. The Pf cheeses had a higher “acetic” (*P* = 0.13) and

Figure 3. Results of descriptive analysis of odor and aroma of 3 Cantalet cheeses manufactured without (control) and with addition of yeasts [*Kluyveromyces lactis* (Kl) and *Pichia fermentans* (Pf)] as adjunct cultures, evaluated after 95 d of ripening. Values are perception frequencies, expressed as percentage [i.e., the number of “presence” responses divided by the maximal number of responses (36: 18 assessors, duplicate evaluations)]. Values of probability of χ^2 test <0.05 are marked by an asterisk.

“potato” ($P = 0.12$) aromas and a lesser “malty” ($P = 0.04$) aroma than the control and Kl cheeses (Figure 3).

DISCUSSION

This study shows that *K. lactis* had a limited but significant influence on some aspects of the ripening process of Cantale cheese. The presence of viable cells of *K. lactis* in Cantale cheese was associated in particular with a longer survival of lactococci, a marked increase in the concentrations of some odorous compounds, and changes in olfactory profiles, but little change in cheese flavor.

The populations of *K. lactis* and *P. fermentans* yeasts were stable at approximately 6 log cfu/g during cheese manufacture and showed variations of ~ 1.5 log cfu/g over the ripening. The 2 yeast strains used as adjunct cultures in the present study were isolated from the dominant yeast population of a Cantal cheese containing $\sim 5 \times 10^4$ yeasts/g. Therefore, their lack of growth was unexpected and may result from their inability to adapt their metabolism to cheese conditions after being grown in a glucose-yeast extract medium and directly inoculated in cheese milk. The growth of yeasts in cheese is highly dependent on the environmental conditions and on the species used. In curd slurries, for example, *K. lactis* grew from 5×10^5 to 1×10^8 cfu/g over 5 d of incubation (Arfi et al., 2004), whereas in Raclette cheese, the population of viable cells of 4 species of adjunct yeasts (*Galactomyces geotrichum*, *Pichia jardinii*, *Y. lipolytica*, and *D. hansenii*) decreased from $\sim 10^5$ to 10^6 cfu/g at the molding step to $< 10^2$ cfu/g after 90 d (Wyder et al., 1999). In contrast, indigenous yeasts grew in the control cheeses during the manufacture and reached 2.7 log cfu/g, as reported in Cheddar cheeses, where populations up to 6 log₁₀ cfu/g in 37-d-old Cheddar were detected (Welthagen and Viljoen, 1999).

Starter cultures may have benefitted from the presence of yeasts at the beginning of ripening, because cultivable lactococcal populations remained greater in both Kl and Pf cheeses than in the control cheeses at 45 d of ripening, in agreement with results reported in Cheddar cheese with *D. hansenii* or *Y. lipolytica* added as adjunct cultures (Ferreira and Viljoen, 2003). A synergistic effect between lactic acid bacteria and many species of yeasts including *K. lactis*, which resulted in a prolonged survival of lactic acid bacteria, has also been observed in cheese slurries (Arfi et al., 2004).

The presence of yeasts did not modify gross cheese composition (Table 1), which can be explained by the fact that the adjunct yeasts did not grow in Cantale cheeses, and thus did not modify the rate of acidification. Moreover, the presence of yeasts did not modify the level and the specificity of lipolysis, and had only a limited impact on the concentrations of FAA. In contrast, the

presence of *K. lactis* in Cantale cheese induced large variations in the concentrations of some volatile odorous compounds. This effect was particularly marked for ethanol, ethyl esters, some aldehydes, branched-chain alcohols (Table 2), branched-chain acids, and 1-octen-3-ol (Figure 2, Table 4). Ethanol was formed at concentrations ~ 200 μ g/g in Kl cheeses [vs. ~ 40 μ g/g in the control cheeses and in raw milk Cantal cheese (De Freitas et al., 2005)]. Such high values are observed in Emmentaler cheese containing obligatory heterofermentative lactobacilli as adjuncts (Chamba, 2000). Ethyl esters were formed at concentrations approximately 5-fold greater in the presence of *K. lactis*, and up to 30-fold greater for ethyl acetate (Table 2). Moreover, a branched-chain ester (3-methylbutyl acetate) was also detected in Kl cheeses only. Only one strain of *K. lactis* was tested in the present study, but the results observed were in general agreement with previous reports regarding the use of *K. lactis* as adjunct. Hence, high levels of ethyl acetate were detected in cheese slurries containing *K. lactis* (Arfi et al., 2004). Various esters including ethyl acetate, ethyl butanoate, and 3-methylbutyl acetate were also produced by *K. marxianus* in cheese slurries (Leclercq-Perlat et al., 2004). The formation of esters in Kl cheeses can result directly from the ester-forming activity of *K. lactis*, because yeasts, and particularly *K. lactis*, are known to produce esters by the acylation of an alcohol by an acylCoA catalyzed by alcohol acyltransferases (Mason and Dufour, 1985). Alternatively, the formation of greater levels of ethyl and 3-methylbutyl esters in Kl cheeses could have simply resulted from the presence of greater concentrations of the corresponding alcohols (ethanol and 3-methylbutanol), because the concentration of alcohols can be a limiting factor of ester synthesis in cheese (Thierry et al., 2006). Branched-chain compounds were detected at concentrations from 2- (acids) to 20-fold (alcohols) greater in Kl cheeses than in the 2 other cheeses, in agreement with previously reported results for *K. lactis* or *D. hansenii* added in model cheese media (Arfi et al., 2004; Leclercq-Perlat et al., 2004). Branched-chain compounds likely originate from the catabolism of branched-chain AA. Several saturated (hexanal, octanal) and unsaturated (2-pentenal, 2-hexenal) aldehydes were also detected in the present study at approximately 4-fold-greater concentrations in the presence of *K. lactis*, in agreement with previous observations in cheese slurries inoculated with *D. hansenii* (Leclercq-Perlat et al., 2004). These results were unexpected because *K. lactis* possess alcohol dehydrogenase activity (Bozzi et al., 1997), which could have enhanced the rate of reduction of aldehydes in Kl cheeses, as generally observed in raw milk cheeses compared with pasteurized or microfiltered milk cheeses (Beuvier and Buchin, 2004). Regarding the formation of sulfur com-

pounds, only dimethyl disulfide was detected in the present study, and its concentration did not vary with the presence of yeasts (data not shown), and no sulfur odors were detected by GC-O. This result is in contrast with previous studies reporting that many yeast species including *K. lactis* are able to produce volatile sulfur compounds from Met (Spinnler et al., 2001). The ability to produce volatile compounds can also depend on the strain, although most of the effects of *K. lactis* on cheese volatiles shown in the present study are in agreement with previously reported results regarding the species *K. lactis*. The effects observed were largely dependent on the species. Hence, at the same populations of viable *K. lactis* and *P. fermentans* over ripening, only the former had an effect on cheese flavor under the experimental conditions used.

The use of the recently developed multicanal olfactometry device (8W-GC-O), in parallel with GC-MS, allowed us to identify most (29 of 33) of the odorous compounds responsible for the olfactory zones detected in Cantalet cheese. Most of the compounds are common in cheese (Maarse et al., 1994) and were previously identified in cheeses by GC-O/MS (Curioni and Bosset, 2002). Three original compounds, however, were detected: 2-pentenal, 2-hexenal, and 2-ethyl-3-methoxypyrazine. 2-Pentenal and 2-hexenal, associated with the fruity odors perceived in peaks 12 and 18, were previously detected by GC/MS in cheese and in butter, respectively (Maarse et al., 1994), but their role in cheese flavor was not previously investigated, to our knowledge. 2-Ethyl-3-methoxypyrazine was tentatively identified as being responsible for the empyreumatic odor perceived in peak 28. Various alkyl-pyrazines and alkyl-methoxy-pyrazines have been detected in cheese, including Cheddar cheese (Curioni and Bosset, 2002; Suriyaphan et al., 2001), but not 2-ethyl-3-methoxypyrazine, to the authors' knowledge.

The main notes of Cantalet cheese flavor appeared to be "fruity" and "lactic-cheesy", from the results of both olfactory profiles and cheese qualitative sensory evaluations. Hence, 16 of the 33 olfactory zones identified in 8W-GC-O profiles were classified in 1 of these 2 families (Table 3), whereas the main attributes of Cantalet cheese odor and aroma were "boiled milk", "butter", "lemon", and "whey" (Figure 3). Regarding the impact of *K. lactis* as an adjunct culture, divergent results were given by 8W-GC-O profiles and sensory evaluations of cheeses. Hence, the 8W-GC-O profiles of KI cheeses differed very significantly from those of the control and Pf cheeses by a greater intensity of perception of 8 olfactory zones: 1 alcohol odor (ethanol), 4 fruity odors associated with esters and unsaturated aldehydes, 2 cheesy odors associated with branched-chain VFA, and 1 empyreumatical odor (peak 32; Figure 2). The ability of *K. lactis* to develop strong fruity olfactory notes was previously reported

(Martin et al., 2002). Regarding sensory evaluations, however, *K. lactis* had only a low impact on Cantalet cheese flavor in the present study. The KI cheeses differed from the other cheeses by the perception of only 2 odor attributes, "acetaldehyde" and "alcohol" (Figure 3), in agreement with the data of concentrations of ethanol and acetaldehyde (ethanal). The other differences detected by DH-GC-MS or GC-O between KI cheeses and the other cheeses did not result in detectable differences in cheese odor and flavor. This result could be because instrumental analyses such as DH-GC-MS have, in most cases, better sensitivity and accuracy than sensory evaluations. Regarding GC-O, this method gives only a first indication of the potent flavor-active compounds of cheese, because it does not make allowance for the effects of combinations of odorous compounds (Delahunty and Piggott, 1995). Moreover, the volatility of compounds is modulated by their interactions with cheese fat and proteins, which differ in the mouth during mastication and in the headspace device. Regarding VFA, their volatility and thus their perception depends on the pH. It could explain why VFA, which were the most potent odorous compounds and differed in CG-O profiles (Figure 2), did not play a role in differentiating the flavor profiles of the whole cheeses. These results also highlight the fact that sensory evaluations are required to characterize the characteristics of cheese flavor.

In conclusion, yeasts added as adjunct cultures can influence the formation of odorous compounds and, even if the overall effects on cheese flavor were quite small in the present study, yeasts could have the potential to impact cheese flavor.

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