# The effect of artificial reduction of the somatic cell count, as a violation of authenticity, on milk quality indicators

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Abstract: The somatic cell count (SCC) in raw milk is an important indicator of health and hygienic quality. Artificial reduction of the SCC (ARSCC) in milk, for the apparent improvement of milk quality for commercial reasons, is an undesirable phenomenon and a violation of authenticity both in the factual sense and legislatively. Analytical methods need to be developed to identify ARSCC as well as to assess the effects that ARSCC may have on milk. The aim of the work was to quantify the effects of ARSCC on cow's milk as a food raw material. The results presented are some of the first on the given problem. Raw bulk tank cow milk was sampled in two experiments, each time for the whole year (2021–2022 and 2023–2024, n = 66 and n = 53, respectively) from herds of Czech Fleckvieh and Holstein cows, 1:1. ARSCC in experiment 1 (n = 66) slightly reduced the fat content, in experiment 2 (n = 53) it did not, otherwise the milk indicators with the exception of SCC were almost (1) and completely (2) identical. All samples were negative for the presence of inhibitory substance residues. ARSCC under the specified technological conditions reduced SCC (1) from  $772 \pm 906 \cdot 10^3$ /ml to  $376 \pm 630 \cdot 10^3$ /ml, by -51.3% (P < 0.001) and (2) from  $592 \pm 798 \cdot 10^3$ /ml to  $304 \pm 468 \cdot 10^3$ /ml, by -48.5% (P < 0.001). Under these circumstances, the milk fermentation ability improved (1) from  $28.52 \pm 4.72$  °SH to  $31.0 \pm 4.65$  °SH, by 8.66% (P < 0.001) and (2) from  $32.51 \pm 2.61$  °SH to 33.80  $\pm$  2.88 °SH, by 3.97% (P < 0.001). Curd firmness was better for SCC  $\leq$  400  $10^3$ /ml compared to higher SCC by 5.26% (P < 0.001). Nevertheless, it is not possible, for hygienic and health reasons, to allow such milk for human consumption and it is necessary to find effective identification analytical methods for ARSCC.

Keywords: centrifugation; milk composition; raw cow's milk; subclinical mastitis; technological properties

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Quality control of raw milk is an important societal task (de la Vara Martinez et al. 2018; Pereira et al. 2020). This supports and defines the general rule against any illegal alteration of the quality of food and food raw material. However, some apparent "quality upgrading" of a proportion of raw cow's milk with increased somatic cell count (SCC) can currently be found in some localities. It is an artificial reduction of SCC in mastitis milk. This is a commercial unethical solution to possible problems with an increased frequency of occurrence, especially of subclinical mastitis in dairy herds. This technology clearly violates the authenticity of the dairy raw material.

However, the problem of the occurrence of mastitis in dairy herds, which is generally permanent (Pyorala 2003; Hisira et al. 2023), must be addressed through prevention and, if necessary, treatment not by artificially reducing the SCC (ARSCC) in raw milk via centrifugation prior to delivery to the dairy. For this purpose, relevant separation technologies have also been developed, for which "an increase in milk quality" is also argued as a reason for ARSCC. However, such a procedure can mean numerous hygienic (quality) risks for processing technologies in the production of dairy foods and is fundamentally unethical towards consumers of dairy products, as it can inadequately increase the proportion of milk processed into food from sick animals. Furthermore, from the point of view of food legislation, Regulation (EU) No. 1308/2013 of the European Parliament and of the Council of December 17th 2013 contains the clause: "Milk means exclusively the normal mammary secretion obtained from one or more milkings without either addition thereto or extraction therefrom." It is from this perspective that it is necessary to view the using of ARSCC technology, quite apart from the possibility that it will lead to untreated cows from an animal welfare point of view and the large production and economic losses associated with increasing SCC levels.

Production disorders of the mammary gland (disorders of milk secretion, subclinical and clinical mastitis) are a constant problem in dairy farming (Pyorala 2003; Pitkala et al. 2004; Kvapilik et al. 2014, 2015; Arikan et al. 2024; Stanek et al. 2024). Hisira et al. (2023) mentioned: "Despite the considerable efforts made by many scientists and experts in mastitis, the disease has not been eliminated on dairy farms and remains a serious problem, causing significant losses to farmers." For a long

time, since the 70s-80s of the last century, SCC has been a globally recognised indicator of the health and hygiene quality of the mammary gland of a dairy cow (quarter and individual mixed milk samples), entire herds of milked cows (bulk tank milk samples) and raw milk for its processing in the dairy plant. This fact is officially captured in international milk quality standards, e.g. Regulation (EC) No. 853/2004 of the European Parliament and of the Council of 29 April 2004, laying down specific hygiene rules for food of animal origin, consolidated version as of 9 May 2024. The quality limit is here  $\leq 400 \ 10^3$ /ml. However, this already corresponds to a loss of milk yield of at least 4%. Furthermore, statistical procedures were developed in detail to interpret SCC with regard to the occurrence of mastitis and their pathogens, loss of milk yield, management of prevention and treatment of milk secretion disorders and improvement of milk quality (Reneau 1986; Wiggans and Shook 1987). Here, the limit of individual (mixed individual milk sample in milk recording) SCC for registration of suspected occurrence of subclinical mastitis in a dairy cow was statistically derived in the value of  $\geq 283 \cdot 10^3$ /ml. However, a truly healthy cow's mammary gland has a SCC of  $\leq 100 \ 10^3/\text{ml}$ . A cut-off limit of 200 10<sup>3</sup>/ml is also sometimes used (Bobbo et al. 2020; Zecconi et al. 2020).

Somatic cells in milk originate mainly from blood (75% to 95%, maximum up to 25% epithelial cells; Sharma et al. 2011) and are an integral part of the immune system of the mammary gland due to their physiological functions (Bobbo et al. 2020). SCC is causally related to the occurrence of numerous mastitis pathogens and its pathological dynamics in dairy herds have been described in detail (Rysanek and Babak 2005; Rysanek et al. 2007). Increased SCC is especially associated with increased losses in the milk yield of cows (Reneau 1986; Kvapilik et al. 2014). It was recently calculated that in the case of clinical mastitis (also severe subclinical mastitis, i.e. treated) the farmer loss in the Czech Republic is CZK 9 000 (EUR 360) per case (Kvapilik et al. 2015). Losses caused by mastitis can vary from CZK 4 000 to CZK 18 000 (EUR 160 to EUR 720) per case. Due to current inflation, these financial losses are now about 1/3 higher. In the distribution of mastitis losses by source, it was estimated that 53% was lower milk sales revenue, 20% higher cow culling (herd renewal), 14% higher cost of drugs and cow treatment, 7% labour in nursing sick cows,

and 6% fines for farmers from the price of milk for a milk quality deficit (Kvapilik et al. 2015).

Increased SCC also brings losses to the quality of milk, e.g. impaired coagulation properties and yield in cheesemaking (Politis and Ng-Kwai-Hang 1988a, b; Bobbo et al. 2016, 2017), or decreased milk fermentation ability (Hanus et al. 1993), which was significantly correlated with SCC (r = -0.23; P < 0.01). The SCC indicator is therefore not only health-wise, but also economically very important. Thus, SCC logically remains at the centre of research attention in the evaluation of dairy cow health and milk quality from various genetic, health, hygienic and economic perspectives (Sharif and Muhammad 2008; Sharma et al. 2011; Bochniarz et al. 2014; Kvapilik et al. 2014, 2015; Costa et al. 2019; Bobbo et al. 2020; Al-Noman et al. 2022; Citek et al. 2022).

The problem with practical dairying is that conventional commercial raw milk quality control systems do not yet have methods that could identify this ARSCC procedure. Such methods have not yet been investigated, or developed. Currently, there are several analytical methods that could potentially identify undesirable changes in the food raw material, but only after research and certain specific modifications. Suitable examples include: flow cytometry (FC), mid-range infrared spectroscopy with a Michelson interferometer and Fourier transform (MIR-FT), colorimetric (Millan-Verdu et al. 2003) methods for determining enzyme activity (for catalase, esterase, lactoperoxidase, lactate dehydrogenase) nuclear magnetic resonance (NMR based on <sup>1</sup>H NMR spectroscopy) or MALDI-TOF mass spectrometry (MALDI, matrix-assisted laser desorption/ionisation, in combination with a TOF, time-of-flight detector; Rysova et al. 2021, 2022). The above is an important subject of necessary current and future research. Among the tested methods for identifying ARSCC, the approximate estimation based on comparing current values of lactoferrin content and somatic cell score with appropriately defined threshold levels in bulk tank milk appears to have practical potential (Hanus et al. 2025; unpublished data), given the significant correlation observed between these milk indicators.

Another topic in this context is relevant research on the effects of ARSCC on other qualitative properties of raw milk and their quantification. This research is now in its early stages (Hanus et al. 2021; other relevant results were not found in the available sources) and has not yet been sufficiently

described, therefore the results of such an evaluation are presented in this work.

This work aims to experimentally determine, describe and possibly statistically quantify the effect of ARSCC in raw cow's milk on its quality characteristics from the point of view of hygiene and processing into dairy foods.

## **MATERIAL AND METHODS**

## Milk samples, experimental conditions

From May to April (2021-2022), experimental bulk tank samples of raw cow's milk were collected in the first experiment. The milk of Czech Fleckvieh and Holstein herds was represented in a ratio of approximately 1:1. The herds of cows were stabled year-round in free housing, at an altitude of 300 m to 550 m, fed with TMR (total mixed feed ration). Summer grazing was practiced in some herds. This corresponded to the standard conditions in dairy farming in the Czech Republic. Some milk samples were selectively (directly at the collection site) violated by individual abnormal milk (discarded milk for subclinical mastitis). This resulted in significantly higher SCC variability and values often higher than the SCC standard  $\leq 400 \, 10^3 / \text{ml}$ . In total, 66 samples were taken. The samples, each in a volume of 2 l, were transported to the laboratory under cold conditions (≤6 °C) and processed and analysed the next day.

Milk samples were collected in a similar manner using the same method in the second experiment (2023–2024). A total of 53 milk samples were collected. The samples, each with a volume of 30 l, were transported to the laboratory in cold conditions ( $\leq$ 6 °C) and processed and analysed the next day.

## Model of violation of the authenticity of the raw milk material, artificial reduction of SCC (ARSCC)

Milk for analysis (first experiment) was sampled as original (subsample 1). Furthermore, the equivalent of the sample (subsample 2) was created and sampled after ARSCC, i.e. after centrifugation (2 l, at a temperature of 25 °C). Cream was then returned to the skimmed milk and mixed in (including the cream stuck in the outlet device of the

centrifuge). This subsample 2 showed a significant reduction in SCC. The ratio of the internal volume of the centrifuge drum to the model-centrifuged volume of milk (2 l) in the simulated, experimental ARSCC was significantly higher than is usually applied in dairy practice. Therefore, a distinct decrease in fat content (F) was logically registered in the modified samples. The residue in the drum was 0.3% to 0.8% of the fat in the milk depending on the quality of the sample. This difference could in the future affect the results of the spectral methods intended for the methodological development of the identification of unwanted ARSCC. Therefore, a theoretical calculation was carried out (based on real numbers of continuous control analysis) to determine that, in this case, centrifuging 30 l (reducing the ratio of the internal volume of the centrifuge drum to the centrifuged volume of milk) in the next procedure will be sufficient to minimise the impact of partial fat loss on the intended identification analyses.

The aforementioned procedure for processing a 30 l milk sample was used in the second experiment. Here, the difference in fat content between the original milk and the milk after ARSCC was logically much smaller for technological reasons, approximately 0.1%. Then, a targeted, calculated, back-adding of fat from identical milk (cream) to the ARSCC-treated milk was carried out to achieve a balance in fat content with the original milk.

## Technological, analytical and statistical methods

For the model centrifugation of SCC, simulating a real technological process in practice, a classic, flow-through, small dairy centrifuge was used (historically, the Alfa Laval construction type).

The technical parameters of the centrifuge used are listed in Table 1.

SCC was determined by the fluoro-opto-electronic flow cytometry method on a Somacount 300 device (Bentley Instruments, Chaska, MN, USA). Calibration and control activities were carried out within the framework of the following standards: CSN EN ISO 13366–1 (57 0531); CSN EN ISO 13366–2 (57 0531).

Milk composition [content: fat (F), crude protein (CP), casein (C), lactose monohydrate (L), solids non fat (SNF), total solids (TS), urea (U), citric acid (CA) and free fatty acids (FFAs)] was determined by MIR-FT spectroscopy on a DairySpec FT (Bentley Instruments, Chaska, MN, USA). Calibration and control activities were carried out within the framework of the following standards: CSN 57 0536; CSN 57 0530; CSN ISO 8196-2 (57 0536); CSN ISO 8196-1 (57 0536); CSN ISO 8196-3 (57 0536). The equivalent of the freezing point of milk (MFP) was also determined on the same device.

The milk freezing point (MFP-CR) was determined using a cryoscope (CryoStar Automatic; Funke-Gerber, Berlin, Germany). The electrical conductivity of milk (EC) was determined on a Hanna Instruments HI5321-02 conductometer (Woonsocket, USA, produced in Romania). The active acidity of milk (pH) was measured using a pH-meter 1100L (VWR pHenomenal pH, Darmstad, Germany). The titration acidity (SH) of milk was measured by titrating 100 ml of milk (Soxhlet-Henkel) using an alkaline solution of NaOH 0.25 N in an indicator medium (phenolphthalein) according to the standard CSN 57 0530 (in °SH = ml × 2.5 mmol/l).

The milk fermentation ability (MFA – yoghurt test) was determined by titration acidity [Soxhlet-Henkel (°SH)] using an alkaline solution of NaOH 0.25 N (M) in an indicator medium (phenolphtha-

Table 1. Technical parameters of the model centrifugation of SCC (ARSCC)

Parameter	Value
Number of plates in drum	12
Number of drum revolutions per minute	11 000
Effective radius of the plate (central disk, distance of the center of the centrifuged material from the center rotation) in mm	25
Dimensionless relative centrifugation force [RCF; acceleration caused by rotation, multiple of gravitational acceleration ( <i>g</i> )] parameter for the applied material	3 500
Dimensionless relative centrifugal force at maximum drum radius	7 000

lein) according to CSN EN ISO 1211 (ON 57 0534). The test was performed with the thermophilic yoghurt culture YC-180, 50U (Chr. Hansen, Denmark), *Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *lactis* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. Residues of inhibitory substances (RIS) in milk were checked using the rapid assay TwinSensor KIT020 (Unisensor, E.U. Regulation).

The cheeseability of milk, as the coagulation time (ECT) of lactoproteins (seconds, until the formation of visible protein flakes), was determined with the addition of the bacterial solubilising enzyme Fromase (Fromase® 220 TL BF, Royal DSM, Heerlen, Netherlands) in 50 ml of milk at 32 °C in a water bath. The time was adjusted empirically to approximately 5 min of exposure. After an hour of syneresis, the firmness of the formed curd cake was recorded – CF; by the drop of the dropped body under standardised conditions in mm [the smaller the value, the better the quality (higher curd firmness)].

Milk samples were (first experiment) divided into groups A ( $\leq 400 \ 10^3/\text{ml}$ ) and B ( $> 400 \ 10^3/\text{ml}$ ) according to SCC in subsample 1 in accordance with the quality limit of European food legislation [Regulation (EC) No. 853/2004, consolidated version as of 9 May 2024]. Mean values (arithmetic and geometric means and medians) and other statistical characteristics (standard deviations, coefficients of variation, minimum and maximum) of basic milk parameters were calculated. Values with usually missing normal data frequency distribution, such as SCC values and enzymatic coagulation time (ECT) were logarithmically transformed ( $log_{10}$ ) to obtain an approximately normal frequency distribution and also for geometric (xg) mean calculations. Unpaired and paired classic *t*-tests (MS Excel, Microsoft, Redmond, WA, USA) were used to test differences between mean values of milk indicators and the validity of null hypotheses. Possible linear regressions of the relationships between milk indicators and sample types were processed in the same program. The same evaluation was performed in the second experiment without dividing the samples into groups  $\leq 400 \ 10^3$ /ml and  $> 400 \ 10^3$ /ml for SCC.

## **RESULTS AND DISCUSSION**

The results of the influence of ARSCC on the composition and properties of milk (Table 2, 3 and 4), especially technological (MFP, pH, SH, ECT, CF

and MFA), were obtained during a trial search for analytical methods with the potential to identify the use of ARSCC in practice. Such an effective method is not yet known. The influence of ARSCC on the properties of milk has not yet been characterised, with the exception of one partial work. Therefore, the results presented are some of the first on the given problem. All milk samples (Table 2, 3 and 4; n = 66, n = 66 and n = 53, respectively) were negative for the examination of the risk of RIS occurrence, which is an important methodological prerequisite for subsequent control of MFA without the risk of interference effects on the results.

In Table 2, it can be seen that the average SCC in the original milk (OM) was  $772 \pm 906 \, 10^3 / \text{ml}$  (vx 117.4%,  $xg 405 10^3/ml$ ). In Table 4, it was  $592 \pm 798 10^3/ml$ (vx 134.8%, xg 340  $10^3$ /ml). These values are significantly higher than the SCC averages in milk quality control in the Czech Republic: 240 10<sup>3</sup>/ml (2015), 223 10<sup>3</sup>/ml, 231 10<sup>3</sup>/ml, 226 10<sup>3</sup>/ml, 221 10<sup>3</sup>/ml, 230 10<sup>3</sup>/ml, 227 10<sup>3</sup>/ml, 235 10<sup>3</sup>/ml, 235 10<sup>3</sup>/ml (2023). This difference was achieved intentionally, by specific milk sampling. The higher SCC values demonstrate the suitability of the milk sample sets (Tables 2 and 4) for the experimental description of the effect of ARSCC on milk properties and the search for a suitable analytical identification method for ARSCC. The ARSCC procedure used (Table 2) reduced SCC to  $376 \pm 630 \ 10^3/\text{ml}$  (vx 167.6%, xg  $107 \cdot 10^3$ /ml), i.e. by  $-396 \pm 396 \cdot 10^3$ /ml, i.e. by -51.3% (P < 0.001). These characteristics also confirm the suitability of the set for the stated purpose of the study. However, the ARSCC technology used (Table 2) also reduced the fat content from  $3.96 \pm 0.90\%$  to  $2.75 \pm 0.85\%$ , by  $-1.21 \pm 0.37\%$ , or relatively by -30.6% (P < 0.001). Considering the known relationships of specific gravity of individual milk components (F < 1, CP, C, L and SNF > 1), the ARSCC process logically led to a relatively slight increase in other milk components: CP by 1.95%; C by 3.09%; L by 1.04%; SNF by 1.22% (*P* < 0.001, due to the very low variability of these differences). Urea in milk decreased, probably due to the properties of the MIR-FT analytical method, by -7.79% (P < 0.001).

Milk properties (Table 2), such as MFP, pH, SH and EC changed relatively little: by 0.15% (P > 0.05); by -0.30% (P < 0.01); by -2.19% (P < 0.05) and by 2.14% (P < 0.001), respectively. For EC, this can be explained by an increase in ion concentration when a certain volume of fat with a specific

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MI (unit)	Milk	$x \pm SD$	(%) xa	gx	ш	Min	Max	d ± SD	(%) p	t	P-value
	OM	3.96 ± 0.90	22.8	ı	3.80	2.38	9.91				
F (%)	ARSCC	$2.75 \pm 0.85$	30.8	ı	2.64	1.43	8.21	$-1.21 \pm 0.37$	-30.6	26.58	<0.001
(8)	OM	$3.59 \pm 0.36$	10.1	ı	3.52	3.04	4.83		l.	1 0 7	0
CF (%)	ARSCC	$3.67 \pm 0.38$	10.4	I	3.59	3.09	4.99	0.07 ± 0.05	1.95	17.7	<0.001
(70)	OM	$2.91 \pm 0.39$	13.3	I	2.83	2.21	4.19	- 000	ć	0	0
(%) )	ARSCC	$3.01 \pm 0.42$	13.9	I	2.94	2.26	4.44	0.09 ± 0.08	5.09	8.90	<0.001
( )0	OM	$4.8 \pm 0.31$	6.4	I	4.9	3.92	5.20	- 100	5	00 71	6
L (%)	ARSCC	$4.85 \pm 0.31$	6.3	I	4.94	3.99	5.27	0.05 ± 0.02	1.04	16.80	<0.001
(70)	OM	$9.05 \pm 0.27$	3.0	ı	9.03	8.45	9.75			0	0
SINF (%)	ARSCC	$9.16 \pm 0.28$	3.1	I	9.13	8.57	88.6	$0.11 \pm 0.05$	1.22	18.8/	<0.001
11 (100 ) 11	OM	$25.8 \pm 11.3$	43.8	I	24.9	4.8	75.1	7 2 4 2 2 2	1	16 50	, 0001
(IIIB/ 100 IIII)	ARSCC	$18.6 \pm 10.4$	55.9	ı	17.6	3.2	57.4	C.C ± 2.7–	61:1-	10.39	< 0.001
MED (C)	OM	$-0.5337 \pm 0.0099$	1.9	ı	-0.5331	-0.568 7	-0.4994	0 600 0 + 0 000 0	71.0	1 70	700
Irr ( C)	ARSCC	$-0.5329 \pm 0.0095$	1.8	ı	-0.532 3	-0.5668	-0.5017	0.000 ± 0.000	0.13	1./0	c0.0<
	OM	$6.67 \pm 0.13$	2.0	I	6.71	6.17	6.83	- 60	Ċ	ć	Ç
hrl	ARSCC	$6.66 \pm 0.13$	1.9	I	89.9	6.21	6.87	-0.02 ± 0.05	-0.30	67.6	<0.01
(1130) 1.	OM	$8.22 \pm 1.68$	20.5	I	7.72	6.49	14.53	- 07	6		Ç
( NC ) NC	ARSCC	$8.05 \pm 1.49$	18.5	I	7.62	6.24	13.83	-0.18 ± 0.55	-2.19	C0.7	<0.0>
(32) 52	OM	$4.67 \pm 0.39$	8.4	ı	4.66	4.03	5.97	010	c 7	11 53	,000
C (ms/cm)	ARSCC	$4.76 \pm 0.42$	8.9	I	4.74	3.96	6.17	0.10 ± 0.0/	<b>7.14</b>	11.32	<0.001
(*) H.J.	OM	$203 \pm 122$	60.1	169	171	30	517	- -	6	0,7	,
(s)	ARSCC	$173 \pm 112$	64.7	142	144	21	540	CC + OC-	-14.0	4.40	<0.001
Ę	OM	$2.2282 \pm 0.2786$	I	I	2.2330	1.477 1	2.713 5	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0			000
IOB ECI	ARSCC	$2.1524 \pm 0.2882$	I	I	2.1584	1.322 2	2.732 4	-0.0/3 8 ± 0.119 4	I	21.0	<0.001
(""")	OM	19 ± 1	5.3	I	20	18	21			,	0
								-		<	1

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MI (unit)	Milk	x ± SD	(%) xA	xg	ш	Min	Max	d ± SD	(%) p	t	t P-value
(103/2-1)	OM	$772 \pm 906$	117.4	405	323	47	4 357	206 1 206	S	70 6	1000
SCC (10-/mi)	ARSCC	$376 \pm 630$	167.6	107	113	2	3 482	-390 ± 390	-51.5	8.00 8.00	<0.001
	OM	$2.607\ 2\pm0.511\ 1$	I	I	2.508 2	1.672 1	3.639 2			1000	000
log sec	ARSCC	$2.027 \ 8 \pm 0.793 \ 7$	I	I	2.052 3	0.3010	3.5418	0.542.0 ± 0.542.0	I	13.00	<0.001
MIN (°CH)	OM	$28.52 \pm 4.72$	16.5	I	28.46	11.98	44.58	4 14 14	99 0	19.70	0000
MFA ( 311)	ARSCC	$31.00 \pm 4.65$	15.0	ı	30.95	11.36	46.67	0C:1 I /4.7	0.00	13.20	<0.001

Fable 2 to be continued

SH = degree by Soxhlet-Henkel (milk titration with NaOH solution in ml × 2.5 mmol/l); ARSCC = artificial reduction of somatic cell count, n = 66; C = casein content; electrical conductivity; ECT = enzymatic coagulation time; F = fat content; L = lactose monohydrate content; log ECT = logarithm of ECT value to the base 10; og SCC = logarithm of SCC value to the base 10; m = median; Max = maximum; MFA = milk fermentationability (yoghurt test); MFP = milk freezing point (MFP-CR); MI = milk indicator; Min = minimum; OM = original milk, n = 66; P-value = significance, or rather probability of the null hypothesis; pH = active acidity pH; SCC = somatic cell count; SD = standard deviation; SH = titration milk acidity; SNF = solids non fat content; t = t-value of the paired t-test criterion; U = urea content; v = t-coefficient CF = curd firmness; CP = crude protein content; d (%) = relative average difference, where 100% = OM; d = average difference (difference, arithmetic mean: ARSCC – OM) The difference between the means of the OM and ARSCC variants is always tested by a paired t-test, d always with SD of variation (%); x = arithmetic mean; xg = geometric mean gravity <1 is lost, as mentioned above. However, the effect of ARSCC on the technological properties of milk, ECT, CF and MFA is interesting: by -14.8% (P < 0.001); by 0,00% (P > 0.05); by 8.66% (P < 0.001), respectively. Curdling improved by shortening ECT without affecting the curd firmness. MFA improved significantly, i.e. increased by 8.66%. This was not entirely expected. However, taking into account the consideration that with the removal of approximately 50% of somatic cells from milk, a decrease in the concentration of immunoglobulins bound to cell membranes can be expected, which can partially inhibit the growth of noble dairy cultures, this surprising result (an increase in MFA by  $2.47 \pm$ 1.50 °SH) ultimately sounds logical, from the biological nature of the matter.

To assess the changes in ARSCC in relation to the official milk quality limits in the EU, the set was divided into classes according to SCC in OM: A with SCC  $\leq 400 \ 10^3$ /ml; B with SCC > 400 10<sup>3</sup>/ml (standard and non-standard milk, Table 3). In class A, ARSCC reduced SCC by -128 ±  $53\ 10^3$ /ml (by -74.4%, P < 0.001) and in B by  $-680 \pm$  $404\ 10^3/\text{ml}$  (by -48.3%, P < 0.001). These results differentiated by SCC suggested other possible connections. Other changes in milk components (Table 3) due to ARSCC logically correspond to the comments in Table 2, e.g. in a certain slight increase in the concentration of the main milk components (such as CP, C, L and SNF) in both classes. However, it turned out (Table 3) that class milk B had, probably due to sample treatment: higher F by 0.27% (relatively by 7.05%, P > 0.05); higher CP by 0.29% (relatively by 8.41%, P < 0.001); higher C and SNF (P < 0.001 and P > 0.05, respectively); logically lower L by -0.31% (relatively by -6.26%, P < 0.001). It is interesting to note that: ECT was shortened relatively equally in both classes A and B due to ARSCC (by -12.9% and -16.8%, respectively), while the phenomenon was lower, but more significant in class A than B (P < 0.001 and P < 0.05, respectively); CF did not change in both classes (P > 0.05); MFA improved similarly in both milk classes (A by 2.81  $\pm$  1.58 °SH, 10.0%, P < 0.001; B by  $2.11 \pm 1.34$  °SH, 7.26%, P < 0.001).

Some results (Table 3) were not in accordance with previous findings (Politis and Ng-Kwai-Hang 1988a, b; Hanus et al. 1993, 1995). For example, in terms of cheesemaking, the ECT in OM was better (shorter) in class B (SCC >  $400\,10^3$ /ml) by -5.74%, although not significantly (P > 0.05). Hanus et al. (1995) re-

Table 3. Comparison of the results of the determination of selected milk indicators, somatic cell count (SCC), cheesemaking and milk fermentation ability (MFA, yoghurt test) in original bulk tank raw cow's milk (OM, regular milk samples and samples with the addition of abnormal milk with an originally higher SCC, samples

MI (unit)	Milk	SCC class	$x \pm SD$	(%) xx	d ± SD	(%) p	t	P-value
	MO	A	$3.83 \pm 0.47$	12.2	1 00 - 0 33	coc	10.01	0000
	ARSCC	A	$2.75 \pm 0.38$	13.9	-1.08 ± 0.32	7.62-	19.03	<0.001
6	OM	В	$4.10 \pm 1.20$	29.2		c	0	0000
F (%)	ARSCC	В	$2.74 \pm 1.16$	42.4	$-1.36 \pm 0.3$	-33.2	20.75	<0.001
	OM	B - A	I	I	0.27	7.05	1.20	>0.05
	ARSCC	B - A	I	I	-0.01	-0.36	0.05	>0.05
	OM	A	$3.45 \pm 0.24$	8.9		1	1	600
	ARSCC	A	$3.52 \pm 0.26$	7.4	0.00 ± 0.00	1.74	7.55	<0.001
(8)	OM	В	$3.74 \pm 0.41$	11.1	- 60	0	Ö	0000
CF (%)	ARSCC	В	$3.83 \pm 0.43$	11.2	0.08 ± 0.05	7.14	9.90	<0.001
	OM	B – A	ı	ı	0.29	8.41	3.47	<0.001
	ARSCC	B - A	I	I	0.31	8.81	3.52	<0.001
	OM	A	$2.76 \pm 0.26$	9.5		o o	7	600
	ARSCC	A	$2.83 \pm 0.30$	10.5	0.08 ± 0.07	7.30	0.4	<0.001
(8)	OM	В	$3.08 \pm 0.43$	14.0	- 11	1	80	0.00
(0)	ARSCC	В	$3.19 \pm 0.45$	14.2	0.11 ± 0.09	70.0	0.88	<0.001
	OM	B - A	I	I	0.32	11.6	3.61	<0.001
	ARSCC	B – A	I	I	0.36	12.7	3.78	<0.001
	OM	A	$4.95 \pm 0.13$	2.6			0) 01	600
	ARSCC	A	$5.00 \pm 0.13$	2.6	$0.05 \pm 0.02$	1.01	15.08	<0.001
S	OM	В	$4.64 \pm 0.36$	7.7			7000	000
L (%)	ARSCC	В	$4.69 \pm 0.36$	7.7	0.06 ± 0.03	1.29	15.30	<0.001
	OM	B - A	ı	I	-0.31	-6.26	4.65	<0.001
	ARSCC	B - A	I	ı	-0.31	-6.20	4.64	<0.001
	OM	A	$9.04 \pm 0.17$	1.9	4 00 0	-	13.06	,
	ARSCC	A	$9.14 \pm 0.19$	2.1	₩0.10 ± 01.0	1111	13.00	100°0>
(NIE (0/)	OM	В	$9.06 \pm 0.35$	3.8	1000	1 00	12.61	000
IF (%)	ARSCC	В	$9.18 \pm 0.36$	3.9	CO.U I 71.0	72.1	15.04	<0.001
	OM	B - A	I	1	0.02	0.22	0.30	>0.05

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Table 3 to be continued

MI (unit)	Milk	SCC class	x ± SD	(%) xA	d ± SD	(%) p	t	P-value
	OM	A	24.2 ± 8.36	34.7		0 00		0000
	ARSCC	А	$16.3 \pm 7.93$	48.5	$-/.8 \pm 5.1$	-32.2	14.45	<0.001
(1 001)) 11	OM	В	$27.5 \pm 13.7$	49.8		000	o c	000
U (mg/100 mi)	ARSCC	В	$21.1 \pm 12.1$	57.3	-0.5 ± 5.9	-23.0	9.78	<0.001
	OM	B - A	ı	I	3.3	13.6	1.17	>0.05
	ARSCC	B – A	I	I	4.5	27.6	1.89	>0.05
	OM	Α	$-0.5326 \pm 0.0094$	1.8		)		,
	ARSCC	А	$-0.5312 \pm 0.0087$	1.6	0.001 4 ± 0.005 5	0.26	7.44	<0.05
Mep (°C)	OM	В	$-0.5349 \pm 0.0103$	1.9	0.0000 ± 0.0000	COC	0.13	200
MFF ( C)	ARSCC	В	$-0.5348 \pm 0.0102$	1.9	0.000 I I 0.004 Z	0.02	0.13	c0:0<
	OM	B - A	I	I	-0.002 3	-0.43	0.93	>0.05
	ARSCC	B - A	ı	I	-0.003 6	-0.68	1.52	>0.05
	OM	A	$6.68 \pm 0.13$	1.9		C C	ć.	,
	ARSCC	А	$6.66 \pm 0.12$	1.7	C0.0 ± 20.0-	06:0-	7:30	<0.05
ב י	OM	В	$6.67 \pm 0.13$	2.0	2004100	, ,	100	200
hп	ARSCC	В	$6.65 \pm 0.14$	2.1	CO.U I IO.U-	CT.0-	CO.1	c0:0<
	OM	B - A	I	I	-0.01	-0.15	0.30	>0.05
	ARSCC	B - A	I	I	-0.01	-0.15	0.31	>0.05
	OM	A	$7.99 \pm 1.66$	20.8	660 + 800	00	1 20	u O
	ARSCC	A	$7.91 \pm 1.59$	20.1	-0.00 ± 0.55	-T:00	1.39	60:0<
(1130) 113	OM	В	$8.47 \pm 1.69$	20.0	0.07	60 0	21.0	, H
) HS ) HS	ARSCC	В	$8.20 \pm 1.39$	16.9	0.77 ± 0.70	co.o_	7.14	60.0>
	OM	B - A	I	I	0.48	6.01	1.14	>0.05
	ARSCC	B – A	I	I	0.29	3.67	0.78	>0.05
	OM	А	$4.50 \pm 0.30$	9.9	90 0 T 80 0	000	000	100.0
	ARSCC	A	$4.60 \pm 0.32$	6.9	0.00 ± 60.00	7.00	0.00	<0.001
(30) 50	OM	В	$4.84 \pm 0.41$	8.5	010+010	0	1	100.0
EC (1113/C111)	ARSCC	В	$4.94 \pm 0.45$	9.1	0.10 ± 0.00	7.07	C7. /	<0.001
	OM	B - A	I	I	0.34	7.56	3.79	<0.001
	ARSCC	B - A	-	1	0.34	7.39	3.51	<0.001

P-value <0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.05 >0.05 >0.05 >0.05 >0.05 >0.05 >0.05 4.43 2.62 0.39 0.64 0.32 4.00 13.87 9.37 7.46 5.15 12.62 14.25 13.74 6.22 0.51 2.51 (%) p -5.74-12.9-16.8-74.4-48.3719 1 557  $-0.3668 \pm 0.1433$  $-0.0817 \pm 0.0754$  $-0.0695 \pm 0.1542$  $-0.7797 \pm 0.3549$ -0.0357-0.0235 $-680 \pm 404$  $-33 \pm 70$ 0.88041.2933 $d \pm SD$  $-27 \pm 35$  $-128 \pm 53$  $0 \pm 1$  $0 \pm 1$ -1264.5 88.6 5.35.05.05.0 104.5  $2.2455 \pm 0.2745$  $2.1638 \pm 0.3113$  $2.1804 \pm 0.2313$  $3.0608 \pm 0.2803$  $2.2098 \pm 0.2861$  $2.1403 \pm 0.2660$  $1.4007 \pm 0.5204$  $2.6940 \pm 0.3781$  $44 \pm 39$  $209 \pm 120$  $182 \pm 122$  $197 \pm 127$  $164 \pm 102$  $1409 \pm 948$  $729 \pm 762$  $172 \pm 84$  $20 \pm 1$  $20 \pm 1$  $20 \pm 1$ ı SCC class B - AB - AARSCC OM ARSCC OM ARSCC OM OM OM ARSCC OM ARSCC OM ARSCC OM ARSCC ARSCC ARSCC ARSCC ARSCC OM ARSCC OM OM OMOM OM SCC (10<sup>3</sup>/ml) MI (unit) CF (mm) log SCC

Table 3 to be continued

P-value < 0.001 <0.001 >0.05 >0.05 0.32 0.91 3.82 7.26 (%) p 1.20 10.0  $2.81 \pm 1.58$  $2.11 \pm 1.34$  $d \pm SD$ 1.07 0.37 17.8 17.7 12.1  $29.08 \pm 5.17$  $30.82 \pm 3.73$  $31.19 \pm 5.51$  $28.01 \pm 4.27$ SCC class ARSCC ARSCC ARSCC OM OM MI (unit)

Fable 3 to be continued

The difference between the means of classes A (SCC  $\leq 400\ 10^3/\text{ml}$ ) and B (> 400\ 10^3/\text{ml}) within the OM and ARSCC variants is always tested by unpaired t-test, d always without SD

value to the base 10; log SCC = logarithm of SCC value to the base 10; MFA = milk fermentationability (yoghurt test); MFP = milk freezing point (MFP-CR); MI = milk indicator; OM = original milk, class A n = 34, class B n = 32; pH = active acidity pH; P-value = significance, or rather probability of the null hypothesis; SCC = somatic SH = degree by Soxhlet-Henkel (milk titration with NaOH solution in ml × 2.5 mmol/l); ARSCC = artificial reduction of somatic cell count, class A n = 34, class B n = 32.; C = casein content; CF = curd firmness; CP = crude protein content; d (%) = relative average difference, where 100% = OM; d = average difference (difference, arithmetic cell count; SD = standard deviation; SH = titration milk acidity; SNF = solids non fat content; t = t-value of the paired t-test criterion; U = urea content; v = t-coefficient mean: ARSCC – OM); EC = milk electrical conductivity; ECT = enzymatic coagulation time; F = fat content; L = lactose monohydrate content; log ECT = logarithm of ECT of variation (%); x = arithmetic mean ported a correlation coefficient of 0.23 for SCC and ECT (P < 0.001). The MFA in OM was also better (higher) in class B by 1.07 °SH, i.e. by 3.82%, although also not significantly (P > 0.05). However, this corresponded to the results of earlier work [MFA < 25 °SH (17.93 °SH) and SCC 320  $\pm$  223  $10^3/\text{ml}$ : > 25 °SH (30.62 °SH) and  $380 \pm 303 \cdot 10^3/\text{ml}$  of Gencurova et al. (1997). In addition, Faria et al. (2020) did not record the effect of SCC on fermentation, but only on the results of yoghurt storage. Nevertheless, the results for ECT and MFA in our work were unexpected and quite surprising. Previously, the opposite correlation coefficient for SCC and MFA of -0.23 (P < 0.01) was recorded (Hanus et al. 1993). This can be explained by the higher frequency of significantly higher SCC values in milk samples in earlier works (Hanus et al. 1993, 1995) as compared to this work. On the other hand (Table 3), within the framework of cheesemaking and OM, better (lower value in mm) curd firmness was found in class A (SCC ≤ 400  $10^3$ /ml): CF better by 5.26% (P < 0.001), which is already in line with earlier results of other works (Politis and Ng-Kwai-Hang 1988a, b; Gencurova et al. 1997). Fernandez et al. (2007) investigated the quality of yoghurt made from whole milk with low  $(147 \ 10^3/\text{ml})$ , medium  $(434 \ 10^3/\text{ml})$  and high SCC (1 943 10<sup>3</sup>/ml). The viscosity of yoghurt with high SCC was higher (P < 0.05) than that of yoghurt with low SCC. This could be in accordance with our results here. Yoghurt (Fernandez et al. 2007) with high SCC also had higher FFAs content (P < 0.05). SCC did not affect the pH, acidity, fat content and proteolysis of yoghurt (P > 0.05). Thus, SCC increases lipolysis in the resulting yoghurt during its storage.

The results of the second experiment, which was carried out with a modified methodological design for the balance of values after ARSCC, especially fat content, are summarised in Table 4. It can be seen that the significant difference between OM and the milk after ARSCC was only in the SCC indicator. The reduction in SCC was by -48.5% (from  $592 \ 10^3/\text{ml}$  to  $304 \ 10^3/\text{ml}$ , i.e. by  $-287 \ 10^3/\text{ml}$ (P < 0.001), in xg from 340  $10^3$ /ml to 158  $10^3$ /ml). In addition to this significant reduction in SCC (from the area >400  $10^3$ /ml to <400  $10^3$ /ml of the EU limit for standard milk), there are slight changes in other milk indicators. Some average differences show, due to the methodological nature of instrumental measurements, low variability (such as C, U, MFP and FFAs) and are therefore statistically significant, others lack statistical significance (CP,

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x ± SD         vx (%)         xg         m         Min         Max         d ± SD         c           3.96 ± 0.52         13.2         -         3.89         2.50         6.00         0.008 ± 0.026           3.96 ± 0.52         13.0         -         3.99         2.51         5.97         0.008 ± 0.026           3.52 ± 0.19         5.5         -         3.51         3.02         3.88         0.003 ± 0.012           2.84 ± 0.23         8.1         -         2.80         2.43         3.44         -0.008 ± 0.016           2.83 ± 0.22         7.9         -         2.79         2.41         3.40         -0.008 ± 0.016           4.81 ± 0.14         3.0         -         2.79         2.41         3.40         -0.008 ± 0.016           4.81 ± 0.14         3.0         -         4.84         4.42         5.13         0.004 ± 0.009           9.02 ± 0.21         2.3         -         9.04         8.46         9.70         0.011 ± 0.069           9.02 ± 0.21         2.3         -         9.04         8.46         9.70         0.011 ± 0.009           13.70 ± 0.23         2.4         -         13.76         11.72         15.78         0.701 ± 0.072	of the somatic cell count (ARSCC, milk adulteration) with a balanced fat content and all other milk components (second experiment, $n = 53$ )	,										
OM   396±6.52   132   - 3.89   2.50   6.00	MI (unit)	Milk	x ± SD	(%) xA	gx	ш	Min	Max	d ± SD	(%) p	t	P-value
ARSCC   3.96 ± 0.52   13.0   - 3.90   2.51   5.97   0.000 =	L (%)	OM	$3.96 \pm 0.52$	13.2	ı	3.89	2.50	6.00	2000 - 8000	000	ccc	, ,
MRSCC 3.52±0.19 5.5 - 3.51 3.03 3.89 3.89 0.003±0.012  ARSCC 2.83±0.23 8.1 - 2.80 2.43 3.44 - 0.008±0.016  ARSCC 2.83±0.22 7.9 - 2.79 2.41 3.40 0.004±0.016  ARSCC 2.83±0.22 7.9 - 2.79 2.41 3.40 0.004±0.009  ARSCC 4.81±0.14 3.0 - 4.84 44.2 5.10  ARSCC 4.81±0.14 3.0 - 4.83 44.3 5.13 0.004±0.009  ARSCC 3.93±0.22 2.4 - 9.05 8.45 9.70 0.011±0.069  OM 3.22±6.88 3.0 - 2.20 4.7 5 13.76 11.71 15.80 0.011±0.069  C) ARSCC 2.1±6.86 3.1 0.0 0.0528 0.050 0.050 0.011±0.069  C) ARSCC 0.527±0.0041 0.8 0.05 0.0528 0.053 0.050 0.008±0.0001  ARSCC 0.527±0.0049 0.8 0.0528 0.053 0.050 0.050 0.008±0.0001  ARSCC 0.527±0.0049 0.8 0.0528 0.053 0.050 0.008±0.0001  ARSCC 0.527±0.0049 0.8 0.0528 0.053 0.050 0.008±0.0001  ARSCC 0.527±0.0049 0.8 0.0528 0.053 0.050 0.008±0.0001  ARSCC 0.527±0.0049 0.8 0.050 0.053 0.050 0.008±0.0001  ARSCC 0.527±0.0049 0.8 0.050 0.050 0.050 0.050 0.008±0.0001  ARSCC 0.527±0.0049 0.8 0.0 0.050 0.050 0.050 0.008±0.0001  ARSCC 0.527±0.0046 0.0 0.050 0.050 0.050 0.008±0.0001  ARSCC 0.575±0.0046 0.0 0.050 0.050 0.008 0.0001  ARSCC 0.575±0.0046 0.0 0.050 0.009 0.000 0.	F (%)	ARSCC	$3.96 \pm 0.52$	13.0	1	3.90	2.51	5.97	0.008 ± 0.028	0.20	77:7	c0.0>
ARSCC 3:52±0.19 5.4 - 3.50 3.02 3.88 0.0003±0.012  ARSCC 2.83±0.22 7.9 - 2.89 2.41 3.44 - 0.008±0.016  OM 4.81±0.14 2.9 - 2.79 2.41 3.40 - 0.008±0.016  ARSCC 4.81±0.14 3.0 - 4.84 4.42 5.10 0.004±0.009  ARSCC 9.03±0.22 2.4 - 9.05 8.45 9.70 0.011±0.069  OM 13.79±0.65 4.7 - 13.76 11.71 15.80 0.012±0.072  C) ARSCC 13.81±0.65 4.7 - 13.76 11.72 15.78 0.012±0.072  C) ARSCC - 0.527±0.0041 0.8 - 0.6228 0.6359 0.6308±0.0001  ARSCC - 0.527±0.0049 7.1 - 0.6228 0.6359 0.6308 0.0008±0.0001  ARSCC 6.90±0.49 7.1 - 6.79 6.65 8.84 0.072±0.278  C) OM 6.83±0.40 5.9 - 6.75 6.65 8.84 0.0004±0.009  ARSCC 6.90±0.49 7.1 - 6.79 6.65 8.88  ARSCC 6.90±0.49 7.1 - 6.79 6.65 8.88  ARSCC 7.055±0.046 8.0 - 0.5276 0.435 0.685  OM 0.19±0.027 14.1 - 0.000 0.12 0.004  ARSCC 10.19±0.027 14.1 - 0.000 0.12 0.001  ARSCC 0.19±0.027 14.1 - 0.000 0.12 0.001  ARSCC 0.19±0.027 14.1 - 0.000 0.12 0.001	(8)	OM	$3.52 \pm 0.19$	5.5	I	3.51	3.03	3.89		S	60	L
MRSCC 283 ± 0.23	CF (%)	ARSCC	$3.52 \pm 0.19$	5.4	ı	3.50	3.02	3.88	$0.003 \pm 0.012$	0.03	1.80	>0.05
MSCC 2.83 ± 0.22 7.9 - 2.79 241 3.40 -0.005 ± 0.010  OM 4.81 ± 0.14 2.9 - 4.84 442 5.10 0.004 ± 0.009  ARSCC 4.81 ± 0.14 3.0 - 4.83 443 5.13 0.004 ± 0.009  OM 9.02 ± 0.21		OM	$2.84 \pm 0.23$	8.1	I	2.80	2.43	3.44	7100	o o	ć	500
OM 4.81 ± 0.14 2.9 - 4.84 442 5.10 0.004 ± 0.009  ARSCC 4.81 ± 0.14 3.0 - 4.83 443 5.13 0.004 ± 0.009  OM 9.02 ± 0.21 2.3 - 9.04 8.46 9.70 0.011 ± 0.069  OM 13.79 ± 0.65 4.7 - 13.76 11.71 15.80 0.012 ± 0.072  OM 22.3 ± 6.88 3.09 - 22.0 4.7 37.2 15.78 0.012 ± 0.072  C) OM - 0.528 ± 0.0041 0.8 - 0.528 0.535 9 0.509 0.0008 ± 0.0001  ARSCC 21.6 ± 6.86 31.8 - 0.652 8 0.535 9 0.509 0.0008 ± 0.0001  OM - 0.528 ± 0.0041 0.8 - 0.527 6 0.535 9 0.509 0.0008 ± 0.0001  ARSCC 6.90 ± 0.49 7.1 - 6.75 6.65 8.84 0.072 ± 0.27 8  OM 4.62 ± 0.23 = 0.004 0.057 0.000 0	(%)	ARSCC	$2.83 \pm 0.22$	7.9	1	2.79	2.41	3.40	-0.008 ± 0.016	-0.28	5.01	<0.001
MSSCC 4.81±0.14 3.0 - 4.83 4.43 5.13 0.004 ± 0.009  ARSCC 9.03±0.22 2.4 - 9.04 8.46 9.70 0.011±0.069  ARSCC 9.03±0.22 2.4 - 9.05 8.45 9.70 0.011±0.069  OM 13.79±0.65 4.7 - 13.76 11.71 15.80 0.012±0.072  ARSCC 13.81±0.65 4.7 - 13.76 11.72 15.78 0.012±0.072  OM 22.3±6.88 30.9 - 22.0 4.7 37.2 0.011±0.653  C) ARSCC 21.6±6.86 31.8 - 0.528 0.536 0.509 0.509 0.509 0.509  OM 0.528 2±0.0041 0.8 - 0.527 6 0.535 2 0.508 6  ARSCC 0.527 4±0.003 0.8 - 0.527 6 0.535 2 0.508 6  OM 46.2±0.23 5.0 - 6.76 6.65 8.84 0.072±0.278  ARSCC 4.60±0.24 5.1 - 6.79 6.65 8.88 0.004±0.008  OM 4.62±0.23 5.0 - 4.55 4.13 5.11 0.018±0.05  OM 0.575±0.046 8.0 - 0.575 0.058 0.058 0.004±0.008  ARSCC 0.579±0.045 7.8 - 0.585 0.450 0.685  OM 0.19±0.027 14.1 - 0.20 0.12 0.23 0.001±0.004  ARSCC 0.599±0.045 7.8 - 0.585 0.450 0.685  OM 0.19±0.027 14.1 - 0.20 0.12 0.23 0.001±0.004	(70)	OM	$4.81 \pm 0.14$	2.9	ı	4.84	4.42	5.10		o o	ć	Č
) OM 9,02±0.21 2.3 - 9,04 8.46 9,70 0.011±0.069 ARSCC 9,03±0.22 2.4 - 9,05 8.45 9,70 0.011±0.069 OM 13.79±0.65 4.7 - 13.76 11.71 15.80 0.012±0.072 OM 22.3±6.88 30.9 - 22.0 4.7 37.2 0.011±0.653 OM 22.3±6.88 30.9 - 22.0 4.7 37.2 0.011±0.653 C) ARSCC 21.6±6.86 31.8 - 0.528 0.535 0.5090 0.0008±0.0001 C) ARSCC -0.527±0.0041 0.8 - 0.528 0.535 0.5090 0.0008±0.0001 OM 6.83±0.40 5.9 - 6.76 6.65 8.84 0.072±0.278 OM 6.83±0.40 5.9 - 6.76 6.65 8.89 0.0008±0.0001 OM 4.62±0.23 5.0 - 6.76 6.65 8.89 0.0008±0.0001 ARSCC 0.575±0.046 5.0 - 4.58 4.16 5.21 0.018±0.050 OM 0.575±0.046 8.0 - 0.575 0.455 0.455 0.455 0.685 OM 0.19±0.027 14.1 - 0.20 0.12 0.23 0.001±0.004 OM 0.575±0.046 8.0 - 0.575 0.455	L (%)	ARSCC	$4.81 \pm 0.14$	3.0	I	4.83	4.43	5.13	$0.004 \pm 0.009$	80:0	3.20	<0.01
MRSCC 9.03 ± 0.22 2.4 - 9.05 8.45 9.70 0011 ± 0.009 and a maol/100 g) ARSCC 1.3.1 ± 0.65 4.7 - 13.76 11.71 15.80 0.012 ± 0.072 and a maol/100 g) ARSCC 1.3.4 ± 0.65 4.7 - 13.76 11.71 15.80 0.012 ± 0.072 and a maol/100 g) ARSCC 0.52 ± 0.004 1 0.8 - 0.527 6 - 0.535 9 0.509 0.0008 ± 0.0001 and a maol/100 g) ARSCC 0.527 ± 0.046 8.0 - 0.527 6 - 0.535 9 0.509 0.0008 ± 0.0001 and a maol/100 g) ARSCC 0.577 ± 0.046 8.0 - 0.575 0.435 0.655 8.84 0.072 ± 0.278 0.000 and a maol/100 g) ARSCC 0.579 ± 0.046 8.0 - 0.575 0.435 0.685 0.004 ± 0.000 0.000 and a maol/100 g) ARSCC 0.579 ± 0.045 7.8 - 0.585 0.450 0.685 0.000 and a maol/100 g) ARSCC 0.579 ± 0.045 0.000 and a maol/100 g) ARSCC 0.579 ± 0.045 0.000 and a maol/100 g) ARSCC 0.579 ± 0.045 0.020 0.12 0.020 0.12 0.001 ± 0.004 and a maol/100 g) ARSCC 0.579 ± 0.045 0.020 0.12 0.001 ± 0.004 and a maol/100 g) ARSCC 0.579 ± 0.045 0.020 0.12 0.020 0.12 0.001 ± 0.004 and a maol/100 g) ARSCC 0.579 ± 0.045 0.020 0.12 0.001 ± 0.004 and a maol/100 g) ARSCC 0.579 ± 0.045 0.020 0.12 0.001 ± 0.004 and a maol/100 g) ARSCC 0.579 ± 0.045 0.020 0.12 0.020 0.12 0.001 ± 0.004 and a maol/100 g) ARSCC 0.579 ± 0.045 0.020 0.12 0.020 0.12 0.001 ± 0.004 and a maol/100 g) ARSCC 0.579 ± 0.045 0.020 0.12 0.020 0.12 0.001 ± 0.004 and a maol/100 g) ARSCC 0.579 ± 0.045 0.059 0.12 0.020 0.12 0.001 ± 0.004 and a maol/100 g) ARSCC 0.579 ± 0.045 0.059 0.12 0.020 0.12 0.001 ± 0.004 and a maol/100 g) ARSCC 0.579 ± 0.045 0.059 0.12 0.020 0.12 0.001 ± 0.004 and a maol/100 g) ARSCC 0.579 0.045 0.059 0.12 0.050 0.12 0.001 ± 0.004 and a maol/100 g) ARSCC 0.579 0.045 0.059 0.059 0.050 0.12 0.050 0.12 0.001 ± 0.004 and a maol/100 g) ARSCC 0.579 0.045 0.050 0.12 0.050 0.12 0.050 0.12 0.050 0.12 0.050 0.12 0.001 ± 0.		MO	$9.02 \pm 0.21$	2.3	ı	9.04	8.46	9.70		6	ì.	i G
ARSCC 13.81 ± 0.65 4.7 - 13.76 11.71 15.80 0.012 ± 0.072 10.012   ARSCC 13.81 ± 0.65 4.7 - 13.76 11.72 15.78 15.78   100 ml) ARSCC 21.6 ± 6.86 31.8 - 22.0 4.7 37.2   ARSCC -0.528 ± 0.0041 0.80.528 8 -0.535 9 0.509 0 0.0008 ± 0.0001   C) ARSCC -0.527 ± 0.003 9 0.80.528 8 -0.535 2 -0.508 6   OM -0.528 ± 0.40	SNF (%)	ARSCC	$9.03 \pm 0.22$	2.4	I	9.05	8.45	9.70	$0.011 \pm 0.069$	0.12	I.I5	>0.05
MRSCC 13.81 ± 0.65 4.7 - 13.76 11.72 15.78 0002 ± 0.0072 c)  OM 22.3 ± 6.88 30.9 - 2.2.0 4.7 37.2	(70) 5H	OM	$13.79 \pm 0.65$	4.7	I	13.76	11.71	15.80	0.000	G G	-	L C
OM         22.3 ± 6.88         30.9         -         22.0         4.7         37.2         -0.711 ± 0.653           ARSCC         21.6 ± 6.86         31.8         -         21.7         6.6         36.6         -0.711 ± 0.653           OM         -0.528 ± 0.0041         0.8         -         -0.528 8         -0.535 2         -0.508 6         0.509 0         0.0008 ± 0.0001           ARSCC         -0.527 ± 0.0049         7.1         -         6.76         6.65         8.84         0.072 ± 0.278           OM         4.62 ± 0.23         5.0         -         6.76         6.65         8.84         0.072 ± 0.278           OM         4.62 ± 0.23         5.0         -         6.79         4.16         5.21         -0.018 ± 0.050           ARSCC         4.60 ± 0.24         5.1         -         4.58         4.16         5.21         -0.018 ± 0.050           OM         0.575 ± 0.046         8.0         -         0.575         0.435         0.685         0.004 ± 0.008           ARSCC         0.579 ± 0.045         7.8         -         0.20         0.12         0.20         0.12         0.20         0.00         0.12         0.00         0.00         0.12         0.00	15 (%)	ARSCC	$13.81 \pm 0.65$	4.7	ı	13.76	11.72	15.78	$0.012 \pm 0.072$	0.09	1.22	>0.05
ARSCC 21.6 ± 6.86 31.8 - 21.7 6.6 360.711 ± 0.053 COM -0.528 2 ± 0.004 1 0.80.528 8 -0.535 9 0.509 0 0.0008 ± 0.0001 COM -0.527 4 ± 0.003 9 0.80.527 6 -0.535 2 -0.508 6 0.0008 ± 0.0001 COM	(1 001/) 11	OM	$22.3 \pm 6.88$	30.9	I	22.0	4.7	37.2	77.	6	1	500
OM 6.528 ± 0.004 1 0.8 - 0.528 8 -0.535 9 0.509 0 0.0008 ± 0.0001  ARSCC -0.527 4 ± 0.003 9 0.8 - 0.527 6 -0.535 2 -0.508 6 0.0008 ± 0.0001  OM 6.83 ± 0.40 5.9 - 6.76 6.65 8.84 0.072 ± 0.278  ARSCC 6.90 ± 0.49 7.1 - 6.79 6.65 8.88 0.072 ± 0.278  OM 4.62 ± 0.23 5.0 - 4.58 4.16 5.21 -0.018 ± 0.050  ARSCC 4.60 ± 0.24 5.1 - 4.55 4.13 5.21 -0.018 ± 0.050  OM 0.575 ± 0.046 8.0 - 0.575 0.450 0.455 0.450 0.685  OM 0.19 ± 0.027 14.1 - 0.200 0.12 0.23 0.001 ± 0.004  ARSCC 0.579 ± 0.045 14.1 - 0.200 0.12 0.23 0.001 ± 0.004  OM 0.19 ± 0.027 14.1 - 0.200 0.12 0.23 0.001 ± 0.004  ARSCC 0.579 ± 0.045 0	∪ (mg/100 mi)	ARSCC	$21.6 \pm 6.86$	31.8	I	21.7	9.9	36.6	$-0.711 \pm 0.655$	-5.19	76:/	<0.001
ARSCC -0.527 4 ± 0.003 9 0.80.527 6 -0.535 2 -0.508 6 0.0008 ± 0.0001  OM 6.83 ± 0.40 5.9 - 6.76 6.65 8.84 0.072 ± 0.278  ARSCC 6.90 ± 0.49 7.1 - 6.79 6.65 8.88 0.072 ± 0.278  OM 4.62 ± 0.23 5.0 - 4.58 4.16 5.21 -0.018 ± 0.050  OM 0.575 ± 0.046 8.0 - 0.575 0.435 0.685 0.004 ± 0.008  OM 0.575 ± 0.046 8.0 - 0.575 0.435 0.685 0.004 ± 0.008  OM 0.19 ± 0.027 14.1 - 0.20 0.12 0.23 -0.001 ± 0.004 - 0.004	()% (1)*	OM	$-0.528\ 2\pm0.004\ 1$	8.0	I	-0.5288	-0.535 9	0.5090		ī	1	500
OM         6.83 ± 0.40         5.9         -         6.76         6.65         8.84         0.072 ± 0.278           ARSCC         6.90 ± 0.49         7.1         -         6.79         6.65         8.88         0.072 ± 0.278           (mS/cm)         4.62 ± 0.23         7.1         -         4.58         4.16         5.21         -0.018 ± 0.050         -           ARSCC         4.60 ± 0.24         5.1         -         4.55         4.13         5.21         -0.018 ± 0.050         -           ARSCC         0.575 ± 0.046         8.0         -         0.575         0.435         0.685         0.085         0.004 ± 0.008           (%)         0.00         0.12         0.20         0.12         0.20         0.12         0.001 ± 0.004         -	MFF ( C)	ARSCC	$-0.5274 \pm 0.0039$	8.0	1	-0.527 6	-0.535 2	-0.5086	0.0008 ± 0.0001	1.51	2.7	<0.001
(mS/cm)	11:	OM	$6.83 \pm 0.40$	5.9	ı	92.9	9.65	8.84	0.000	-	107	L
ARSCC A.60 ± 0.23 5.0 - 4.58 4.16 5.21 -0.018 ± 0.050 - 4.55 4.13 5.21 -0.018 ± 0.050 - 5.21 $-0.018 \pm 0.050$ - 0.004 ± 0.008 - 0.575 ± 0.046 8.0 - 0.575 ± 0.045 0.045 0.085 0.004 ± 0.008 ARSCC 0.579 ± 0.045 7.8 - 0.585 0.450 0.685 0.03 - 0.001 ± 0.004 = 0.009 0.13 0.03 0.03 0.03 0.004 ± 0.004 0.00	пq	ARSCC	$6.90 \pm 0.49$	7.1	1	6.79	9.65	8.88	0.072 ± 0.278	1.05	1.84	>0.05
ARSCC $4.60 \pm 0.24$ $5.1$ $ 4.55$ $4.13$ $5.21$ $-0.018 \pm 0.030$ $ 0.575 \pm 0.046$ $8.0$ $ 0.575$ $0.435$ $0.685$ $0.004 \pm 0.008$ ARSCC $0.579 \pm 0.045$ $7.8$ $ 0.585$ $0.450$ $0.685$ $0.004 \pm 0.008$ OM $0.19 \pm 0.027$ $14.1$ $ 0.20$ $0.12$ $0.23$ $-0.001 \pm 0.004$ $-$	(/3)	OM	$4.62 \pm 0.23$	5.0	ı	4.58	4.16	5.21	0100	Ċ	c	ć
OM $0.575 \pm 0.046$ $8.0$ $ 0.575$ $0.435$ $0.685$ $0.004 \pm 0.008$ ARSCC $0.579 \pm 0.045$ $7.8$ $ 0.585$ $0.450$ $0.685$ $0.004 \pm 0.008$ OM $0.19 \pm 0.027$ $14.1$ $ 0.20$ $0.12$ $0.23$ $-0.001 \pm 0.004$ $-$	EC (IIIS/CIII)	ARSCC	$4.60 \pm 0.24$	5.1	I	4.55	4.13	5.21	0000 ± 0000	-0.39	£6.7	<0.01
ARSCC 0.579 $\pm$ 0.045 7.8 - 0.585 0.450 0.685 0.00 $\pm$ 0.000 $\pm$ 0.000 OM 0.19 $\pm$ 0.027 14.1 - 0.20 0.12 0.23 -0.001 $\pm$ 0.004 - 0.007 0.13 0.23 0.001 $\pm$ 0.004 0.10 0.10 0.10 0.10 0.10 0.10 0.1	EE A. (mm.01/100 g)	OM	$0.575 \pm 0.046$	8.0	ı	0.575	0.435	0.685	900 0 + 700 0	0.70	9 61	000
OM $0.19 \pm 0.027$ $14.1$ - $0.20$ $0.12$ $0.23$ $-0.001 \pm 0.004$	(g 001 (million) syrr	ARSCC	$0.579 \pm 0.045$	7.8	I	0.585	0.450	0.685	0000	2	70.0	100.07
ABSCC 010+0038 147 030 013 032 -C:001±0:00#	(%) <b>V</b>	OM	$0.19 \pm 0.027$	14.1	I	0.20	0.12	0.23	0.001 + 0.004	0 53	1 80	30.05
0.17 ± 0.020 = 14.7 = 0.12	(70)	ARSCC	$0.19 \pm 0.028$	14.7	ı	0.20	0.12	0.23	F00:0 - 100:0	70.0	7:00	50.0

https://doi.org/10.17221/90/2025-CJAS

MI (unit)	Milk	$x \pm SD$	(%) xA	xg	m	Min	Max	d ± SD	(%) p	t	t P-value
(1) [0]	OM	$592 \pm 798$	134.8	340	292	49	3 483	170		ŗ	000
SCC (10 <sup>-</sup> /mi)	ARSCC	$304 \pm 468$	154.0	158	163	111	2 388	-28/ ± 301	-48.5	5.81	<0.001
	OM	$2.5320 \pm 0.4331$	I	I	2.4654	1.6902	3.5420	0 0110 - 7 7000		2	1000
10g SCC	ARSCC	$2.1976 \pm 0.4822$	I	I	2.212.2	1.0414	3.3780	-0.3344±0.1188	I	20.44	<0.001
(H3°) V1)V	OM	$32.51 \pm 2.61$	8.0	ı	32.32	27.93	38.18	(Figure 1)	0 0	0	100.0
MFA ( 3H)	ARSCC	$33.80 \pm 2.88$	8.5	ı	33.42	28.83	41.75	$1.29 \pm 1.00$	76.0	7.0%	<0.001

Fable 4 to be continued

acidity pH; P-value = significance, or rather probability of the null hypothesis; SCC = somatic cell count; SD = standard deviation; SH = titration milk acidity; SNF= MFA = milk fermentationability (yoghurt test); MFP = milk freezing point (MFP-CR); MI = milk indicator; min = minimum; OM = original milk, n = 53; pH = active SH = degree by Soxhlet-Henkel (milk titration with NaOH solution in ml × 2.5 mmol/l); ARSCC = artificial reduction of somatic cell count, n = 53; C = casein content; CA = citric acid concentration; CF = curd firmness; CP = crude protein content; d (%) = relative average difference, where 100% = OM; d = average difference (difference, arithmetic mean: ARSCC – OM); EC = milk electrical conductivity; ECT = enzymatic coagulation time; F = fat content; FFAs = content of free fatty acids in milk fat; L = lactose monohydrate content; log ECT = logarithm of ECT value to the base 10; log SCC = logarithm of SCC value to the base 10; m = median; max = maximum; solids non fat content; t = t-value of the paired t-test criterion; TS = t total solids content; U = t urea content; t = t-value of the paired t-test criterion; t = t-value of the paired t-test criterion t-test criterion. geometric mean TS, CA). Overall, it can be stated that, apart from U and especially MFA, all average relative differences (d %) of milk indicators (naturally with the exception of SCC) vary around 1% or less. These changes through ARSCC for the 12 other controlled milk indicators can be considered practically negligible. For this reason, except for SCC, milk before and after ARSCC can be considered identical in terms of components and properties (MFP, pH and EC). For MFA, an increase (Table 4; improvement of fermentation quality) from 32.51 °SH to 33.80 °SH, i.e. by 1.29 °SH (by 3.97%; P < 0.001) was found. The *t*-value for the test criterion, in terms of significance, was the highest for SCC, log SCC and MFA (5.81, 20.44 and 9.07, respectively). The above finding of improvement of MFA of milk from a technological point of view through ARSCC confirms the result from the experiment 1 and thus proves that reduced fat did not affect this result. It can be hypothetically mentioned that the effect of a potential reduction in gamma globulins on the membranes (immunoglobulins from B-lymphocytes) of removed somatic cells (ARSCC) may be the cause of this quite unexpected phenomenon (Figure 1). Similar information has not been reported in the scientific literature so far.

On the topic of the influence of ARSCC (only one work was found) on the technological properties of milk, Carmo et al. (2024) found that milk with SCC  $\leq 200 \ 10^3$ /ml showed higher yields in cheesemaking and although centrifugation and microfiltration reduced the content of milk fat (centrifugation from 3.3% to 2.9%), total solids (centrifugation from 11.8% to 11.27%) and SCC (centrifugation from 221 10<sup>3</sup>/ml to 122 10<sup>3</sup>/ml), these processes did not affect the yield of fresh cheese. However, this finding was made at lower levels of SCC. Silva et al. (2012) evaluated the effect of SCC and TCM of raw milk on cheese yield. They used three levels of SCC (below 200 10<sup>3</sup>/ml; from  $200 \, 10^3 / \text{ml}$  to  $750 \, 10^3 / \text{ml}$ ; above  $750 \, 10^3 / \text{ml}$ ). Increasing SCC in raw milk led to increased protein loss in whey. High SCC (above 200 10<sup>3</sup>/ml) in milk correlated with reduced dry matter yield.

With the specified parameters for centrifugal separation of somatic cells from milk, it was logically confirmed (Hanus et al. 2021) that the total number of microorganisms in milk (TCM) is not reduced by the ARSCC process and was not significantly affected from a practical point of view [xg before (OM) and after ARSCC 179  $10^3$  CFU/ml and  $213 \ 10^3$  CFU/ml; P > 0.05 for TCM]. Furthermore,

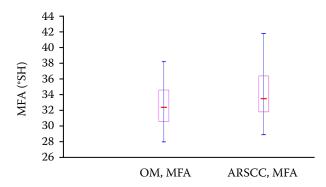


Figure 1. Comparison of frequency distribution of MFA (°SH), medians, variability and variation range before and after ARSCC (32.32 °SH and 33.42 °SH; P < 0.001; Table 4) with milk otherwise identical in composition and properties

Construction of box graph: the file median (the central short horizontal line); the top edge of 1<sup>st</sup> and 3<sup>rd</sup> quartile (the tetragon); the variation range as difference between maximum an minimum (the vertical line)

ARSCC = artificial reduction of the somatic cell count; MFA = milk fermentation ability (yoghurt test); OM = original milk

if mastitis milk, as a result of the activity of bacterial pathogens, contains their metabolites and toxins, then the reduction of SCC itself does not reduce these toxins in milk. Subsequent pasteurisation usually eliminates pathogens to a large extent. However, because many enterotoxins can be heat-stable, their potential for health risk persists even after pasteurisation, and mastitis milk naturally contains more pathogens with a higher potential for the presence of these toxins. It can therefore be reasonably assumed that ARSCC will not improve the quality of raw milk, possibly already damaged by mastitis during its secretion, from a health point of view. For the above reasons, it is necessary to investigate, evaluate and describe the effect of ARSCC on milk as a raw material and to further search for and develop suitable analytical methods for identifying the ARSCC process, in order to improve the quality control of raw milk supplies for processing in dairies.

When testing the ARSCC process under laboratory conditions, the centrifuge drum was finally disassembled after ARSCC and carefully washed using mechanical cleaning with relevant milk with reduced SCC to achieve maximum recovery of SCC in the treated milk (Hanus et al. 2021), relative to the original milk. This repaired milk was again measured for SCC after gentle but thorough homoge-

nisation. In this process, the recovery of SCC (its return by washing) from the centrifuge drum after ARSCC to the treated milk achieved values similar to the original milk – SCC in the original milk 850  $10^3$ /ml (xg 505  $10^3$ /ml), after ARSCC 310  $10^3$ /ml (xg 163  $10^3$ /ml) a reduction in SCC by -63.5%, and after recovery again 867  $10^3$ /ml (P > 0.05). This also means that there was no physical disintegration of somatic cells when milk was loaded by the ARSCC.

As early as Papajova (1983), inhibitors of milk fermentation processes were reported to include not only residues of pharmaceuticals and disinfectants, but also immunoglobulins (gamma globulins) and other natural substances with antibacterial activity, produced during inflammation of the mammary gland as part of the udder's defense mechanisms. A correlation was found between the occurrence of mastitis and inhibitory substances in milk, i.e. a decrease in the fermentation ability of milk at high SCC (correlation coefficient –0.35). Further a decreased fermentation ability of milk with increased SCC was found. On the contrary it has also been sporadically pointed out that even milk with high SCC can be a suitable nutrient medium for cultivation, and that mastitis itself does not have a decisive influence.

## **CONCLUSION**

The previous suspicion that adulteration technology and violation of the authenticity of milk raw material by the method of artificial reduction of somatic cell count (ARSCC) deteriorates the technological properties of cow's milk (cheesemaking and fermentation ability) has not been confirmed. On the contrary, the fermentation ability of raw milk improves after ARSCC and the cheesemaking also improves slightly due to the shortening of the milk coagulation time.

Despite these results, it is necessary to look for analytical methods with the potential to detect this illegal practice (ARSCC) when supplying milk for processing to dairies for reasons of: *i*) health risks (penetration of higher pathogen loads and occurrence of their toxins); *ii*) legislative and ethical requirements. Logically, milk subjected to ARSCC is not suitable as a raw material for technological processing intended for human consumption and therefore should not be delivered to the dairy.

## **Conflict of interest**

The authors declare no conflict of interest.

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