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ORIGINAL ARTICLE

# DEGRADATION OF MILK PROTEIN FRACTIONS DUE TO *PSEUDOMONAS* SPP. IN THE DAIRY INDUSTRY

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**Ethical considerations:** When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published in July 14, 2020 (https://doi.org/10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

## ABSTRACT

**Milk is a nutritionally important food, particularly due to its milk protein content. The representation and degradation of individual protein fractions in raw cow's milk were monitored using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), with a focus on the presence of undesirable milk microbiota and somatic cell count (SCC). Raw cow's milk samples (n = 240) were collected from three farms in accordance with the principles of the international standard STN EN ISO 707 during the autumn 2022/winter 2022/spring 2023/summer 2023. After cultivating microorganisms (ISO/TS 11059), 73 isolates of *Pseudomonas* spp. were identified using the polymerase chain reaction (PCR). Subsequently, qualitative parameters, including somatic cell count, protein content, and protein profile, were analyzed. The results showed statistically significant differences ($p < 0.001$) in relation to seasonal changes. The findings indicate significant interactions between the presence of *Pseudomonas* spp., somatic cell count, seasonality, and the content and composition of milk proteins ($p < 0.001$). The presence of *Pseudomonas* spp. and a high somatic cell count contributed to a decrease in the $\alpha_{s2}$-casein, $\alpha_{s1}$-casein, and ß-casein fractions, accompanied by an increase in serum proteins β-lactoglobulin and α-lactalbumin ($p < 0.001$). The negative impact of these changes in protein composition affects the further processing of milk in the dairy industry, particularly in cheese production. The aim of this work was to determine the level of the protein fractions degradation caused by the presence of undesirable dairy *Pseudomonas* spp. using proteomic methods, considering SCC.**

**Keywords: electrophoresis; microorganisms; milk; proteins; somatic cells**

## INTRODUCTION

Milk is not only an important source of protein in the human diet but also serves as a crucial source of protein for the food industry [1]. Milk proteins make up approximately 3.2–3.8 % of the total milk composition. They are categorized into two groups based on their reaction during the acidification of milk to a pH value of 4.6. The first group consists of stable micelles that form a complex of caseins $\alpha_{s1}$-casein ($\alpha_{s1}$-CN); $\alpha_{s2}$-casein (αs2-CN); β-casein (β-CN); and κ-casein (κ-CN) [2]. The second group consists of whey proteins, which, unlike caseins, are soluble. Key whey proteins include α-lactoalbumin (α-LA) and β-lactoglobulin (β-LG) [3]. All these protein fractions can be separated using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). After treatment with SDS, regardless of their native charge, all proteins acquire a high negative charge. Denaturation of proteins is accomplished by heating them in a buffer containing a soluble thiol reducing agent (2-mercaptoethanol; dithiothreitol) and SDS. Mercaptoethanol reduces all disulfide bonds of cysteine residues to free sulfhydryl groups, and heating in SDS disrupts all intramolecular and intermolecular protein interactions. Denatured proteins are subsequently separated electrophoretically strictly according to their molecular size in a polyacrylamide gel [4].

The composition of milk significantly influences its physicochemical properties, yield, and the quality of dairy products. Casein is the primary protein needed for cheese production. During the technological process of cheese making, most whey proteins transition into liquid form of whey [5]. The casein content, along with factors such as somatic cell count (SCC), temperature, the amount of soluble $Ca^{2+}$ ions, and titration acidity (up to 8 °SH), greatly influences milk coagulation, forming the curd. The yield and composition of the main proteins in cow's milk determine the value of the milk depending on its intended use. High-quality milk with a high casein content, especially κ-CN, is necessary for dairy production especially cheese production. Processing such high-quality milk results in increased yield, stronger curd formation, and minimal casein loss in the whey [1,6].

Psychrotrophic bacteria are ubiquitous and can grow at low temperatures (< 7 °C), often multiplying during the storage of raw milk at refrigerated temperatures [7]. The group of psychrotrophic microorganisms consists of Gram-positive microorganisms, such as *Aeromonas hydrophila* a Yersinia enterocolitica and Gram-negative microorganisms, such as *Pseudomonas* spp., *Listeria monocytogenes* and *Bacillus cereus* that is particularly important from a food point of view, since the storage of many foods at low temperatures is a common practice during production, processing and food transport [8].

The genus *Pseudomonas*, dominate the microbial community in stored, refrigerated raw cow milk, and may also be a contaminant of pasteurised milk. The proteolytic activity of *Pseudomonas* spp. in raw cow's milk is an important factor affecting the final quality of dairy products. Milk contains microbial proteases that can cause casein degradation and thus affect the processing of dairy products. The breakdown of milk proteins by proteases negatively affects milk coagulation (coagulation, gelatinization), cheese ripening, ultra-high temperature (UHT) milk shelf life, flavor formation and the texture of dairy products. Microbial proteases cleave milk protein fractions with varying intensity. Whey proteins are resistant to proteases, as indicated by their minimal degradation [9, 10, 11]. Determining the microbiological quality of food provides important information for assessing food safety and serves as an essential indicator for studying protein quality in milk and dairy products [12].

The increase in the presence of *Pseudomonas* spp. is also accompanied by an increase in the number of somatic cells, which indicate the health status of dairy cows. Somatic cells are naturally present in small amounts in milk. Their primary function is to fight disease and help repair damaged tissue. Any intramammary infection (mastitis) leads to an increase in these cells in the milk [13]. The quality of raw cow's milk intended for processing for human consumption is very important, and therefore raw cow's milk must meet certain minimum criteria for the number of somatic cells and the total number of microorganisms. The number of somatic cells at 30 °C in 1 $cm^3$ may not exceed 400,000 colony forming units (CFU). The criteria for the total number of microorganisms determined at 30 °C in 1 $cm^3$ may not exceed 100,000 CFU [14].

The aim of this work was to determine the protein fractions degradation using proteomic methods caused by the presence of *Pseudomonas* spp., taking SCC into account.

## MATERIALS AND METHODS

### Collection of samples

Raw cow's milk samples were collected from three farms (FMa, FMb, FMc) located in the Slovak Republic regions Abov, Spiš and Zemplín. The experimental period spanned from autumn 2022/ winter 2022/ spring 2023/ summer 2023, with a total of 240 samples taken. On each farm an experimental group of 20 Slovakian spotted dairy cows was selected. Samples were collected quarterly, during each season (80 raw cow's milk samples from each farm in total), obtained from morning milking. The farms were selected based on identical housing, husbandry practices, and feeding methods. Sample collection adhered to the principles of STN EN ISO 707 [15], conducted by the farm's zootechnician. The milk samples were transported to the Department of Hygiene, Technology and Food Safety of the Faculty of Veterinary Medicine in Košice. During transport and until the time of analysis, the raw cow's milk samples were stored at a temperature of 6 °C. The samples were subjected to microbiological analysis within three hours and subsequently to physicochemical determination (SCC, protein content and SDS-PAGE). Microbiological analysis was aimed at determining the presence of bacteria of the genus *Pseudomonas*, which are an indicator of the hygienic quality of milk and can also contribute to the development of inflammation of the mammary gland.

### Cultivation and isolation of microorganisms of the genus *Pseudomonas*

According to the principles of STN EN ISO 6887-5 [16], suitable tenfold dilutions were prepared from the raw cow's milk samples, which were inoculated in an amount of 0.1 ml onto the surface of the pre-dried selective diagnostic medium Pseudomonas Agar Base (Hi-Media, India) supplemented with penicillin (100,000 IU/l, Dr. Ehrenstorfer GmbH, Augsburg, Germany) and pimaricin (0.01 g/l, Dr. Ehrenstorfer GmbH), which is used for selective isolation of *Pseudomonas* bacteria. After the end of incubation for 48 hours at 25 °C by ISO/TS 11059 [17], 5 identical solitary colonies from each plate were used for further identification.

### Identification of isolates of *Pseudomonas* spp.

The isolated strains were primarily identified based on their phenotypic characteristics. Strains that were positive for oxidase and catalase tests, and partially negative for Gram staining and glucose fermentation were evaluated and identified as *Pseudomonas* spp. [18,19]. These strains were subsequently subjected to identification using the polymerase chain reaction (PCR) method [20]. The reference strain *Pseudomonas aeruginosa* CCM 1960T (Czech Collection of Microorganisms, Brno, Czech Republic) was used as a positive control.

### Determination of somatic cell count by fluorescence microscopy

A milk sample of 50 ml was transferred to a glass tube, at room temperature, which was then mixed using Vortex. Next, 100 µl of milk sample was taken and transferred to a microtube with lyophilization dye (Sofia Green). Subsequently, the sample was mixed, left to rest, mixed again and an amount of 8 µl of the prepared sample was transferred to the LACTOCHIPx4 (Milkotronic Ltd., Nova Zogora, Bulgaria) [21]. The results of SCC determination are given in log units.

### Determination of protein content

The milk protein content (%) was determined using the Lactoscan MCCW device (Milkotronic Ltd., Nova Zogora, Bulgaria). [21].

### Detection of protein fractions by SDS-PAGE electrophoretic method

Milk samples were stored at -18 °C until SDS-PAGE electrophoresis was performed. Before use, milk samples were thawed, diluted, and adjusted for protein content (5 µl) using a NanoDrop 2000 Spectrophotometer [22]. Electrophoretic detection of protein fractions was performed using a vertical electrophoretic system (Bio-Rad, California, USA). A combination of two gels (14 % separation gel and 4 % focusing gel) with a thickness of 0.75 mm was used to separate the protein fractions. After the polymerization time, the tested raw cow's milk samples (20 µl) were placed in the formed wells. 180 V, 100 mA for 1.5 h was used for focus of the samples. Subsequently, the gel was fixed, stained and destained. Using GelAnalyzer 19.1 software, the relative amounts of individual protein fractions were analysed and determined [23, 24, 25]. Two molecular weight markers from 6.5 to 66 kDa and one molecular weight marker from 14 to 66 kDa (Serva, iBioTech) were used to determine the molecular weight of pro-

teins. The following protein fraction standards were used: bovine milk lactoferrin, casein $\alpha_{s1}$, casein $\alpha_{s2}$, casein-β, casein-κ, β-lactoglobulin, α-lactalbumin, and serum albumin (Sigma-Aldrich, Germany).

**Statistical evaluation of experiment results**

One-way analysis of variance (ANOVA) and Tukey's test for multiple comparisons with a 95 % confidence interval were used for evaluation using GraphPad Prism 8.3.0.538 statistical software (GraphPad Software, San Diego, CA, USA) [21].

## RESULTS

Raw cow's milk samples were collected quarterly during the experimental period (autumn 2022/ winter 2022/ spring 2023/ summer 2023) from three farms. From total of 240 milk samples taken, 151 isolates were confirmed based on phenotypic characteristics which showed a positive oxidase and catalase reaction, and at the same time were negative for Gram staining and glucose fermentation. Isolates (n = 151) obtained from milk samples originating from FMa, FMb and FMc were subsequently subjected to identification using the PCR method, which was aimed at determining the presence of the 16S rRNA gene characteristic of the genus Pseudomonas, which was confirmed in 73 isolates.

The quantitative representation of isolates on individual farms also showed differences in their presence in relation to the season. On FM*a*, we captured a total of 17 isolates (23.29 %), of which the following amount was collected: spring (6 isolates; 35.29 %), summer (8 isolates; 47.06 %), autumn (1 strain; 5.88 %), winter (2 isolates; 11.76 %). On FM*b*, we obtained a total of 21 isolates (29 %), which were isolated during the following seasons: spring (7 isolates; 33.33 %), summer (9 isolates; 42.86 %), autumn (3 isolates; 14.29 %), winter (2 isolates; 9.52 %). Despite the identical management of dairy cattle breeding on farms, we noted an increased number of monitored bacteria of the genus *Pseudomonas* on FM*c*, where we obtained a total of 35 isolates (47.95 %), of which: spring (12 isolates; 34.29 %), summer (15 isolates; 42.86 %), autumn (4 isolates; 11.43%), winter (4 isolates; 11.43 %).

Using the Lactoscan SCC device, quantitative determination of SCC was performed in samples of raw cow's milk originating from FM*a*, FM*b* and FM*c* (Table 1). The results of the determination of SCC indicate significant differences in the milk quality of the sampled farms and also indicate a statistically significant effect of the change in the season $p < 0.0001$.

The protein content was determined on the farms quarterly during the entire experimental period, on the basis of which we were able to record one of the important factors affecting the protein content, i.e. change of seasons (autumn, winter, spring, summer) and associated SCC. Despite identical characteristics (breeding management, breed of cattle, feed ration), different protein content values were determined on the farms (Table 2). These differences were most pronounced ($p < 0.0001$) during the spring season between FMa (3.44 ± 0.10 %) and FMc (3.59 ± 0.11 %) and also between FMb (3.43 ± 0.14 %) and FMc (3.59 ± 0.11 %). Dairy cows with SCC more than 5.60 log exceed the maximum permitted values set by Regulation (EC) 853/2004.

Based on the results of determining the somatic cell count, the samples of raw cow's milk were divided into four experimental groups (ExG): ExG-1: SCC < 5.3 log (n = 20), ExG-2: SCC > 5.3 log < 5.6 log (n = 20), ExG-3: SCC > 5.6 log (n = 20), ExG-4: SCC > 5.3 log with the presence *Pseudomonas* spp. (n = 20). In the investigated milk samples (n = 80), considerable differences were noted in the presence and amount of individual protein (casein and whey) fractions (Table 3). Experimental group InG-4 contained samples of naturally contaminated milk with the presence of *Pseudomonas* spp. confirmed by PCR method. Samples in this group were selected from all farms we investigated, with the SCC in this group being higher than 5.6 log in n = 7 milk samples.

A detailed analysis of the protein fractions: SA, $\alpha_{s2}$-CN, $\alpha_{s1}$-CN, β-CN, κ-CN, β-LG, α-LA, which was carried out by SDS-PAGE electrophoresis, showed us the relationship between the relative amount of protein fractions, protein content (%), SCC (log) and, above all, the microbiological (presence of *Pseudomonas* spp.) milk quality. As can be seen from Fig. 1, the content of milk proteins varied from 3.18 % to 3.59 %, but we observed more significant changes in the representation of individual protein fractions.

In the experimental group ExG-1 and ExG-2, where SCC was below 5.6 log, the relative amount of protein fractions of caseins SA, $\alpha_{s2}$-CN, $\alpha_{s1}$-CN, β-CN was significantly dominant. We noted a significant decrease and

**Table 1. SCC (log ± sd) determined on farms (FM*a*, FM*b*, FM*c*) during the experimental period (autumn, winter, spring, summer)**

| Farm | SCC [log] | | | | |
|---|---|---|---|---|---|
| | Autum | Winter | Spring | Summer | p value |
| FM*a* | 5.11 ± 2.21$^{b,2}$ | 5.07 ± 2.21$^{b,2}$ | 5.52 ± 2.89$^{ab,2}$ | 5.71 ± 3.03$^{a}$ | < 0.001 |
| FM*b* | 6.01 ± 3.08$^{1}$ | 5.98 ± 2.91$^{1}$ | 5.92 ± 3.05$^{1}$ | 6.00 ± 3.13 | > 0.05 |
| FM*c* | 5.91 ± 3.32$^{1}$ | 5.79 ± 3.23$^{1}$ | 5.65 ± 2.99$^{1,2}$ | 5.80 ± 3.17 | > 0.05 |
| p value | < 0.001 | < 0.0001 | < 0.05 | > 0.05 | |

$^{a, b, c}$ - values in rows with different superscripts are statistically different (p < 0.05)
$^{1,2,3}$ - values in columns with different superscripts are statistically different (p < 0.05)
sd - standard deviation

**Table 2. Percentage protein content (mean ± sd) determined on farms (FM*a*, FM*b*, FM*c*) during the experimental period (autumn, winter, spring, summer)**

| Farm | Protein content [%] | | | | |
|---|---|---|---|---|---|
| | Autumn | Winter | Spring | Summer | p value |
| FM*a* | 3.38 ± 0.08$^{a,b}$ | 3.45 ± 0.11$^{a,2}$ | 3.44 ± 0.10$^{a,2}$ | 3.30 ± 0.12$^{b,c}$ | < 0.0001 |
| FM*b* | 3.36 ± 0.15$^{a,b}$ | 3.36 ± 0.18$^{a,b,1}$ | 3.43 ± 0.14$^{b,2}$ | 3.30 ± 0.11$^{a}$ | < 0.05 |
| FM*c* | 3.40 ± 0.15$^{b}$ | 3.53 ± 0.21$^{a,b,2}$ | 3.59 ± 0.11$^{a,1}$ | 3.18 ± 0.25$^{c}$ | < 0.0001 |
| p value | > 0.05 | < 0.001 | < 0.0001 | > 0.05 | |

$^{a, b, c}$ - values in rows with different superscripts are statistically different (p < 0.05)
$^{1,2,3}$ - values in columns with different superscripts are statistically different (p < 0.05)
sd - standard deviation

**Table 3. Representation of relative amounts of protein fractions (mean ± sd) on farms (FM*a*, FM*b*, FM*c*)**

| ExG | SA | $\alpha_{s2}$-CN | $\alpha_{s1}$-CN | β-CN | κ-CN | β-LG | α-LA |
|---|---|---|---|---|---|---|---|
| ExG-1 | 5.70 ± 0.45$^{a}$ | 15.56 ± 1.13$^{a}$ | 9.23 ± 0.69$^{a}$ | 39.73 ± 0.83$^{a}$ | 10.38 ± 0.51$^{a}$ | 15.50 ± 0.55$^{a}$ | 7.56 ± 0.42$^{a}$ |
| EXG-2 | 4.97 ± 0.62$^{b}$ | 14.10 ± 2.85$^{a}$ | 8.80 ± 0.42$^{a}$ | 42.03 ± 1.33$^{b}$ | 13.79 ± 2.02$^{b}$ | 22.13 ± 1.21$^{b}$ | 11.57 ± 1.06$^{b}$ |
| exg-3 | 3.62 ± 0.40$^{c}$ | 9.91 ± 1.16$^{b}$ | 7.47 ± 0.56$^{b}$ | 30.77 ± 1.17$^{c}$ | 12.97 ± 0.78$^{b}$ | 32.60 ± 1.70$^{c}$ | 16.17 ± 0.88$^{c}$ |
| exg-4 | 3.61 ± 0.65$^{c}$ | 8.71 ± 0.65$^{b}$ | 6.15 ± 0.85$^{c}$ | 32.11 ± 1.50$^{c}$ | 12.15 ± 0.88$^{b}$ | 33.15 ± 1.35$^{c}$ | 16.65 ± 0.87$^{c}$ |
| *p* value | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 |

$^{a, b, c}$ - values in columns with different superscripts are statistically different (*p* < 0.05).
SA = serum albumine; $\alpha_{s2}$-CN = $\alpha_{s2}$-casein; $\alpha_{s1}$-CN = $\alpha_{s1}$-casein; β-CN = β-casein; κ-CN = κ-casein; β-LG = β-lactoglobulin; α-LA = α-lactoalbumin
sd - standard deviation.

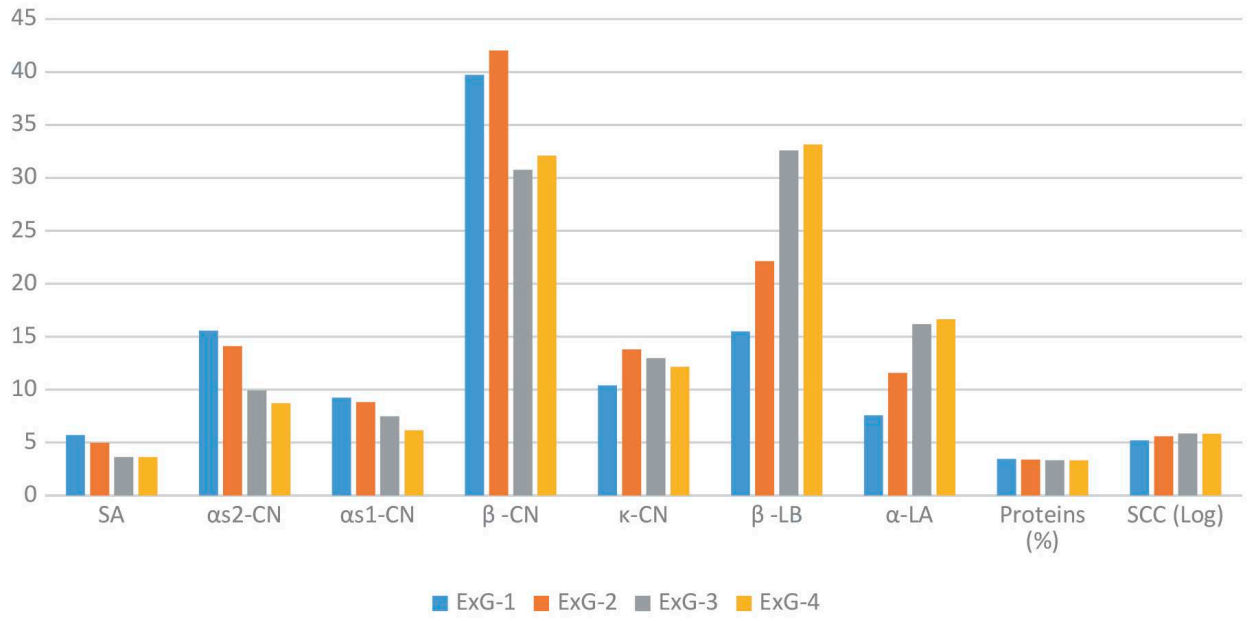


**Fig. 1. The effect of *Pseudomonas* spp. and SCC (log) on milk protein content (%) and the relative amount of protein fractions determined in experimental groups (ExG-1, ExG-2, ExG-3 and ExG-4)**

increase ($p < 0.001$) of β-LG and α-LA in groups ExG-3 and ExG-4, which contained high SCC. In the experimental group ExG-4, which contained *Pseudomonas* spp., the lowest relative amount of SA, $\alpha_{s2}$-CN and $\alpha_{s1}$-CN was detected, as well as the highest representation of β-LG and α-LA, which clearly points to the negative impact of undesirable microorganisms in the dairy industry and their impact on the quality of milk as well as its further processing.

## DISCUSSION

Dairy products are extremely diverse due to the nutritionally rich composition of milk and the presence of species of microorganisms that can grow in it and affect its physicochemical and sensory properties [26].

The microbiological quality of milk is linked to the hygiene of milk collection and the health status of cattle. With insufficient hygiene during milking and poor health (inflammation of the mammary gland), there is a prevalence of undesirable dairy microorganisms that degrade the quality of milk and its suitability for further technological processing. The somatic cell count in milk is one of the main criteria used to assess the intramammary health status, both in individual animals and in milk tanks, because there is a direct correlation between the technological quality of milk (protein fractions), the microbiological quality and SCC [27].

The aim of this work was to determine the undesirable degradation of protein fractions caused by the presence of *Pseudomonas* spp., considering SCC and using proteomic methods. Cheese is a globally consumed dairy product produced by various technological processes that provide specific properties of individual types of cheese. The quality of cheese is mainly linked to the quality of the raw milk used for its production. However, when using milk containing undesirable dairy bacteria (*Pseudomonas* spp.) and high SCC, cheeses with changed properties can be produced. The use of milk with a high somatic cell count can negatively affect coagulation, ripening, final yield, chemical composition, overall structure and development of undesirable cheese flavors [28].

B o m b a d e et al. [29] found a slight seasonal variation in SCC from bovine milk, reporting higher SCC in summer than in winter or spring, which correlates with results of this study, where maximum SCC values were detected in farms FM*a* and FM*b* during the summer season. FM*c* farm showed the highest SCC during autumn. Milk with high SCC adversely affects cheese yield and cheese production efficiency, mainly due to the loss of casein to the whey [28].

Undesirable dairy microorganisms of the genus *Pseudomonas* producing heat-stable enzymes, the so-called proteases, cause spoilage of milk stored at refrigerated temperatures. In this study 240 samples of raw cow's milk from farms FM*a* (n = 80), FM*b* (n = 80) and FM*c* (n = 80) were analyzed, from which 73 isolates of *Pseudomonas* spp. were obtained. The results of the experimental work carried out by Z h a n g et al. [30] point to the preservation of 55-100 % of the activity of proteolytic enzymes in milk heated to 141 °C/10 s., which contained the presence of *Pseudomonas* spp., while heat treatment at 160 °C/20 s. saw a decrease in residual proteolytic activity to 9 % [31]. M e n g et al. [32] confirmed that 143 isolates of *Pseudomonas* spp. were found to be randomly distributed among the different farms.

N a r v h u s et al. [9] in their study analysed isolates of Pseudomonas spp. from cold-stored raw milk. The variation in proteolytic and lipolytic properties was observed and they also observed that, in general, the caseins were hydrolysed faster, and to a greater extent, than the whey proteins.

A high level of proteolysis can negatively affect the production of dairy products and the related sensory properties of the final product. In the case of cheese production, the activity of bacterial proteases can affect the casein content, which negatively causes low yield, off-flavor (i.e. bitterness) and textural defects in the cheese. P a l u d e t t i et al. [33] analysed the effect of proteases on milk coagulation in the cheese production process. The results of their experimental work show a negative correlation between the concentration of $\alpha_{S2}$-CN and β-CN and the storage time of milk. Proteolytic hydrolysis of the $\alpha_{S1}$-CN casein fraction was determined to be minimal.

The SDS-PAGE electrophoretic method performed in our study determined that in the experimental group ExG-4, (group of raw cow's milk with the presence of *Pseudomonas* spp.) the lowest relative amount of SA, $\alpha_{s2}$-CN and $\alpha_{s1}$-CN, and the highest representation of β-LG and α-LA were detected at the same time By this, we demonstrated the negative impact of undesirable microorganisms

in the dairy industry. β-CN together with $\alpha_S$-CN form the basic microstructure of cheese, and their reduced concentrations in milk could affect milk coagulation and curd formation [34]. As reported by L a m i c h h a n e et al. [35], the hydrolysis of $\alpha_S$-CN and β-CN affects the consistency and related deformation of cheeses.

The composition and interactions of proteins in cow's milk and the modifications resulting from storage, milk processing and above all the presence of microorganisms represent a complicated complex issue [36]. As our results show, even if the husbandry conditions and the type of cattle (dairy cows) are the same, it is important to focus on each production farm individually due to the different management practices implemented at the farm level.

## CONCLUSIONS

The presence and enzymatic activity of undesirable microorganisms in dairy products significantly affect the suitability of milk for further processing, as well as its technological, nutritional, and sensory properties. The aim of this study was to investigate the degradation of protein fractions, as observed through SDS-PAGE, caused by the presence of undesirable dairy microbiota *Pseudomonas* spp., while considering somatic cell count levels. The results of this experiment demonstrate that increased SCC and the presence of *Pseudomonas* spp. have a significantly negative impact on the casein protein fractions of milk, with caseins ($\alpha_{S1}$-CN, $\alpha_{S2}$-CN, and β-CN) being degraded more than individual whey proteins. Controlling the potential influences on protein fraction degradation, such as the presence of undesirable microbiota and increased SCC, has significant implications for the further processing of raw cow's milk.

## DATA AVAILABILITY STATEMENT

The raw data of this article will be made available by the authors, without undue reservation.

## ETHICAL STATEMENT

No ethical approval was needed for this study.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.


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## GENERATIVE AI STATEMENT

The authors declare that no Gen AI was used in the creation of this manuscript.

## AUTHORS CONTRIBUTIONS

Conceptualization, M.K.; Methodology, J.V..; Software, M.K.; Formal analysis, J.V.; Investigation and data curation, J.V., M.K.; Writing—original draft preparation, M.K.; Writing—review and editing, M.K.; Supervision, J.V.; Project administration, J.V.

All authors have read and agreed to the published version of the manuscript.

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