ABSTRACT

The objective of this work was to compare goat cheeses obtained from milk previously subjected to high pressure homogenization (1000 bar) with those produced from untreated milk and milk subjected to sanitization (61°C; 20 min) or to pasteurization (72°C; 15 s).

The pressure homogenization treatment had both direct and indirect effects on cheese characteristics and their evolution during ripening. The direct effects were principally linked to the change in water-binding capacity of proteins as shown also by the lower whey separation. The indirect effects involved the microbial growth or activity and, particularly, modifications of the population of the lactic acid bacteria that occurred naturally and their evolution as well as a more precocious yeast and mold growth with a consequent rapid rise in pH.

Although the treatment proved to enhance both proteolytic and lipolytic activities according to Fourier transform infrared analysis, which was used to obtain a rapid description of the biochemical modification, the cheeses homogenized under high pressure showed relevant qualitative differences only in the zone corresponding to amide I and amide II signals of proteins. The activation of these enzymatic activities observed in the homogenized cheeses could be either an indirect effect of the shift of the microbial population or a consequence of a different exposure of the macromolecules to the enzymatic activity.

Scanning electron microscopy analyses of goat cheeses revealed that cheeses homogenized under high pressure had a more homogeneous microstructure than did the others.

(Key words: high pressure homogenization, goat cheese, microbial and chemico-physical characteristics, enzymatic activities)

INTRODUCTION

Milk treatment based on hydrostatic pressure remarkably reduces spoilage and the viability of potentially pathogenic microorganisms (13). In addition to the effects on microorganisms, milk constituents, especially proteins, undergo physico-chemical modifications leading to changes in their functionality (5, 23, 24).

High pressure homogenization (HPH) of up to 1500 bar has been proposed as an effective alternative to pasteurization in milk sanitization (17) and probably shares some action mechanisms with hydrostatic pressure treatment. However, the dynamics of the process do not coincide with those described for the hydrostatic pressure treatments (15, 16). In fact, the fluid is forced through a narrow gap, after which it is subjected to an ultrarapid depression. In particular, when the local pressure in a liquid is reduced without temperature change, gas-filled bubbles (or cavities) nucleate and grow within the body of liquid. The collapse of such cavities could transmit several localized forces to surfaces or particles, including the microbial cell (16). High pressure homogenizers that are able to process fluid or pumpable food systems with up to 2000 bar of pressure are now available (16, 17). Their use as an alternative to pasteurization for improved safety and enforced microbiological quality of milk and whole liquid eggs has been proposed (10, 11, 16, 17).

Numerous reports have been made on the effect of homogenization on milk components and on enzymatic clotting although the pressure applied generally did not exceed 200 to 300 bar (22). Humbert et al. (14), who used a pressure of 600 bar, found that HPH of whole and skim milks modified the ratios of nitrogen fractions and soluble forms of calcium and...
phosphorous. Curd strength was affected with a reduction in coagulation time, an acceleration of the rate of curd firmness formation, and a greater final curd strength.

From previous investigations (12, 16, 17), it was found that homogenization pressures greater than 800 bar were able to induce two practical effects in the processed food system. An especially significant reduction occurred in the microbial population, and modification also occurred in the functional properties of proteins and some microstructural characters.

This work was based on the characterization of caprine cheeses produced with milk previously subjected to HPH (1000 bar) in comparison with cheeses obtained from raw milk subjected to pasteurization (72°C; 15 s) or sanitization (61°C; 20 min). Our research focused on identification of comparative effects of treatments on 1) selective action on the naturally occurring microbial population, 2) enzymatic and microbial proteolytic and lipolytic activities and their products, and 3) modifications of some microstructural and organoleptic characters of cheeses during ripening.

**MATERIALS AND METHODS**

**Milk Treatments and Cheesemaking**

Cheeses, obtained from a small farm in Siena (Italy) were manufactured with raw, whole caprine milk that was filtered through cheesecloth and then divided into four batches. Three of the milk samples were subjected to different treatments (i.e., homogenization at 1000 bar, pasteurization at 72°C for 15 s, or sanitization at 61°C for 20 min). The untreated and treated milks were coagulated at 18°C with 10 ml of commercial calf rennet (strength 1/10,000) for each 100 L of milk and were left to rennet until the next morning. The coagulum was taken from the vat in thin slices with a ladle and distributed into deep plastic molds (8 cm in diameter × 15 high), where it was left to drain. The drained curds were then turned and salted by adding solid salt. Samples of curd and cheese were ripened for 15, 30, 45, and 89 d. The ripening process took place at a temperature of 10 to 15°C and a relative humidity of 70 to 85%.

Four different types of cheeses were obtained. For each type, 25 cheeses were subjected to the ripening process. The four types of cheese were subjected to microbiological, physical-chemical, and sensory analysis during ripening. Five cheeses for each type were used for sensory analysis, and five cheese repetitions of each type were subjected to physical-chemical analyses after 15, 30, 45, and 89 d of ripening. The microbiological analyses were performed on three samples.

**Determination of Homofermentative and Heterofermentative Metabolism of Lactic Acid Bacteria**

The homofermentative and heterofermentative metabolism of the lactic acid bacteria isolated was determined with the method of Leveau et al. (20) and was based on the CO2 detection in tubes of liquid MRS (Oxoid, Basingstoke, England) with the aid of Durham microtubes (Marchi, Bologna, Italy).

**Cheese Yield**

The cheese yields were expressed as the ratio between the curd obtained after 2 d of draining and the milk used.

**Enumeration of Microorganisms in Milk and in Cheeses**

Ten milliliters of the four different milks or 10 g of each sample were homogenized with 90 ml of a sterile solution of 2% (wt/vol) sodium citrate with a Stomacher (Lab Blender 80; Seward Medical, London, United Kingdom) for 2 min. Blended samples were then serially diluted 10-fold and plated in duplicate on selective media. Lactobacilli were enumerated on MRS (Oxoid) plates after incubation for 72 h at 37°C in anaerobic Gas Pack jars (Oxoid) in an H2 plus CO2 atmosphere. The selectivity of the growth conditions and media was checked by the microscopic appearance of the colonies. Streptococci were counted on M17 (Oxoid) plates after incubation for 48 h at 37°C under anaerobiosis. Plate count agar (Oxoid) was used for mesophilic aerobic bacteria incubated at 30°C for 48 h. Violet red bile agar (Oxoid) was used for total and fecal coliforms incubated at 37 and 44°C, respectively, for 24 h. Slanetz and Bartley (Oxoid) agar was used for enterococci incubated at 44°C for 24 h. Sabauraud dextrose agar (Oxoid) was used for yeasts and molds incubated at 28°C for 48 h. Plate count agar (Oxoid) with 1 ml/L of crystal violet and 5 ml/L of 2,3,5-triphenyl-tetrazolium chloride (TTC) was used for Gram-negative bacteria incubated at 30°C for 48 h. The results were the mean of three repetitions.

**Determination of the Aroma Compounds in the Head Space**

For gas chromatographic analyses of the aroma compounds in the head space of sealed vials contain-
ing 5 g of cheese, a gas chromatograph (GC 6000 Vega Series; Carlo Erba Inst., Milano, Italy) equipped with an FID detector and a 2-M × 2-mm glass column packed with 80/120 Carbopack B/6.6% Carbowax 20 M (Supelco, Bellefonte, PA) was used. The conditions of each analysis was as follows: column temperature increased from 80 to 180°C at a rate of 4°C/min, injector temperature was 200°C, detector temperature was 200°C, carrier gas was N₂, and flow rate was 20 ml/min. The gas chromatograph was connected to a head space autosampler (HW 250; Carlo Erba Inst.) equipped with a gas-tight syringe (Hamilton, Bonaduz, Switzerland). The analysis temperature of the samples (conditioned at 60°C for 1 h) was 60°C. The aroma compounds were identified on the basis of their retention times compared with those of standard compounds. The amounts of the identified compounds in the head space were calculated on the basis of previously prepared calibration curves.

**Analysis of Free Fatty Acids**

Free fatty acids were extracted and quantified according to the method of Caboni et al. (2). Analysis of free fatty acids, such as methyl esters, was carried out using a Carlo Erba HRGC 5160 Mega Series gas chromatograph equipped with a fused silica capillary column (30 m × 0.32-mm i.d.) coated with a film of 0.20-μm thickness (Supelco); the oven temperature was programmed from 30°C to 320°C with an increase of 10°C/min, and the detector (FID) temperature was 350°C.

The data were the mean of five repetitions for each cheese type.

**FTIR Spectroscopic Analysis**

An ATI Mattson Fourier transform infrared (FTIR) spectrometer, equipped with a horizontal zinc selenide (ZnSe) ATR sampling accessory in the sample compartment, was used. Each spectrum, recorded between 700 and 4000 cm⁻¹ at a resolution of 4 cm⁻¹, consisted of 200 interferograms. For each sample, the spectra were recorded in triplicate as sample single-beam spectra and ratioed against the single-beam spectrum of water before conversion into absorbance units. Triangular apodization was employed. Bands were identified using known standards and by acquiring spectral data on standard sets (6).

**pH and Water Activity Determinations**

The water activity was analyzed with an Aqualab CX2 Instrument (Decagon Devices, Inc., Pullman, WA) calibrated with a standard solution of sodium chloride and distilled water at a constant temperature. The pH measurements were obtained with a pH meter (model 2001; Crison Instruments, Barcelona, Spain) calibrated with two standard solutions buffered at pH 4.00 and 7.02.

**Organoleptic Assessment**

The quality of the cheeses was assessed after 15 d by 18 untrained evaluators at DIPROVAL (Dipartimento di Prote Zione E Valorizzazione Agroalimentare, Bologna, Italy). Samples were served at 25°C during sessions in which five samples were considered.

A sensory vocabulary comprising five attributes was used. The assessors were asked to rate each attribute on a 0- to 5-point scale. In addition, the assessors were asked to rate each product in terms of overall acceptability. The ANOVA was performed with the Statistics for Windows (25). This test enabled us to quantify the respective influence of the various milk treatments on the characteristics of the experimental cheeses.

**Scanning Electron Microscopy Analysis**

Samples for study with an electron microscopy (6400; JEOL USA, Peabody, MA) were prepared using the method of Westall et al. (28).

**RESULTS AND DISCUSSION**

**Microbiological Characteristics of the Four Types of Cheeses**

The HPH and the two thermal treatments to the milk reduced the naturally occurring psychrotrophic aerobic cells by about 2 log₁₀ cfu/ml with respect to the untreated milk. However the homogenization treatment was less active against enterococci, which accounted for 4.06 and 0.92 log₁₀ cfu/g in the control and in the homogenized milk, respectively, but they were not detectable in 1 ml of the heat-treated milks. The coliforms were absent in 1 ml of the processed and unprocessed milks.

The growth after manufacture and during ripening at 12 to 15°C of total mesophilic bacteria, coliforms, *Staphylococcus* spp., yeasts, and lactic acid bacteria was analyzed. A low concentration of coliforms and yeasts was observed in all of the curds. However, the yeasts could exceed 7 log₁₀ cfu/g after 15 d in the cheeses from HPH milk. Their growth rate seemed to be favored by the homogenization treatment,
although they could attain $7 \log_{10} \text{cfu/g}$ in the cheeses from sanitized milk but only after 45 d. The coliforms showed an increase during ripening, but they did not exceed $6 \log_{10} \text{cfu/g}$ for the entire period.

The initial concentrations of enterococci were highest in the untreated milk, curd, and cheeses and in the HPH samples. However, enterococci were able to grow in all four types of cheeses to a concentration of $6 \log_{10} \text{cfu/g}$ (Figure 1a). According to Litopoulou-Tzanetataky and Tzanetakis (21), enterococci appear to be an important group in the microflora of goat cheeses. Coagulase-positive staphylococci, present at higher concentrations in the curd from untreated milk, increased during ripening (Figure 1b) to values similar to those reported by Fatichenti et al. (7) and Fontecha et al. (8) for caprine cheeses.

High counts of lactic acid bacteria were recorded during ripening of the four cheese types (Figure 1c). They presented the highest values in curd from HPH milk. In the various cheeses, they tended to stabilize at a level of about $8 \log_{10} \text{cfu/g}$. This concentration was constant in all the samples after a decrease during the first 15 d; they increased again in the pasteurized and sanitized cheeses.

Colonies (~15 to 20) both from MRS and M17 plates have been isolated from each of the curds obtained from the three processed and the control milks. A total of 72 isolates have been identified for evaluation of a possible selective action of treatments. The homogenization treatment seemed to favor the proliferation of heterolactic types. A rapid screening for the detection of CO$_2$ production indicated that in the curds from the control and heat-treated milks all of the colonies of cocci and rods were homofermentative types; whereas, 33% of the rod colonies and 18% of the coccus colonies isolated from the curd from HPH milk were heterolactic.

### Chemical-Physical and Biochemical Features of the Cheeses

The quantity of serum released from the curds made from the differently processed milks depended on the treatments. The actual cheese yields, expressed as the ratio between the curd obtained and the milk used, were 0.16, 0.207, 0.203, and 0.32, respectively, for the curds from the milks that were untreated, sanitized, pasteurized, or HPH. As an indirect measure of the water content, Table 1 shows the absorbances obtained with an FTIR spectrometer of the band at 3300 cm$^{-1}$, which corresponds to water, for the curds and cheeses during ripening. The absorbance values of the curd and cheeses from HPH milk were higher than those of the other samples. The table also shows the water loss (percentage) during ripening. Although in HPH cheeses the water loss rate during ripening was remarkably higher, the weight loss after 3 mo was comparatively lower.

The evolution of the water activity of the cheeses during ripening (Figure 2a) suggested a different water retention ability of the proteins and their
TABLE 1. Influence of milk treatment on the evolution of water loss and water absorbance at 3300 cm⁻¹ in cheese.

<table>
<thead>
<tr>
<th>Time (d)</th>
<th>UTR</th>
<th>SAN</th>
<th>PAST</th>
<th>HPH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water retention²</td>
<td>Water absorbance³</td>
<td>Water absorbance</td>
<td>Water absorbance</td>
</tr>
<tr>
<td>15</td>
<td>92.00</td>
<td>0.84</td>
<td>92.60</td>
<td>0.950</td>
</tr>
<tr>
<td>30</td>
<td>90.50</td>
<td>0.50</td>
<td>88.67</td>
<td>0.820</td>
</tr>
<tr>
<td>45</td>
<td>89.46</td>
<td>0.57</td>
<td>85.95</td>
<td>0.630</td>
</tr>
</tbody>
</table>

¹UTR = untreated control; SAN = sanitization at 61°C for 20 min, PAST = pasteurization at 72°C for 15 s, HPH = homogenization at 1000 bar.

²Water retention = moisture content at specified day × 100 (SE < 1%) initial moisture content.

³Water absorbance measured with a Fourier transform infrared spectrometer at 3300 cm⁻¹. Mean of three repetitions for 5 cheeses of each type (SE < 0.004).

hydrolysis products. Although the initial water activity of the four curds showed similar values, the four types of cheeses presented a different evolution of this variable. In particular, the water activity of the inner part and of the rind of the HPH cheeses, which initially were the highest, tended to decrease more precociously than did those of the other samples, as indicated in Figure 2a.

Figure 2b shows data of the pH changes of the rind and of the inner part of four cheeses during ripening. As expected, the pH of the rind of HPH cheeses remarkably increased over time because of the effect of the more rapid yeast and mold growth.

The HPH cheeses presented the peculiarity of a precocious increase in the inner pH within the first 30 d of ripening in concomitance with the drop in water activity. Because of the usually limited growth of yeast and mold in the inner part of cheeses, this more rapid pH increase could be attributed to a higher protein hydrolysis with consequent formation of hydrolysis products. Moreover, the peptides or amino acids formed could contribute to the decrease in water activity. In this period, the inner pH of the untreated, sanitized, and pasteurized cheeses increased more slowly.

FTIR Analysis

Samples of curd and of the inner part of the various cheeses were analyzed with an FTIR spectrometer to obtain a contemporaneous description of the changes in the principal components of the systems. This technique can provide spectral data concerning water, proteins, acids, lipids, and carbohydrates with a single analysis (18, 27). Moreover, on the basis of the comparison with pure compounds and of literature spectra, several absorption lines can be associated to functional groups, which can be taken also as a measure of metabolic or enzymatic activities (26, 27). The superimposition of the spectra recorded from the curd and from 15-, 30-, and 45-d-old cheeses are reported in Figure 3 (untreated cheese, a; HPH cheese, b; sanitized cheese, c; and pasteurized cheese, d).

The absorbance of the band at 1740 cm⁻¹, associated with both lactic acid and free fatty acid content (26), was higher in the sanitized and pasteurized cheeses than in the HPH cheeses. This band attained a maximum level after 30 d after which it decreased, probably because of lactic acid consumption.

The major differences for the various absorbances can be observed in the zone between 1700 and 1330 cm⁻¹. The bands, which corresponded to amide I vibration of α-helix and amide II vibration of β-sheet of proteins, increased during ripening in all the samples because of proteolytic activity. However their absorbance values, which were related to the presence of soluble peptides, continued to increase in the HPH cheeses after 45 d and decreased in all of the other cheeses. In particular, after 45 d of ripening, only the HPH samples presented a remarkable absorbance at 1650 cm⁻¹, which corresponded to the amide I vibration of the α-helix of proteins. In this ripening phase, the cheeses that were sanitized and pasteurized and the untreated cheese, showed a relevant absorbance decrease below the baseline. It is likely that some peptides formed during protein hydrolysis were highly hydrophobic and no longer water-soluble. As a result, the amide absorbance decreased. Therefore, two FTIR patterns could be identified; the first corresponded to the HPH cheeses, in which the absorbance of the typical bands of amides continued to increase overtime, and the second corresponded to the untreated, sanitized, and pasteurized cheeses, for
which, after an initial increase, a sudden drop in the
band absorbance occurred. The same two patterns,
which should be investigated further, have already
been observed in bovine cheeses that were obtained
by using as ripening agents strains of *Yarrowia
lipolytica* that had different proteinase activities.
These different hydrolysis models were confirmed by
different UREA-PAGE electrophoretic patterns and
reverse phase HPLC profiles of soluble peptides (10,
11).

An important difference in the FTIR spectra of the
HPH cheeses with respect to the others could be
observed in the zone between 1300 cm\(^{-1}\) and 1000
cm\(^{-1}\) and included the bending vibration of the groups
C-O-C or CH\(_2\)-O of the carbohydrates. In all of the
cheese samples, the absorbance of the bands at 1240,
1160, and 1100 cm\(^{-1}\) increased over time with respect
to the curds. However, the spectra of HPH cheeses showed the lowest absorbance values of the three bands and a different relative ratio between the absorbance values of the peaks at 1160 and 1100 cm$^{-1}$. These bands could account also for lipids and particularly for free fatty acids released by lipids. The lowest absorbance values of the bands in the zone between 1300 cm$^{-1}$ and 1000 cm$^{-1}$ for 45-d-old HPH cheeses were in agreement with the lowest absorbance values at 1740 cm$^{-1}$ and in the zone between 2800 and 3100 cm$^{-1}$, which corresponded to C-H stretching vibrations of fatty acids. These results can be associated with the higher water content of curd and HPH cheeses as also indicated by the absorbance values at 3300 cm$^{-1}$ (Table 1).

These preliminary observations suggest that FTIR spectroscopy, in comparison with other techniques such as low and high resolution nuclear magnetic resonance, could be a useful key to understand the pressure-induced modifications of tertiary and quaternary structures and particularly the stability of the $\alpha$- $\beta$-domains of casein. The FTIR profiles provided a rapid insight into the differences and indicated that the protein hydrolysis in HPH cheeses presented remarkable peculiarities with respect to the other cheeses (M. E. Guerzoni, 1999, unpublished).

**Lipids**

As a consequence of the higher water retention of the curd from HPH milk, the total lipid content did not exceed a level of 18% after 45 d (Table 2).

The lipolysis dynamics, which were studied on the basis of the release of the total and of the individual free fatty acids, presented remarkable differences and depended on the previous milk processing. The total free fatty acids, and particularly their proportion with respect to the total lipids, suggests that the lipolysis

Figure 3. Superimposition of the Fourier transform infrared spectra recorded on the curd (– –) and on 15-, 30-, and 45- d-old cheeses obtained from untreated milk (A), high pressure homogenized milk at 1000 bar (B), milk sanitized at 61°C for 20 min (C), and pasteurized milk at 72°C for 15 s (D).
was favored in the cheeses from HPH milk. Another feature of these products was the high percentage of short-chain fatty acids such as $C_{10}$ and $C_{12}$, which have been reported to have a relevant effect on the flavor of caprine cheese (1). The $C_{16:0}$ fatty acid was dominant in all the samples, although it has been reported that in caprine milk triglycerides the mean percentage of the two most important acyl residues $C_{16:0}$ and $C_{18:1}$ are, respectively, 21.1 and 20.8%. The microbial lipolytic enzymes that were involved acted specifically on $C_{16:0}$ ester linkages rather than on the $C_{18:1}$ linkages. The standard deviations between the five repetitions for each cheese type were <10 mg/kg and <0.15% for total free fatty acids and individual free fatty acids, respectively.

In addition to a direct effect of the microbial population size and composition, the increased lipolysis extent in the HPH cheeses could be associated with the effect of high pressure treatment, which resulted in smaller and highly emulsified fat globules within the curd (19).

**Microstructural Feature**

Figure 4 shows the microstructure of the 30-d-old untreated, sanitized, and HPH cheeses observed by scanning electron microscopy. The microstructure of the HPH cheese appears as a flexible polymer network, whereas the untreated and sanitized cheeses appear as an agglomeration of strands of caseins. The pasteurized cheeses showed a microstructure similar to that of sanitized cheeses.

The spongy appearance of the HPH cheeses can account for the higher water retention with respect to the other cheese types and reflects the supposed modifications of the functional properties of the milk protein as a consequence of the high pressure treatment. The texture of this cheese after 30 d of ripening was creamier and more spreadable. However, it is difficult to establish whether such texture characteristics were attributable to the direct effect on protein of homogenization or to its indirect effect on lipolytic and proteolytic activities.

**Aroma Compounds**

The presence and evolution over time of some volatile metabolites in the various cheeses during ripening can account for a different ratio between homofermentative types (lactococci, thermophilic lactobacilli, and *Streptococcus thermophilus*) and heterofermentative types (*Leuconostoc* spp. and many *Lactobacillus* spp.), particularly during the fermentation or the

Figure 4. Scanning electron micrographs of 30-d-old cheeses obtained from untreated milk (top), milk sanitized at 61°C for 20 min (middle) and high pressure homogenized milk at 1000 bar (bottom).
TABLE 2. Changes of total lipid content and of individual free fatty acids during ripening of cheeses obtained from milk that was subjected to different treatments.  

<table>
<thead>
<tr>
<th></th>
<th>UTR ripening phase (d)</th>
<th>HPH ripening phase (d)</th>
<th>SAN ripening phase (d)</th>
<th>PAST ripening phase (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 30 45</td>
<td>15 30 45</td>
<td>15 30 45</td>
<td>15 30 45</td>
</tr>
<tr>
<td>TL2 (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17.50</td>
<td>19.07</td>
<td>20.115</td>
<td>10.45</td>
<td>14.47</td>
</tr>
<tr>
<td>Total FFA (mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2889.39</td>
<td>3258.71</td>
<td>5248.64</td>
<td>2232.06</td>
<td>4784.70</td>
</tr>
<tr>
<td>Individual FFA, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C10</td>
<td>2.10</td>
<td>1.70</td>
<td>3.61</td>
<td>4.04</td>
</tr>
<tr>
<td>C16</td>
<td>43.60</td>
<td>38.20</td>
<td>37.86</td>
<td>38.36</td>
</tr>
<tr>
<td>C16:1</td>
<td>0.62</td>
<td>2.80</td>
<td>2.792</td>
<td>2.71</td>
</tr>
<tr>
<td>C18</td>
<td>4.55</td>
<td>4.05</td>
<td>4.00</td>
<td>3.86</td>
</tr>
<tr>
<td>C18:1</td>
<td>20.99</td>
<td>20.02</td>
<td>18.91</td>
<td>17.81</td>
</tr>
<tr>
<td>C18:2</td>
<td>2.18</td>
<td>2.39</td>
<td>2.20</td>
<td>2.28</td>
</tr>
<tr>
<td>C18:3</td>
<td>0.40</td>
<td>0.81</td>
<td>0.77</td>
<td>0.91</td>
</tr>
<tr>
<td>C10 + C12 (mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>155.14</td>
<td>342.90</td>
<td>678.04</td>
<td>297.80</td>
<td>524.22</td>
</tr>
<tr>
<td>Total FFA/TL, mg/g</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16.51</td>
<td>17.10</td>
<td>26.09</td>
<td>21.36</td>
<td>33.07</td>
</tr>
</tbody>
</table>

1Milk treatments: UTR = untreated control, HPH = homogenized at 1000 bar, SAN = sanitized at 61°C for 20 min, PAST = pasteurized at 72°C for 15 s.  
2Total lipids.

early phases of the ripening. In the 15-d-old HPH cheeses, the presence of acetaldehyde and ethanol was higher than in the other cheese types (Table 3). This result is in agreement with the major incidence of heterolactic types in the curd from HPH milk. Alternatively, the acetaldehyde and ethanol in the HPH cheeses could result from pyruvate metabolism by lactococci (3, 9).

The ethyl acetate, which can be attributed to yeast growth, slowly increased over time in the untreated and pasteurized cheeses. However, its occurrence was more precocious and more relevant in the HPH cheeses and, to a lesser extent, in the sanitized cheeses. In both products (sanitized and HPH cheeses), this metabolite (ethyl acetate) attained a maximum content after 1 mo, after which it decreased.

The sensory assessment of the four types of cheese is presented in Table 4. The organoleptic properties were evaluated in 30-d-old cheeses. The HPH cheese received a significantly higher overall grade than did the other samples. The taste, particularly the piquant flavor and the lack of bitter aftertaste, and the texture were the sensory attributes that contributed more to the rating of the HPH cheeses. These characteristics can be associated with an enhanced protein or lipid hydrolysis and with a texture that reflects the observed differences of the microstructure of cheeses. Both of these modifications can be attributed to the direct or indirect effect of the HPH. The creamy texture and piquant flavor can be considered desirable features for these types of caprine cheeses. However, for certain cheeses, it can be assumed that the HPH treatment can induce changes that make them unsuitable for meeting sensory criteria required to achieve specific attributes.

CONCLUSIONS

The HPH had both direct and indirect effects on cheese characteristics and their evolution during ripening. The direct effects were principally linked to the change in water-binding capacity of proteins as shown by the reduced whey separation.

The indirect effects involved microbial growth or activity, particularly 1) modifications of the lactic acid bacterial population that occurred naturally and its evolution, and 2) a more precocious yeast and mold growth with a consequent rapid rise in pH.

The higher initial concentration of the various microbial groups in the curd from HPH milk could, in part, be associated with the characteristics of the curd and, particularly, to the strong reduction in whey separation. The more precocious presence of free fatty acids, particularly short chain, could have an important role in the shift of the lactic acid bacterial population. Growth of homolactic species, such as Lac-
TABLE 3. Evolution of aroma compounds of cheeses obtained from milk subjected to different treatments.1

<table>
<thead>
<tr>
<th>Treatment2</th>
<th>Ripening phase</th>
<th>Acetaldehyde (µg/g)</th>
<th>Acetone (µg/g)</th>
<th>Ethanol (µg/g)</th>
<th>Ethylacetate (µg/g)</th>
<th>Diacetyl (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UTR</td>
<td>15</td>
<td>0.005</td>
<td>0.136</td>
<td>0.109</td>
<td>0.104</td>
<td>ND3</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.005</td>
<td>0.126</td>
<td>0.110</td>
<td>0.072</td>
<td>ND</td>
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<td>0.392</td>
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</table>

1Each value is the mean of five repetitions (SE < 0.005).
2Milk treatments: UTR = untreated control, HPH = homogenized at 1000 bar, SAN = sanitized at 61°C for 20 min, PAST = pasteurized at 72°C for 15 s.
3Not detectable with instrument.

Both these complex enzymatic activities were enhanced by HPH. However, the patterns of lipolysis did not show relevant qualitative differences, whereas the proteolytic activities gave rise to different hydrolysis patterns as evidenced by FTIR spectroscopy. The FTIR profiles of HPH cheeses in the zone of protein can be considered as a fingerprint of the hydrolysis pattern. In particular, in the ripened cheese (45 d), the profile between 1500 and 1700 cm⁻¹ of the HPH

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tobacillus helveticus, Streptococcus thermophilus, Lactococcus lactis subsp. cremoris, and Lactobacillus delbrueckii subsp. bulgaricus, have been reported (4) to be inhibited by oleic, caproic, and linoleic acids.

The activation of proteolytic and lipolytic activities observed in the HPH cheeses could be either an indirect effect on the shift of microbial population or a consequence of a different exposure of the macromolecules to enzymatic activity.

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TABLE 4. Influence of the milk treatments on sensory evaluation scores1 of cheeses2.

<table>
<thead>
<tr>
<th>Sensory criteria</th>
<th>UTR</th>
<th>HPH</th>
<th>SAN</th>
<th>PAST</th>
<th>F value</th>
<th>P value</th>
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<tbody>
<tr>
<td>Flavor and aroma</td>
<td>4.22a</td>
<td>4.33a</td>
<td>4.22a</td>
<td>4.28a</td>
<td>0.13</td>
<td>0.939470</td>
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<td>Texture</td>
<td>1.50b</td>
<td>4.61a</td>
<td>1.94b</td>
<td>2.27b</td>
<td>70.83</td>
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<tr>
<td>Creamy</td>
<td>3.05b</td>
<td>4.50a</td>
<td>2.77b</td>
<td>2.89b</td>
<td>35.54</td>
<td>0.000001</td>
</tr>
<tr>
<td>Bitter</td>
<td>3.99a</td>
<td>4.08a</td>
<td>3.84a</td>
<td>4.00a</td>
<td>1.65</td>
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<tr>
<td>Piquant</td>
<td>4.00a</td>
<td>4.11a</td>
<td>3.88a</td>
<td>4.17a</td>
<td>1.64</td>
<td>0.5767010</td>
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<tr>
<td>Overall assessment</td>
<td>1.44b</td>
<td>4.72a</td>
<td>1.88b</td>
<td>2.17b</td>
<td>82.33</td>
<td>0.000001</td>
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</tbody>
</table>

1Five-point sensory scale (0 = low intensity to 5 = high intensity, except bitter where 0 = high intensity and 5 = low intensity).
2The statistical indices reported are the F value of the overall model and the relative P value.
3UTR = untreated control, HPH = high pressure homogenization at 1000 bar, SAN = sanitization at 61°C for 15 min, PAST = pasteurization at 72°C for 15 s.
4Means with different superscripts in the same row differ (P < 0.05).
cheeses had a completely different pattern than did the other cheeses. These differences, observed only when the proteolysis was in a late phase, could account for conformational changes caused by pressure treatment.

At a molecular level, application of HPH might induce increased exposure of hydrophobic regions of protein, as happens with the high hydrostatic pressure, because of volume changes associated with their formation. In casein micelles, the large supramolecular structure is probably disrupted under high pressure and allows the components to move freely and become independent of the original structure. When the pressure is instantaneously released, interactions can reform, but, because of the independent movements of the components, the original structure is not reformed. Moreover, HPH probably has cavitation phenomena in common with ultrasound technology (16). However, this hypothesis requires further investigation.

This work, which used a semicontinuous homogenizer at 1500 bar, did not consider the molecular aspects of treatment and was focused on a preliminary study of the direct and indirect effects of the treatment on microbiological, biochemical, and microstructural characteristics of caprino, the traditional Italian goat cheese. Although some differences with respect to the control or heat-treated samples have been explained, the treatment proved to remarkably affect the cheese yield, the composition of the curd, and the ripening process.

Product innovation in the cheese industry is generally pursued by use of new ingredients, such as herbs or ham, or by an appropriate combination of starter cultures. The HPH used in combination with other process variables and appropriate starters culture can contribute to the production of new types of cheese or cheese products that have different textures, lipid contents, and redesigned proteolytic and hydrolytic patterns.

REFERENCES