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Detection of milk manipulation for the purpose of targeted reduction of somatic cell count using the MALDI-TOF MS method

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Abstract: This study examines methods for detecting illegal manipulation of raw cow's milk aimed at artificially reducing the somatic cell count (SCC) through centrifugation. This practice, motivated by economic gain, compromises the authenticity of the raw material and masks the true health status of the mammary gland. An experimental analysis of 68 samples demonstrated that centrifugation reduces somatic cell count by an average of 45.9 ± 11.6%. The MALDI-TOF MS method was used to identify changes in the peptide profile in the 500–4 000 Da range. Although no unique peaks specific to adulterated samples were detected, Pearson's correlation analysis revealed significant relationships between the intensity of specific peptides and SCC values. The strongest positive correlation was identified for peaks in the m/z 2 922 Da region (r = 0.69). Other relevant markers include fragments with m/z 1 768 Da (r = 0.51) and 901 Da (r = 0.49). The results confirm that monitoring quantitative changes in the intensity of specific peptides using mass spectrometry is a promising tool for identifying changes in the milk peptidome associated with variations in SCC, including those induced by technological manipulation.

Keywords: dairy authenticity; MALDI-TOF MS; proteomic profiling; udder health indicators

Food adulteration is a widespread activity motivated primarily by economic gain, resulting in products whose actual value is lower than their declared quality. The situation is no different in the milk and dairy products sector, where, in addition to traditional forms of adulteration aimed at increasing the profitability of milk production, there is also the problem of manipulation of raw milk health quality indicators. However, these modifications to milk can not only render it unsuitable for technological

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processing but also pose health risks to consumers (Ionescu et al. 2023). The term milk adulteration was defined by Talkhan (2015) as the removal or replacement of milk components with foreign substances without the consumer's knowledge. Common methods of milk adulteration include the deliberate addition of water, non-dairy proteins, melamine, urea, sodium hydroxide, formaldehyde, animal fat, or synthetic milk (Poonia et al. 2017).

However, a novel method of adulteration has recently been described, leading to an apparent improvement in the quality of raw cow's milk. Through artificial centrifugation, the globally recognised indicator of milk hygienic quality, the somatic cell count (SCC), is reduced. As a result of this fraudulent practice, raw cow's milk that would otherwise fail to meet the SCC criteria stipulated by Regulation (EC) No. 853/2004 of the European Parliament and of the Council of April 29 (2004) can be placed on the market (European Commission 2004). This is an unacceptable solution to possible problems with increased incidence, especially subclinical mastitis in dairy herds. This technology clearly violates the authenticity of raw milk (Gwardys et al. 2025; Hanus et al. 2025).

The somatic cell count (SCC) is a key indicator of the health of the mammary gland and the hygienic quality of milk, and at the same time, significantly affects its purchase price. Exceeding legislative limits leads to financial sanctions or rejection of the raw material. This may motivate some producers to use the aforementioned technological or physical interventions aimed at reducing the declared SCC without actual improving the health of the herd. Such actions represent a specific form of adulteration because a qualitative parameter that is essential for the safety and technological quality of milk is manipulated. Similar to the addition of milk from another animal species, this form of adulteration can also have health and technological consequences. An increased SCC is often associated with subclinical or clinical mastitis and with changes in milk composition (higher enzyme content, changes in protein fractions, poorer cheese yield). Artificial reduction of SCC by centrifugation can conceal the true health status of milk production and allow the introduction of technologically inferior or health-risk raw materials into the food chain. Similarly, it is necessary to develop analytical approaches aimed at revealing manipulation of the SCC, for example, by monitoring changes in enzymatic activity, protein spectrum, or physicochemical properties of milk after technological intervention (Carmo et al. 2024; Gwardys et al. 2025). Experience from the melamine incidents of 2007 and 2008 showed how far the ingenuity of fraudsters can go (Andel and Dlouhy 2009).

The spectrum of bioactive peptides that may occur naturally in milk or be produced through artificial modifications to milk's structure is broad, and studying it can provide a better understanding of how milk functions. Milk and dairy products are a significant source of peptides that may offer nutritional benefits, but some secondary peptides may also have undesirable effects. Changes that have already been identified include, for example, the partial breakdown of proteins during heat treatment. The analysis of short and endogenous peptides can be difficult, but this area can be explored using modern systems such as MALDI-TOF MS or Mass Spectrometry. Detecting manipulation of SCC is analytically more demanding than standard SCC determination, but the integration of multiple methods (e.g. including MALDI-TOF MS followed by statistical analysis) can significantly increase the number of detected samples with artificially reduced SCC and contribute to ensuring the authenticity and safety of dairy products (Meltretter et al. 2008; Capriotti et al. 2016; Punia et al. 2020).

The detection of milk adulteration using MALDI-TOF MS is an advanced analytical approach that relies on the detailed characterisation of the protein and peptide profiles of milk samples (Rysova et al. 2022). MALDI-TOF MS is primarily used to detect changes in the composition of major protein fractions, such as caseins (α_{S1}-, α_{S2}-, β-, and κ-casein) and whey proteins (β-lactoglobulin, α-lactalbumin). Milk adulteration, such as the addition of milk from another animal species (e.g. goat or sheep milk to cow's milk), also leads to characteristic changes in spectral profiles. Chemometrics also plays an important role, where data from various analytical methods are combined and evaluated using multidimensional statistical models (e.g. Principal Component Analysis, Partial Least-Squares Discriminant Analysis), enabling more reliable identification of adulteration (Garcia et al. 2012; Calvano et al. 2013; Song et al. 2024; Roumani et al. 2026).

The latest tool in the fight against milk adulteration is the integration of artificial intelligence into analytical methods for detecting fraud. Automated

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systems are already widely used, for example, in the evaluation of spectra or chromatograms. The potential of machine learning to identify specific differences when analysing adulterated and genuine samples represents another step that can be applied across analytical methods. The foundation lies in creating a sufficiently large database of samples with the required differences to serve as the basis for machine recognition. The expansion of this automation can increase the number of detected counterfeits, primarily due to greater efficiency and reduced staff requirements (Aqeel et al. 2025; Khan et al. 2026).

This study aimed to evaluate the potential of MALDI-TOF MS to detect the artificial reduction of somatic cell count in raw cow's milk caused by centrifugation and to identify peptide markers associated with this form of adulteration.

MATERIAL AND METHODS

Collection of milk samples. During 2023 and 2024, raw milk samples were collected from four dairy cow herds. The herds consisted of the two predominant dairy breeds raised in the Czech Republic, Holstein and Czech Fleckvieh, each representing approximately 50% of the animals. Herd sizes ranged from 100 to 400 cows. Milking on all farms was carried out using machine milking systems. The cows were fed a total mixed ration, which was supplemented with concentrate feed depending on the level of milk production. Some farms also applied a seasonal grazing system. To account for possible seasonal variation, milk samples were collected throughout the year.

The samples were transported to the laboratory under refrigerated conditions, maintaining a temperature below 6 °C. For each sample, at least 30 l of milk was available to perform the artificial centrifugation described later. Altogether, 68 milk samples were used for analysis. Two types of basic samples were obtained and prepared: *i*) original samples (approx. 60%, number) – raw bulk tank cow's milk (herd of cows) with the assumption of a normal SCC of up to 400 thousand/ml; *ii*) modified samples (approx. 40%) based on raw bulk tank cow's milk (30–70% by volume) with the addition of selected individual milk with subclinical mastitis (70–30% by volume), SCC above 600 thousand/ml, to obtain the necessary variation range of SCC in the set of control samples, for relevant tests in the logic of the thesis topic.

SCC analysis. SCC was determined using two analytical approaches: *i*) flow cytometry, in which cell nuclei were stained with ethidium bromide and analysed using a SomaCount 300 instrument (Bentley Instruments, USA), and *ii*) a fluorescence-based method, where DNA was stained with propidium iodide and measured using a DeLaval Cell Counter (DeLaval, Sweden). The final SCC value was calculated as the arithmetic mean of the results obtained from both methods.

Centrifugal treatment of milk samples. To reduce the SCC, all collected milk samples were subjected to a centrifugation procedure. Before this treatment, the entire set of 68 samples was classified as the Control group. From each 30 l sample, a 1 l portion was first taken for the SCC analysis of untreated milk, while the remaining 29 l were processed by centrifugation to reduce SCC. After this procedure, the processed milk samples were categorised as the Adulterated group.

The SCC reduction was performed using a small continuous-flow cream separator corresponding to the original DeLaval separator design. The centrifuge was equipped with a drum containing 12 plates and had a diameter of 100 mm. The separation process was carried out under the following operational conditions: milk temperature between 10 and 15 °C, rotational speed of 11 000 rpm, relative centrifugal force (RCF) of 3 500 × *g*, and a maximum centrifugal force at the outer radius of the drum reaching 7 000 × *g*.

During centrifugation, a small amount of cream (approximately 20 ml) was occasionally separated. In such cases, the cream fraction was returned to the skimmed milk. This was done using a cross-rule calculation to ensure that the fat content and other major components of the centrifuged milk corresponded to the original level of these components measured in the Control group.

Milk sampling for MALDI TOF MS analysis. After the artificial centrifugation, both Control and Adulterated milk samples were homogenised, and a minimum of 2 ml was collected. One millilitre was used for the main analysis. Samples were transported at a temperature of <4 °C to prevent the development of pathogenic microorganisms or other physicochemical changes.

Sample preparation for MALDI-TOF MS analysis. The milk sample was prepared for analysis

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by MALDI-TOF MS using a standardised procedure. First, 1 ml of the sample was pipetted into a centrifuge tube and centrifuged at a relative centrifugal force of 24 900 × *g* for 15 min at 4 °C to effectively separate the milk fat. After centrifugation, a representative fraction of the milk plasma was collected and subsequently diluted with ultrapure water in a ratio of 1 : 1 (v/v). The mixture prepared in this way was homogenised for 10 s using a laboratory homogeniser. When processing a larger number of samples, dilution could be carried out in microtiter plates. 1 μl of the homogenised mixture was then applied to a clean MALDI target plate and allowed to dry at laboratory (room) temperature. After complete drying, the sample was covered with 1 μl of a suitable matrix solution and allowed to dry again at room temperature. Immediately after the matrix dried, the MALDI plate was mounted in a steel carrier, and mass spectra were acquired. Throughout the entire sample preparation, laboratory safety rules were strictly followed, and appropriate personal protective equipment was used.

MALDI-TOF MS analysis. MALDI-TOF MS analysis was performed using an Autoflex Speed instrument (Bruker Daltonics) equipped with a SmartBeamTM II laser (355 nm) and flexControl software (v3.4 Build 135, Bruker Daltonics). Two linear positive methods with different mass ranges and different spectral accumulation were developed and used for the measurements. Specifically, the range 500–4 000 Da was measured. The molecular weight protein method was measured with the following parameters: IS1 19 890 V, IS2 18 450 V, lens 6 000 V; 6 000 shots in 200 steps. Each milk sample was measured in duplicate within a single replicate. Bruker Bacterial Test Standard (Bruker Daltonics) and Peptide Calibration Standard II (Bruker Daltonics) were used to calibrate the method; the ppm error was 1–7. Both standards together covered the entire measurement range from 500 Da to 4 000 Da.

Spectrum processing. All spectra were automatically processed by flexAnalysis software (Bruker Daltonics). The flexAnalysis software (Bruker Daltonics) was utilised for the automated evaluation of the acquired spectra. Initially, the software identified and removed high-frequency background noise and established a baseline for each individual spectrum. Following this pre-processing, the Sophisticated Numerical Annotation Procedure (SNAP) method was applied to detect individual peaks. A specific threshold value was determined by comparing the signal intensity to the noise level, and a final peak list was generated for further statistical analysis.

Statistical analysis. To verify the effectiveness of adjusting the SCC, a graphic display was used. For this analysis, all types of samples (n = 68) – both Control and Adulterated – were used. The degree of linear dependence between peak area and SCC was analysed using Pearson's correlation coefficient. Statistical significance was tested at the α = 0.05 level. The strength of the observed relationship was interpreted based on the absolute value of the coefficient as follows:

- 0.00–0.19 = Very weak
- 0.20–0.39 = Weak
- 0.40–0.59 = Moderate
- 0.60–0.79 = Strong
- 0.80–1.00 = Very strong

If the coefficient is positive, the correlation is direct; if it is negative, the correlation is inverse. The statistical analysis was performed by a special script based on the Python programming language to generate Excel results tables. This created program was used for data sorting and statistical results.

RESULTS AND DISCUSSION

The evaluation of the study began with an assessment of the centrifugation system's effectiveness in removing somatic cells. The SCC results for all samples can be found in Supplementary Table 1. A comparison of the results for the control and adulterated samples can be found in Figures 1 and 2. Figure 1 shows all samples (control and spiked) along with their SCC. Figure 2 shows the distribution test for all samples. The average percentage decrease in SCC for all pairs is 45.9 ± 11.6%. Similar efficacy was achieved by (Carmo et al. 2024), where a 55% decrease in SCC was observed after centrifugation.

The next step involved analysing the obtained spectra from various angles. First, all peaks (i.e. individual peptides) were detected in all samples [results available in Electronic Supplementary Materials (ESM), files 2 and 3]. Values from this database were used for the subsequent search for differences between control and adulterated samples.

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Table 1. Results of Pearson's correlation analysis of peak areas depending on changes in somatic cell count (SCC) (n = 68)

m/z (Da)	2 960.89	2 960.2	2 933.96	**2 922.16**	2 922.75	1 786.56	2 261.17	2 943.75	2 795.75	2 261.75	939.746	939.098	1 635.41	2 908.12	1 777.69
Correlation (Area vs SCC)	0.848 2	0.845 7	0.701 9	**0.693 9**	0.690 5	0.642 8	0.636 3	0.632 3	0.619 5	0.603 0	0.596 3	0.5 941	0.5 939	0.5 862	0.5 860
Peak found in x samples	13	14	8	**117**	117	4	21	6	4	21	22	22	2	4	2
m/z (Da)	2 785.17	1 055.32	1 055.75	2 907.17	2 934.84	2 804.11	3 477.44	1 192.84	1 192.47	**1 768.32**	1 768.69	3 516.36	1 807.01	1 806.42	3 517.35
Correlation (Area vs SCC)	0.574 8	0.548 6	0.547 4	0.529 6	0.525 6	0.524 2	0.523 0	0.516 3	0.515 7	**0.513 4**	0.512 9	0.510 5	0.505 7	0.504 1	0.499 1
Peak found in x samples	4	75	75	3	6	2	2	55	56	**133**	133	3	31	31	3
m/z (Da)	**901.15**	1 488.5	1 886.04	1 886.41	2 813.77	2 805.61	2 113.52	2 805.14	839.758	1 093.15	1 790.27	3 478.57	2 221.95	2 811.58	1 999.72
Correlation (Area vs SCC)	**0.485 2**	0.482 0	0.481 7	0.470 1	0.462 9	0.450 4	0.450 2	0.447 0	0.440 2	0.440 2	0.440 2	0.4 307	0.4 165	0.4 146	0.4 143
Peak found in x samples	**118**	2	12	10	3	3	5	3	1	1	1	3	8	2	2
m/z (Da)	2 905.99	2 267.57	1 168.7	1 911.13	897.922	**1 154.38**	2 908.81	897.013	1 450.88	1 882.27	1 882.99	802.421	801.732	2 813.1	1 998.13
Correlation (Area vs SCC)	0.406 6	0.405 8	0.400 4	0.400 4	0.385 0	**0.384 4**	0.380 6	0.340 5	0.338 0	0.295 6	0.293 1	0.290 0	0.287 0	0.279 7	0.257 4
Peak found in x samples	2	3	1	1	3	**136**	2	4	3	8	8	73	81	6	22
m/z (Da)	2 176.32	2 437.56	1 459.4	1 622.56	1 388.73	1 722.74	1 722.29	782.674	1 623.43	1 225.55	1 350.58	3 250.7	2 837.11	2 055.04	923.233
Correlation (Area vs SCC)	0.244 2	0.229 1	0.212 1	0.209 0	0.201 4	0.181 8	0.181 5	0.178 5	0.152 6	0.146 8	0.118 9	0.117 0	0.102 0	0.099 9	0.090 6
Peak found in x samples	15	1	4	16	5	101	102	1	15	2	10	1	2	8	1
m/z (Da)	1 453.06	2 479.2	2 489.87	1 380.87	2 489.32	1 901.66	1 539.4	3 876.81	3 877.33	2 479.67	1 200.69	1 395.76	1 494.94	2 382.94	3 261.89
Correlation (Area vs SCC)	0.090 6	0.080 0	0.077 4	0.067 8	0.065 7	0.059 0	0.057 5	0.054 4	0.032 2	0.030 1	0.016 9	0.012 3	0.012 0	0.007 1	0.003 6
Peak found in x samples	1	78	6	9	7	1	5	51	51	77	5	1	122	3	12
m/z (Da)	1 631.55	1 268.7	1 632.07	1 386.05	1 267.66	747.934	1 494.27	2 838.47	2 774.01	1 684.05	2 332.23	930.659	3 041.74	1 602.61	792.156
Correlation (Area vs SCC)	−0.001 4	−0.002 7	−0.006 7	−0.010 2	−0.010 3	−0.011 2	−0.011 5	−0.016 9	−0.017 7	−0.024 5	−0.029 5	−0.032 1	−0.037 4	−0.040 8	−0.042 6
Peak found in x samples	36	79	35	2	136	63	125	53	136	1	1	1	3	2	1
m/z (Da)	1 181.46	1 441.62	1 489.66	1 589.01	1 646.39	1 736.45	3 742.88	3 131.73	1 603.67	933.248	948.954	2 315.84	2 481.3	1 583.72	1 063.37
Correlation (Area vs SCC)	−0.044 1	−0.045 1	−0.045 1	−0.045 1	−0.045 1	−0.045 1	−0.045 1	−0.046 1	−0.046 7	−0.047 7	−0.047 7	−0.047 7	−0.047 7	−0.049 4	−0.049 5
Peak found in x samples	1	1	1	1	1	1	1	16	2	1	1	1	1	1	1
m/z (Da)	878.216	2 709.14	525.839	570.415	649.623	684.425	690.155	830.54	1 260.77	829.276	2 839.18	828.83	1 933.06	1 739.52	3 364.19
Correlation (Area vs SCC)	−0.050 0	−0.050 3	−0.054 9	−0.054 9	−0.054 9	−0.054 9	−0.054 9	−0.055 2	−0.056 2	−0.059 9	−0.064 8	−0.067 3	−0.067 7	−0.074 9	−0.075 0
Peak found in x samples	2	3	1	1	1	1	1	2	1	84	51	87	9	5	5
m/z (Da)	3 413.5	2 348.06	1 740.83	765.979	1 418.01	775.051	2 285.59	3 132.49	708.605	2 774.6	1 742.4	1 593.74	3 412.77	2 506.93	2 575.31
Correlation (Area vs SCC)	−0.076 0	−0.077 9	−0.078 0	−0.084 2	−0.086 5	−0.089 6	−0.093 0	−0.095 3	−0.098 3	−0.099 3	−0.106 4	−0.113 0	−0.114 8	−0.119 5	−0.120 2
Peak found in x samples	19	5	4	7	17	11	3	16	11	135	6	136	21	121	8
m/z (Da)	**2 507.41**	2 241.64	2 241.06	1 594.48	1 312.01	3 145.21	2 136.13	2 135.42	1 131.25	1 015.3	3 144.7	1 254.67	1 340.5	1 997.17	1 984
Correlation (Area vs SCC)	**−0.121 9**	−0.129 9	−0.130 6	−0.131 6	−0.138 7	−0.146 1	−0.146 6	−0.153 1	−0.155 9	−0.161 2	−0.163 2	−0.166 7	−0.172 3	−0.185 4	−0.188 5
Peak found in x samples	**121**	61	62	134	13	15	16	18	10	23	18	136	15	121	18

Important and widely discussed results are highlighted in bold

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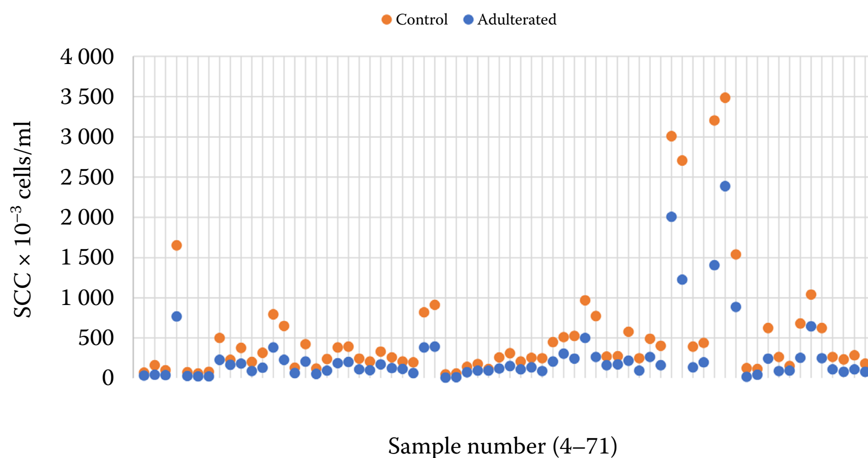

Figure 1. Differences in samples before and after adulteration using centrifugation

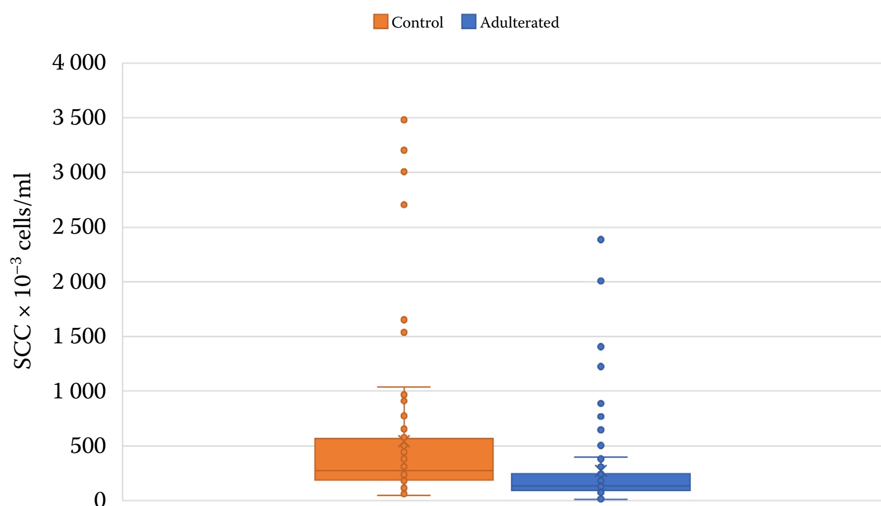

Figure 2. Test of sample distribution before and after centrifugation

First, the results were examined for specific peaks for a single group of samples (results available in ESM, file 4). It was found that none of the peaks were characteristic of only one group of samples. For this reason, we compared peak sizes between the control and adulterated samples. Here, we achieved certain results. When considering the greatest possible representation of peaks across as many samples as possible, it is worth noting the 901 Da peptide, where a 41% decrease in content was observed in the adulterated sample compared to the control. A similarly significant result can be seen in the 1 768 Da compound, which shows an increase of just under 29%. In both cases, the literature agrees on the nature of these peptides. The 901 Da compound is most likely a peptide fragment of one of the milk casein proteins, while the 1 768 Da peak is apparently one of the intact proteins of the casein fraction (Wolk et al. 2020; Zenk et al. 2024). Overall, these results suggest that monitoring changes in the quantitative composition of peptides is more suitable for detecting adulteration than simply detecting their presence. The identified differences could serve as potential indicators of adulteration; however, for their reliable use, it would be necessary to verify their reproducibility on a larger sample set and to supplement the identification of specific peptide sequences.

Due to the lack of statistically significant differences between the groups being compared, it was decided to take a different approach. The mass spectra were statistically evaluated based on the SCC in the sample, rather than solely on group affiliation. Peaks that had already been detected and their areas entered into the database were compared with changes in SCC using correlation analysis. This approach yielded significantly clearer and more verifiable results. Table 1 presents a correlation analysis of the peaks as a function of changes in SCC. The colour scale in the table denotes

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the correlation strength between SCC and peak area, while the number of samples containing the peak provides another key piece of information. Relevant differences in relation to SCC were evaluated by combining the correlation coefficient with the proportion of samples exhibiting each peak.

No peaks were identified that were unique to a single group of samples, which is consistent with findings in the field of peptidomics. Milk peptide profiles are highly complex, and individual peptides commonly occur across different sample types, with differences manifesting more in their relative abundance than in their presence or absence (Dallas et al. 2014a; Khaldi et al. 2014; Valletta et al. 2021).

The highest positive correlations were achieved for peptides with m/z around 2 922 Da (n = 117; r = 0.69), which were also present in a large number of samples, and the correlation can be described as strong. These peptides can be considered potentially robust markers of the monitored parameter. Similar mass ranges have been described as typical for casein fragments formed by enzymatic proteolysis during milk processing. Proteolytic cleavage of caseins (especially β- and α_{S1}-casein) leads to the formation of a wide spectrum of peptides with various molecular weights, with the 2 000–3 000 Da range being particularly common (Ning et al. 2022; Meleti et al. 2025).

Another significant finding is the high number of peaks with lower correlation coefficients, described as moderate, such as m/z 1 768 Da (n = 133; r = 0.51) or 901 Da (n = 118; r = 0.49). These peptides can be considered part of the so-called "core peptidome," i.e. a group of peptides commonly present in most milk samples (Dallas et al. 2013; Dallas et al. 2014b; Weber et al. 2025). Peptides in this mass range (approx. 800–2 000 Da) correspond to short sequences of approximately 7–16 amino acids and are primarily formed by enzymatic cleavage of caseins (Nielsen et al. 2024). Changes in their intensity may be caused by differences in enzymatic activity, processing methods, or the presence of foreign components in the samples.

Conversely, peaks with low frequency of occurrence have limited informative value, even though they may achieve relatively higher correlations. A low number of detections increases the likelihood of statistical randomness and limits their use as reliable markers. This phenomenon is commonly described in peptidomic studies, where variability between samples and the sensitivity of analytical methods play a crucial role (Guerrero et al. 2014).

Another interesting aspect is the presence of negative correlations for some peaks (m/z 2 507 Da, r = −0.12), which may indicate a decrease in the concentration of these peptides as SCC values increase. This trend may be associated with their further degradation or conversion into smaller fragments as a result of ongoing proteolysis (Meleti et al. 2025). The dynamics of peptide formation and degradation are, in fact, highly complex in dairy systems and depend on the activity of endogenous enzymes such as plasmin or cathepsins.

Overall, the results confirm that distinguishing between samples should not rely on the presence of specific peaks, but rather on quantitative changes in their intensity. This approach is widely used in peptidomics, where relative changes in peptide abundance serve as a key tool for identifying changes in the system during the SCC centrifugation.

CONCLUSION

The present study confirmed that technological manipulation of raw cow's milk through centrifugation represents an effective yet illegitimate method of artificially reducing the SCC, which directly compromises the authenticity and declared health quality of the raw material. Experimental data demonstrated that this process leads to a significant decrease in SCC by an average of 45.9 ± 11.6%, which may mask subclinical mastitis in herds and result in the introduction of technologically inferior milk into the food chain.

In this context, the use of MALDI-TOF MS has proven to be an effective tool for characterising changes in the peptide and protein profile of milk. The results of spectral analysis in the 500–4 000 Da range suggest that the detection of this form of adulteration cannot be based on the identification of unique peaks. However, the decisive factor for distinguishing adulterated milk is quantitative changes in the intensity of specific peaks.

The key scientific contribution of this study is the identification of peptides with m/z values in the 2 922 Da range, which show a strong positive correlation (r = 0.69) with changes in SCC and are consistently present in a large number of samples (n = 117), making them robust analytical markers. These fragments, likely arising from the proteo-

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lytic cleavage of casein fractions, complement other significant correlations at the 1 768 Da (r = 0.51) and 901 Da (r = 0.49) peaks. The observed dynamics of changes in the intensity of these peptides suggest that centrifugation selectively affects the distribution of low-molecular-weight nitrogenous compounds in milk plasma.

In conclusion, the MALDI-TOF MS method with statistical analysis represents a promising approach for identifying changes in milk peptidome associated with variations in SCC, including those induced by technological manipulation. For future application in routine laboratory practice, it is desirable to focus on the precise sequencing of identified markers and the use of machine learning methods that would enable automated classification of samples based on comprehensive spectral fingerprinting.

Conflict of interest

The authors declare no conflict of interest.

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