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Original article

Subclinical udder infection with *Streptococcus dysgalactiae* impairs milk coagulation properties: The emerging role of proteose peptones

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Abstract – Subclinical mastitis, caused by different bacteria with similar milk composition and somatic cell count, impairs milk quality and its products differently through increased release of deteriorative enzymes into the milk. Milk from glands infected with *Streptococcus dysgalactiae* was almost identical in gross composition to milk from uninfected glands. However, yogurt and cheese made from commingled milk from the infected quarters exhibited inferior texture compared to yogurt and cheese made from uninfected ones. Proteose peptone was size-fractionated by gel filtration and the various fractions of milk from the infected glands were added to uninfected milk. This study demonstrated for the first time that addition of certain fractions to milk from uninfected glands resulted in altered milk coagulation properties. It is hypothesized that the infecting bacteria influence the immune system of the udder, which then impairs the qualities of the milk from infected quarters that is conventionally used for manufacturing dairy products.

intramammary infection / bacteria / milk quality / yogurt / cheese

摘要 – 停乳链球菌 (*Streptococcus dysgalactiae*) 引起的亚临床乳房感染对牛乳凝乳特性的影响。对于乳的组成和体细胞数量相近的牛乳, 由不同细菌引起的亚临床乳房炎对乳和乳制品的质量有着不同的影响, 这种现象主要是由于引起乳变质的酶的释放量增加而造成的。从感染停乳链球菌 (*Streptococcus dysgalactiae*) 的乳腺中分泌出的乳汁 (感染过的牛乳) 与未感染过牛乳的组成成分几乎相同。然而,用感染过和未感染过的原料乳生产的酸奶和干酪, 前者的质构性质明显地低于后者。采用凝胶过滤方法从感染过的牛乳中分离出示蛋白胨, 然后将分离出不同馏分分别加到未经感染的牛乳中, 试验结果证明, 由于含有示蛋白胨馏分的加入使得未感染牛乳的凝乳特性发生了改变。因此, 可以假设由于细菌的感染而影响了乳房的免疫系统, 结果影响到了分泌乳汁的质量, 进而影响到乳制品的质量。

乳腺炎 / 细菌 / 乳质量 / 酸奶 / 干酪

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Résumé – L'infection mammaire subclinique à *Streptococcus dysgalactiae* détériore les propriétés de coagulation du lait : rôle émergent des protéose-peptones. Les mammites subcliniques provoquées par différentes bactéries, à composition du lait et nombre de cellules somatiques similaires, détériorent d'une manière différente la qualité du lait et ses produits par relargage accru d'enzymes de dégradation dans le lait. Le lait des glandes infectées par *Streptococcus dysgalactiae* était pratiquement identique en composition brute à celui des glandes non infectées. Cependant, le yaourt et le fromage produits à partir du mélange des laits provenant des quartiers infectés présentaient une texture inférieure comparée à celle des yaourt et fromage produits à partir de lait des quartiers non infectés. La protéose-peptone était fractionnée par gel filtration et les différentes fractions du lait des glandes infectées étaient ajoutées au lait non infecté. Cette étude a démontré pour la première fois que l'addition de certaines fractions au lait de glandes non infectées altérait ses propriétés de coagulation. On suppose que les bactéries infectantes influencent le système immunitaire de la mammelle, qui ensuite détériore les qualités du lait des quartiers infectés qui est utilisé de façon conventionnelle pour fabriquer des produits laitiers.

infection intramammaire / bactérie / qualité du lait / yaourt / fromage

1. INTRODUCTION

It is well documented that mastitis, even in its predominant, subclinical form, impairs milk quality through the activation of the immune system, which increases the release of deteriorative enzymes into the milk, whose quality is subsequently affected by their action [6, 20, 26, 28]. High somatic cell counts (SCCs) are blamed for prolonged rennet clotting times and a weak coagulum, which, in turn, lead to increased moisture content in the coagulum and an overall lower cheese yield [4, 5, 7, 9, 18, 26, 29, 32, 34]. There is a complicated relationship between cheese yield and quality, on the one hand, and the factors involved in intramammary infection (IMI), such as bacterial species, inflammatory response and proteolysis of casein, on the other hand [23, 26]. Infecting bacteria may affect proteolysis of casein by secreting extracellular enzymes and promoting enzyme activity. Different bacteria may cause different types of physico-chemical damage to the milk [24, 26, 40]. Moreover, by activation of the host's innate immune system, milk from udders infected with different types of bacteria but with similar SCCs may have diverse characteristics related to a bacteria-specific mixture of leukocyte populations and leucocyte-associated proteases [12, 26]. Proteolysis of casein leads to increased levels of γ-caseins and proteose peptones (p-p) [4, 7, 13, 21, 22, 39].

Studies, on the individual gland level, of milk from sheep, goats and cows have demonstrated that curd parameters were directly related to measures of subclinical infection [25–27]. The poor quality of the curd strongly suggested that the damage caused to the casein micelle as a consequence of the bacterial infection was irreversible. Oxidative stress may enhance the susceptibility of casein to proteolytic activity, which was found to be particularly high in the case of infections with *Escherichia coli* and *Streptococcus dysgalactiae* [26, 37]. The innate immune system of the mammary gland also includes many non-cellular elements, such as lactoferrin [15], albumin [36] and various types of gangliosides [10, 30]. Xanthine oxidase and lactoperoxidase – enzymes capable of forming an oxidizing substance (hydrogen peroxide) and free radicals (nitric dioxide) – were recently identified as important components of the innate immunity system of the mammary gland [36, 38]. Thus, although these substances play a major role in antibacterial defense against pathogens such as *E. coli* and *Staphylococcus aureus*, they can also impair milk quality by oxidizing its fat and protein components, as well as causing substantial tissue injury.

In the present study, the influence of subclinical IMI caused by *S. dysgalactiae* on milk composition, milk clotting properties, and on the quality and quantity of products made from it were investigated. Proteose peptone fractions were isolated from the uninfected and *S. dysgalactiae*-infected milk and its influence on milk clotting parameters was evaluated.

2. MATERIALS AND METHODS

2.1. Animals

At the morning milking, milk was collected from Israeli-Holstein dairy cows, in each of which one quarter was chronically infected with *S. dysgalactiae* without clinical symptoms. All infected quarters were monitored for bacterial condition for 2–3 months prior to the beginning of the study. The cows involved in this study were in the mid- to late lactation stage, yielding between 28 and 52 L·day^{-1}. Prior to each test, each cow's bacterial status was confirmed on the basis of aseptic quarter foremilk samples that were taken three times at 2-day intervals and were sent to the laboratory for analysis within 1 h [33]. Testing was based on the animal model, in which the milk from an uninfected quarter was compared with that from the infected quarter [26]. On the production day every milk sample (of single-quarter milk or of commingled quarter milk) was subjected to SCC with the Fossomatic 360 (Foss Electric, Hillerod, Denmark), and the gross milk composition, i.e., protein, casein, fat and lactose contents, was determined with the Milkoscan FT6000 (Foss Electric) at the Israel Cattle Breeders' Association Laboratory (Caesarea, Israel). Curd firmness (Cf) and clotting time (Tc) were determined with the Optigraph (Ysebaert, Frepillon, France) as described by Leitner et al. [26].

2.2. Proteose peptone preparation and fractionation

Proteose peptone fractions from uninfected and infected milk were prepared according to Andrews [2]. To determine the influence of p-p on milk-coagulating factors, 0.5–2.0 mg·mL^{-1} size-separated fractions of the p-p, prepared by gel filtration on FPLC (see below), were added to milk from uninfected quarters, which were subjected to the Optigraph test. The assay was repeated three times with milk from different healthy quarters. The cows were milked thrice daily, at 05:00, 12:00 and 20:00, and were fed a typical Israeli total mixed ration comprising 65% concentrate (17% protein) and 35% forage.

Protein concentrations in the p-p fraction were determined according to Bradford [8], as adapted to ELISA plates. Alternatively, the optical absorbance at 280 nm was determined spectrophotometrically. Size-separation of the peptides by gel filtration was performed on precalibrated Superdex 75 (30 × 10) and Superdex Peptide (PE 7.5 × 300) Columns with an AKTA-FPLC System (Amersham, Uppsala, Sweden).

2.3. Yogurt-making

Yogurt was made from milk of eight cows infected with *S. dysgalactiae* in one quarter. The quarters were milked separately into quarter milk containers from the infected and the uninfected control quarters. The entire yogurt was made in duplicate and the process was repeated twice on two different dates from the same animal. Yogurt was made from milk that had been pasteurized at 90 °C for 3 min, by inoculating with a yogurt starter culture (YC-180; Chr. Hansen A/S, Hoersholm, Denmark). The mixture was poured into cups and incubated at 42 °C until it reached pH 5.2, after 6–7 h. The cups were then transferred

to 4 °C for stabilization and held at that temperature for 21 days pending testing.

2.4. Cheesemaking

Cheese was made from commingled milk obtained from one quarter of each of three cows infected with *S. dysgalactiae* and from three uninfected quarters. All the cheeses were made in duplicate from the same milk on the same day and the process was repeated two or three times with milk taken from different animals. Cheese was produced in a small dairy from milk that had been pasteurized at 70 °C for 1 min with no added starter culture. The pasteurized milk was heated to 28 °C and left to coagulate after addition of 0.7 mL of chymosin (Maxiren; DSM, Delft, The Netherlands) diluted with 25 mL of distilled water, to coagulate 10 L of milk in about 30 min. The curd was cut into 1-cm^3 cubes and allowed to set, to promote curd firmness, for 40 min. It was then stirred for 30 min, with gradual heating to 42 °C, after which the curd was poured into cylindrical perforated plastic molds. The cheese blocks (5 from every milk origin), each weighing about 300 g and measuring about 100 mm in diameter and about 50 mm in height, were incubated for 48 h at 14 °C, and then packed in vacuum in plastic bags and left for 30 days for ripening at 14 °C at a relative humidity of 70–80%, with periodical turning over. On day 31, whey was drained out of the bags and weighed, and the blocks were repacked in vacuum and stored under refrigeration at 4 °C for final ripening. At 2, 14, 33 and 144 days of ripening, a single block from each cheese was taken for immediate analysis.

2.5. Cheese and yogurt analysis

The total nitrogen (TN) content of the cheese was determined by micro-Kjeldahl [17] using a Kjeltec system 1002 (Tecator, Höganäs, Sweden). Fat was determined in 3-g cheese samples with a Van Gulik butyrometer. All determinations were made in duplicate.

Water-soluble nitrogen (WSN) was determined essentially according to Fox and Kuchroo [19]. The ripening extension index (represented by the ratio WSN/TN), the ripening depth index (represented by the ratio TCA-soluble N/TN) [16] and the free amino acid index (represented by the ratio PTA-soluble N/TN) [3] were calculated. All determinations were performed in duplicate.

Fractions of p-p from milk samples of both uninfected quarters and *S. dysgalactiae*-infected quarters were each size-fractionated by gel filtration on Superdex 75 and Peptide columns, using FPLC.

The textures of the yogurt and cheese were evaluated with a Texture Analyzer TA XT2 (Stable Micro Systems, Godalming, Surrey, UK). The probe was made of stainless steel cut by laser to a spiral form measuring 240 mm in length, 40 mm maximum diameter, with a cross-section of 3 mm height and 2 mm width. Cheese texture was measured by using a puncture test with a 2-mm round probe applied to 2-cm^3 cheese samples. The probe penetrated 20 mm into the yogurt sample and 10 mm into the cheese at 1 $mm \cdot s^{-1}$. All analyses were carried out in five replicates and the results were recorded continuously on a computer and are presented as the force in Newtons (N), averaged with respect to the penetration distance of the probe.

2.6. Statistical analysis

The measured data (log SCC, p-p, Tc, Cf, and milk composition as fat, protein and lactose) were analyzed according to a model that takes into account the cow and the infection status of the quarter, i.e., uninfected, or infected by S. *dysgalactiae*, applying a two-way ANOVA design by using

Table I. Average and standard error of log SCC, fat, protein, lactose, clotting time and curd firmness with statistical significance of milk from 22 quarter-udders (11 cows), uninfected and infected with *S. dysgalactiae*.

	Uninfected quarter	*S. dysgalactiae*-infected quarter	*P* [F]
log SCC	4.77 ± 0.3^{c}	6.74 ± 0.7^{a}	< 0.001
Fat ($g \cdot L^{-1}$)	33.6 ± 3.8	34.0 ± 4.1	NS
protein ($g \cdot L^{-1}$)	34.5 ± 3.1	34.7 ± 3.0	NS
lactose ($g \cdot L^{-1}$)	49.7 ± 0.8^{a}	44.1 ± 0.7^{b}	< 0.001
Proteose peptone ($\mu g \cdot mL^{-1}$)	470 ± 5.0^{a}	1030 ± 12.0^{c}	< 0.001
Clotting time (s)	1179 ± 152.0^{b}	2940 ± 183.0^{a}	< 0.001
Curd firmness (V)	10.9 ± 1.2^{a}	3.9 ± 2.1^{c}	< 0.001

[a–c] Means within a row with no common superscript differ significantly ($P < 0.05$).
NS: Not significant.

the following linear model: $Y_{ijk} = \mu + \alpha_i + \beta_j + e_{ijk}$, in which μ is the grand mean; α_i is the effect of the *i*th cow; β_j is the effect of the *j*th infection status; and e_{ij} represents the residual between-quarters error. Comparisons between infection group's pairs were made by t-test using the Tukey-Kramer HSD. All statistical analyses were carried out with JMP software [35].

3. RESULTS

The data on the milk utilized for yogurt and cheese production are summarized in Table I; it includes mean and SE values of log SCC, fat, protein and lactose, Tc and Cf with statistical significance, of 22 udder-quarters (11 cows) uninfected, or infected with *S. dysgalactiae*. The log SCC was overall significantly higher in the milk of the infected quarters. No difference was found between the fat and protein contents of milk from infected and uninfected glands, whereas lactose was significantly lower in the milk from the infected quarters. The Tc was significantly longer in milk from uninfected glands and Cf was significantly lower in the milk from the infected quarters (Tab. I).

3.1. Proteose peptone fractions

Addition of lyophilized powder of p-p from healthy quarters to uninfected milk and to milk from *S. dysgalactiae*-infected quarters resulted in increased Tc and decreased Cf in a dose-dependent manner (Fig. 1). The addition of p-p powder from milk from *S. dysgalactiae*-infected quarters had a similar effect but to a greater extent, with larger increases in Tc and reductions in Cf at the same concentrations.

Proteose peptone fractions obtained by gel filtration were divided into five zones, A–E (Fig. 2, zone D is not marked since no peptides were detected in it). As shown in Figure 2, no marked difference was observed between the infected and uninfected p-p in zone A. In contrast, marked differences were observed in zones B, C and E. In fact, peptides in zone C were observed only in p-p derived from milk from *S. dysgalactiae*-infected quarters, and were absent from the uninfected quarters. The concentrations of peptides of zone E were also higher in p-p from infected quarters than in p-p from healthy quarters. This zone also contained residual acetone, which was added during p-p preparation, and was removed by evaporation prior to further studies of Fraction E. Fractions in each of zones A, B, C and E were pooled separately for further analysis.

The effect of each of the pooled fractions A, B, C and E on milk clotting parameters was determined by addition of samples of each of these fractions to

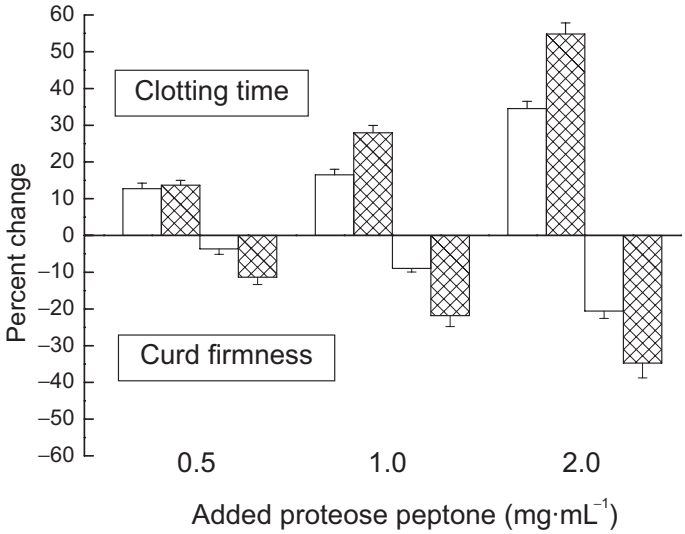

Figure 1. The influence of addition of proteose peptone powder from uninfected quarters (open bars) and from *S. dysgalactiae*-infected quarters (hatched bars) to cow's milk free of udder infection, on clotting time and curd firmness, as measured by the Optigraph. Percent change was calculated from values of the uninfected milk.

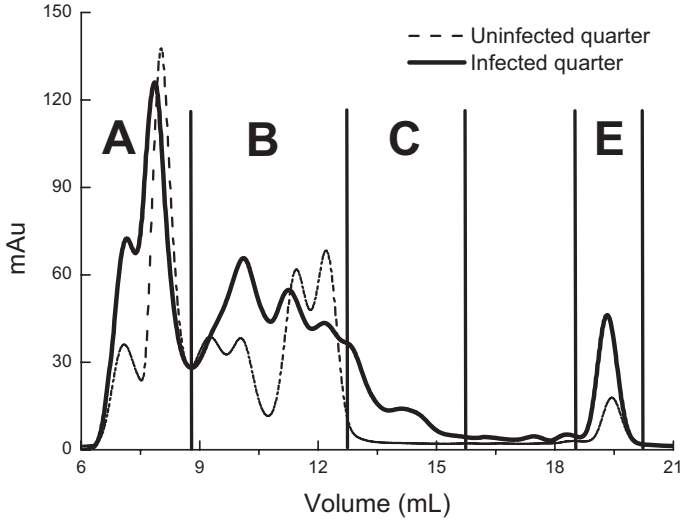

Figure 2. FPLC fractions of proteose peptone prepared from milk of uninfected quarters and *S. dysgalactiae*-infected quarters.

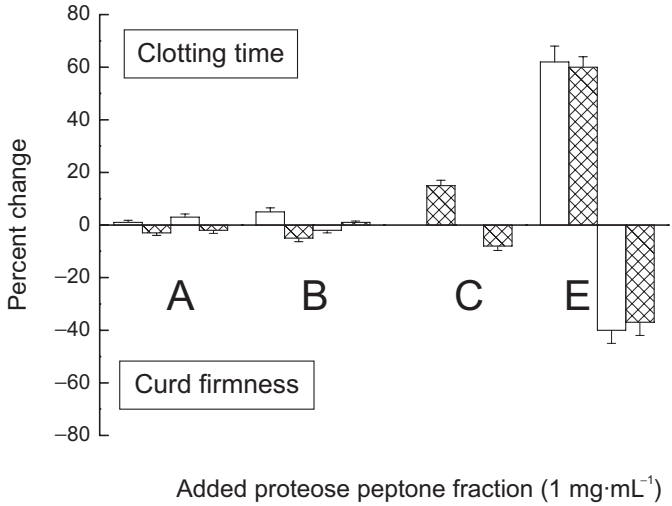

Figure 3. Effect of addition of proteose peptone fractions A, B, C and E from milk obtained from uninfected quarters (open bars) and from *S. dysgalactiae*-infected quarters (hatched bars) to cow's milk free of udder infection, on clotting time and curd firmness, as assessed by the Optigraph. Percent change was calculated from values of the uninfected milk.

milk from uninfected quarters at about 500 μg·mL^{-1}, and then applying an Optigraph test (Fig. 3). Addition of fractions A and B caused hardly any change in coagulation parameters of the milk. Fraction C had a moderate negative effect on clotting parameters, increasing Tc and reducing Cf. In contrast, addition of fraction E, regardless of its origin – infected- or uninfected-quarter milk – resulted in marked changes in coagulation parameters: increasing Tc and reducing Cf.

In conclusion, it was noted that excess of p-p added to milk caused changes in its clotting parameters, increasing Tc and decreasing Cf. These changes were greater with p-p from *S. dysgalactiae*-infected milk than with that from uninfected milk. Apparently, this effect is not caused by the high-molecular-weight fractions – A and B – but, in part at least, by the low-molecular-weight fractions, C and E. The specific effect of fraction C, which was not found in p-p of uninfected milk, on milk clotting parameters remains to be investigated.

3.2. Yogurt production and testing

Yogurt was made from *S. dysgalactiae*-infected milk, and was compared with that made from uninfected milk. Measurement of pH change revealed no difference in development of acidity, between milk from the two sources in the same animal, but the texture analyzer results showed the yogurt made from milk originated in *S. dysgalactiae*-infected glands was softer than that made from uninfected-quarter milk (Fig. 4). No difference was found between day 2 and day 21.

3.3. Cheese production and evolution of cheese yield and quality

Clotting time was almost 2.5 times longer for the milk from the *S. dysgalactiae*-infected quarters, and after cutting,

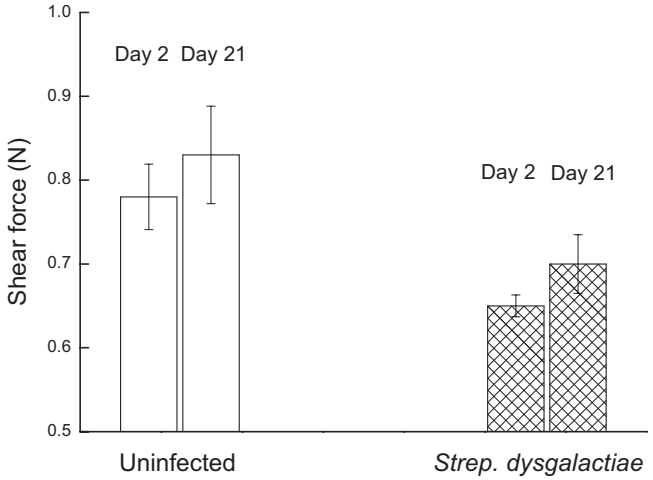

Figure 4. Shear force in yogurt produced from milk from uninfected quarters and from *S. dysgalactiae*-infected quarters on two testing dates.

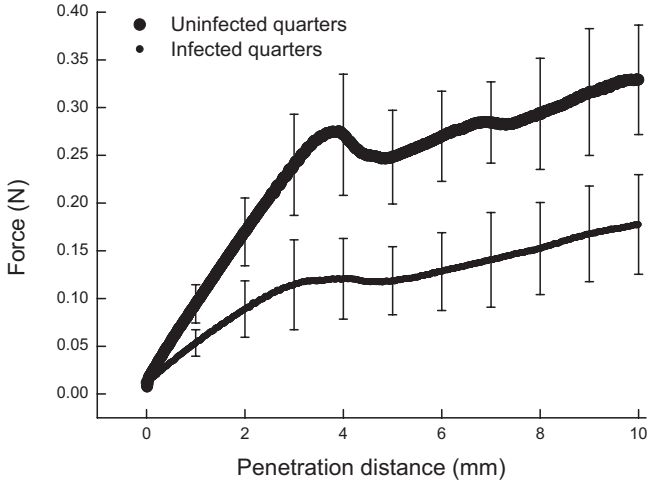

Figure 5. Measurement of cheese firmness using the Texture analyzer, performed on cheese blocks two days after production.

the curd made from these quarters had a fragile texture, which resulted in a noticeable amount of curd fines and, consequently, curd losses. During the two days before vacuum packing, whey from the healthy quarters was transparent, whereas that from the infected quarters was cloudy. Texture analysis performed on the cheese blocks before packing (two days) showed that the fresh cheese prepared from the *S. dysgalactiae*-infected quarters was much softer than that prepared from uninfected quarters (Fig. 5).

Cheese yield from milk from uninfected quarters was 7.6% higher than that from *S. dysgalactiae*-infected quarters, as

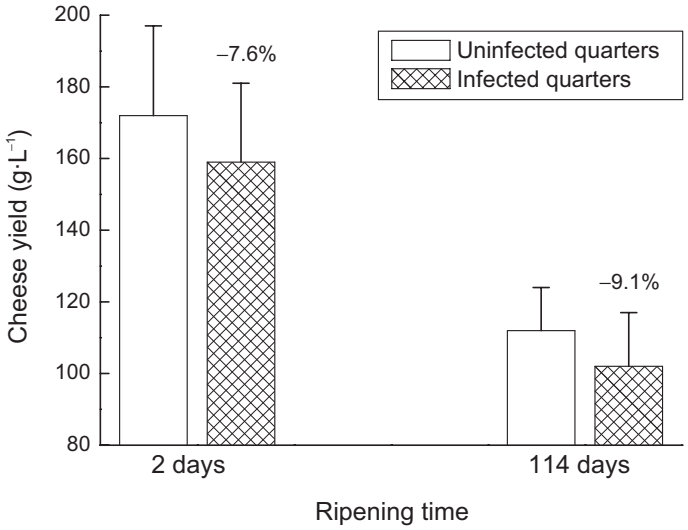

Figure 6. Cheese yield of the two milk sources, i.e., uninfected and infected with *S. dysgalactiae*, calculated from the cheese blocks' weight.

indicated by weighing the cheese blocks after two days (Fig. 6). In the first 33 days of ripening, syneresis was more pronounced in the cheese made from milk from the uninfected quarters than in that made from milk from *S. dysgalactiae*-infected quarters, which resulted in higher loss of whey. However, at the end of maturation (114 days), the difference between the cheese yield of uninfected quarters and that of *S. dysgalactiae*-infected quarters increased to 9.1% (Fig. 6), because of greater evaporation. A significant difference in yield ($P < 0.05$) was found only after 114 days.

Fat and dry matter contents were insignificantly lower at all stages of maturation in cheese that originated in *S. dysgalactiae*-infected quarters than in that from uninfected quarters, although the fat and protein contents in the raw milk were similar (Tab. II). The relevant indices dramatically indicated that proteolysis was much more intense in the cheese made from milk that came from *S. dysgalactiae*-infected quarters than in that from healthy quarters; there were great differences between the two sources of cheeses in their indices of ripening (WSN), depth of ripening (TCAN) and level of free amino acids (PTAN).

4. DISCUSSION

In an earlier study [26] it was reported that certain bacteria caused marked deteriorations in curd yield and curd firmness in milk from infected glands, while other bacteria had only minor effects, and that these effects were species-specific. In the present study, it was further demonstrated that such deteriorative measures also appeared in yogurt and cheese made from milk of *S. dysgalactiae*-infected quarters. These findings suggest that Ct and Cf observed in milk from udder-quarters infected by different bacteria, as reported by Leitner et al. [26], show a similar trend in milk of *S. dysgalactiae*-infected quarters and also result in lower yield and quality of yogurt and cheese.

Table II. Average parameters of cheese composition: fat, dry matter, total nitrogen (TN) and water-soluble nitrogen (WSN); trichloroacetic acid-soluble nitrogen (TCAN) and phosphotungstic acid-soluble nitrogen (PTAN) during cheese ripening in cheese from uninfected quarters and quarters infected with *S. dysgalactiae*.

	Uninfected quarters				*S. dysgalactiae*-infected quarters			
Ripening days	2	14	33	114	2	14	33	114
Fat ($g \cdot L^{-1}$)	18.0^{C}	26.8^{B}	31.5^{A}	31.5^{A}	17.8^{C}	22.2^{B}	30.5^{A}	30.5^{A}
Dry matter ($g \cdot L^{-1}$)	34.51^{C}	47.15^{B}	47.47^{B}	52.93^{A}	32.80^{C}	43.45^{B}	46.15^{B}	49.27^{A}
TN (%)	2.38^{B}	2.91^{A}	3.02^{A}	3.39^{A}	2.19^{C}	2.83^{B}	3.18^{B}	3.52^{A}
WSN·TN^{-1} (%)	8.82^{C}	15.46^{B}	17.61^{B}	34.86^{bA}	13.70^{C}	17.31^{B}	20.00^{B}	52.76^{aA}
TCAN·TN^{-1} (%)	3.36^{C}	10.65^{B}	10.93^{B}	17.43^{bA}	5.02^{C}	10.60^{B}	11.32^{B}	34.97^{aA}
PTAN·TN^{-1} (%)	0.38^{B}	0.45^{B}	0.53^{B}	2.90^{bA}	0.41^{B}	0.49^{B}	0.79^{B}	16.11^{aA}

$^{A–C}$ Means within a row with no common superscript differ significantly ($P < 0.05$) between ripening days.

$^{a–c}$ Means within a row with no common superscript differ significantly ($P < 0.05$) between uninfected and infected quarters in the course of ripening.

It is well documented that high SCC affects cheese quality [1, 4, 7, 31]. However, so far, the changes in quality parameters have not been related to the bacterial species, but rather to the number of somatic cells and to milk indigenous enzymes [11, 29]. It is therefore of utmost importance to note that SCC actually reflects herd udder health status, and thus, the deleterious effect is not the number of cells present in the milk itself, but rather the IMI status of the herd milked into the bulk tank. This point is strengthened by the findings that the plasmin level is almost doubled in infected quarters in comparison with uninfected ones and is similar among the various bacteria species [26]. It was also noted that no relation was found between SCC in the bulk milk tank ($200–300 \times 10^3 \cdot mL^{-1}$) and the deteriorative measurements of milk clotting parameters [28]. It is suggested that part of the difference in the quality parameters of the cheese is related to the interactions between the bacteria and the host's innate immune system, as can be seen from the increases in all the soluble nitrogen fractions. It is well established that during cheese maturation, caseins undergo proteolysis, and that the released peptides contribute significantly to the maturation process. Our data clearly indicate that excessive degradation of caseins occurs during the maturation of cheese prepared from glands infected with *S. dysgalactiae*, leading to what may be characterized as over-ripening. The excessive degradation of casein is most likely associated with liberation of a large amount of peptides from the C-terminal end of α_{s1}- and β-caseins, which are known to be hydrophobic and bitter [14], although this aspect was not investigated.

The FPLC profile of milk that originated in *S. dysgalactiae*-infected glands revealed several p-p fractions not present in milk that originated in uninfected glands, or fractions that increased in the milk from *S. dysgalactiae*-infected glands. Addition of fractions C (which appears only in infected glands) and E to milk from uninfected glands resulted in increased Tc and decreased Cf. It is important to note that fraction E, which contained low-molecular-weight peptides, was also present in high-quality milk from uninfected glands, but at low concentrations. These findings point to the effect of *S. dysgalactiae* as an initiator or precursor of the

formation of short-chain peptides, which could interfere with the coagulation process. The present study has shown for the first time, to the best of our knowledge, that peptides that are probably liberated from casein during proteolysis of milk have negative effects on clotting time and curd firmness. Thus, proteose peptones, which are classically recognized as casein derivates that are liberated during storage of milk for cheese processing, are in addition, a source of material that quite dramatically impedes the curdling process. Furthermore, these peptides and/or morphological changes in the remaining casein hamper cheese maturation, as reflected here by the excessive casein degradation in cheese made from glands infected with *S. dysgalactiae*.

5. CONCLUSIONS

This study demonstrated once again that infection of the mammary gland with *S. dysgalactiae* is specifically devastating with regard to milk properties relevant to cheese production. The study also highlights a novel phenomenon, i.e., that the excessive degradation of casein, noted previously in raw milk, continues during cheese maturation, leading to over-mature cheese with defective texture and appearance, attributed to the excessive liberation of peptides from casein. These properties of the cheese are most likely a result of the biochemical changes that occur during the storage of the milk in the gland (between milking), and that are caused by the presence of the bacteria. Thus, the model of the present study offers an experimental approach for further investigation of the interrelationship between different IMI-causing bacteria and these putative biochemical changes and the biochemistry of cheese maturation.

In light of these findings it is time to revise the long-lasting approach of reducing the number of SCC in the bulk milk tank below $250 \times 10^3 \cdot mL^{-1}$ and to pioneer new management procedures by preventing milk containing certain IMI-causing bacteria from entering the bulk milk tank.

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