



# Article Farm Level Milk Adulteration: Changes in the Physicochemical Properties of Raw Cow's Milk after the Addition of Water and NaCl

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**Abstract:** Sustainable food security assumes the elimination of food resources adulteration that is already present on farms. This paper is focused on changes in physical and chemical properties of raw cow's milk treated by the addition of water and NaCl. The main studied factor is the freezing point of milk, which is strongly influenced by the chosen treatment. Adulteration of milk by water can be detected by the changed freezing point of the milk, but this can be brought within the range of standardized limits by the addition of NaCl. Determining the concentration of chloride ions in milk by the titration method is a proxy for the added NaCl. The analysis of raw cow's milk from 17 agricultural farms in Southwest Slovakia revealed a negative correlation between the content of chlorides and the freezing point. In another laboratory experiment, the differences in the milk freezing points were statistically significant in the samples treated with different amounts of NaCl. The relationship of chlorides and the freezing point to other milk components (minerals, lipids, proteins, solids-not-fat, lactose, pH, and milk acidity after Soxhlet–Henkel) were analysed, as well. The results showed that the chosen method of chlorides detection to identify the adulteration of milk, by added water and NaCl, was not effective due to the unstable composition of milk and uncertainty in measurements (the coefficient of determination was very low,  $R^2 = 0.3022$ ).

**Keywords:** sustainable food security; milk adulteration; raw cow's milk; freezing point of milk; milk quality; sodium chloride; NaCl

### 1. Introduction

There have been great improvements in sustainable food security [1–3] where the farm has an irreplaceable function [4,5]. Food is very diverse and only a few resources are as valuable as milk. On the other hand, there is scepticism by the public about the health effects of milk and dairy products, which is reflected in an increasing intake of plant-based drinks (from soy, rice, oat etc.). However, but the available scientific research shows that intake of milk and dairy products contributes to meeting nutrient recommendations, and may protect against the most prevalent chronic diseases, whereas very few adverse effects have been reported [6]. Besides some accidental negative factors affecting the quality of milk, we also encounter its deliberate adulteration [7–17]. The adulteration is being motivated by economic reasons and the situation is worse in less developed countries due to insufficient monitoring systems [6]. Milk adulteration detection should be comprehensive and effective focusing on the relevant properties of milk and other dairy products [18–20]. Basic parameters that are checked in milk samples are fat percentage, solid-not-fat (SNF)



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**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). percentage, protein content and the freezing point (it detects the content of water) [21]. Adulterants in milk include addition of foreign protein, milk from other species, whey and water. Some of the adulterants have a possible health impact [18,21,22].

Milk is characterized by a cryoscopic effect, which means that the freezing temperature of the milk is lower than the freezing temperature of the solvent, i.e., water (<0 °C). The freezing temperature of milk in Slovakia is around -0.524 °C [23]. The method of determining the freezing temperature of milk has been used for more than half a century to detect the adulteration of milk with water [24]. The changed freezing temperature of watered milk can be adjusted to acceptable values by the addition of NaCl. If NaCl is added to unconcentrated cow's milk, the level of some non-sedimentable elements will increase, e.g., Ca<sup>2+</sup> and Mg<sup>2+</sup>, and the stability of casein micelles will change [25–30]. NaCl will lower the pH of milk [25,26,29,31,32] and increase micelle hydration [25,26,33]. However, the addition of 50–100 mmol NaCl will increase the maximum heat coagulation time (HCT) of unconcentrated milk and the pH will be higher [25,34]. A dose of  $\geq$ 200 mmol NaCl is destabilizing over a wide range of pH 6.4–7.4 [25,35,36]. The minimum pH–HCT was not observed, even at 420 mmol NaCl [25,34,36]. The determination of the freezing point depression (FDP) has been tested most often using NaCl solutions, as well [37,38].

The fact that the addition of NaCl to milk lowers the pH and increases the calcium concentration in the dissolved phase is explained by the replacement of calcium with sodium in casein micelles and the release of calcium into the solution [39–42]. During the lactation period, the salt concentration in milk increases. Na<sup>+</sup> and Cl<sup>-</sup> are among the most important ions influencing the physicochemical properties of milk. A higher NaCl content means more soluble calcium, however, the soluble phosphorus remains the same. As many changes during the lactation period are similar to those caused by the increased NaCl content, we can assume a strong effect of higher NaCl concentration on the physicochemical properties of milk [25]. The key mechanisms in the physicochemical stability of casein micelles caused by the added NaCl are probably related to the charge of casein micelles. Huppertz and Fox [34] and Sbodio et al. [43] recorded the effect of enzymes together with NaCl on the coagulation properties of milk (although enzymes had a significant and NaCl less significant effect). NaCl also affects the solubility of selected proteins during milk storage, or in modified milk [44,45].

The main reason for milk adulteration by water is to increase production volume [23,46–48]. Watered milk can be identified by determining its freezing temperature according to the valid Slovak standard STN EN ISO 5764 (2009) Milk. The most common, freezing point is determined by thermistor cryoscope method (reference method) in laboratory practice. According to the working hypothesis of this paper, the freezing temperature of watered milk can be adjusted to the original or to standardized values by the addition of NaCl. The first partial goal of the paper was to determine the relationship between the natural NaCl content and other selected characteristics of raw untreated cow's milk from farms in SW Slovakia; the second partial goal was to find out how the addition of NaCl affects the freezing point of milk and other selected chemical properties.

#### 2. Materials and Methods

The milk analyses were performed at the accredited laboratory of the Veterinary and Food Institute in Bratislava, at the National Reference Laboratory for Milk and Dairy Products in Nitra, Slovakia. To determine the concentration of chloride ions in milk, a total of 17 samples were taken from various farms (information stored in the non-public archive of the mentioned institute). The samples were collected between 30 July and 28 August in an amount of 1000 mL and transported to the laboratory at 5 °C. Samples of raw milk were taken from a mixture of different breeds, of which the main breeds were Slovak spotted and black Holstein cattle with accompanying breeds Ayshire, Jersey, black-spotted lowland cattle and Pinzgau.

#### 2.1. Determination of Chloride Ions

The concentration of chloride ions was determined according to Article 54 of the valid Slovak standard STN 57 05 30 (1972) Methods of testing milk and liquid dairy products. All chemicals were of analytical grade and were purchased from Centralchem, Bratislava, Slovakia.

#### 2.2. Determination of Other Physical and Chemical Characteristics of Milk

Milk freezing temperature: The freezing temperature of the milk was determined with a special apparatus Cryostar I (Funke-Gerber, Funke—Dr. N. Gerber Labortechnik GmbH., Berlin, Germany) [24]. Certified calibration standards -0.504 °C, -0.512 °C, and -0.560 °C were purchased from Funke-Gerber, Berlin, Germany. The instrument was operated in plateau seeking mode.

Milk acidity expressed in Soxhlet-Henkel degrees (°SH): The acidity of the milk was determined according to Article 58 of the valid Slovak standard STN 57 05 30 (1972) Methods of testing milk and liquid dairy products. All chemicals were of analytical grade and were purchased from Centralchem, Bratislava, Slovakia.

Milk acidity expressed by pH scale: The milk acidity was determined according to Article 59 of the valid Slovak standard STN 57 05 30 (1972) Methods of testing milk and liquid dairy products. We used the pH meter HI 3220 (Hanna Instruments, Woonsocket, RI, USA) and certified pH calibration solutions pH 4.0, 7.0 and, 10.0 (Hach, Slovakia)

Determination of milk composition (fat, proteins, lactose, solids-not-fat, total solids): The number of monitored components in milk was determined using a Milcoscan FT 120 infrared absorption analyser (Foss, Hilleroad, Denmark). The Slovak standard STN 57 05 36 Determination of milk composition by infrared absorption instrument and the international standard ISO 9622:2013 Milk and liquid milk products—Guidelines for the application of mid-infrared spectrometry were used. The certified calibration samples were obtained from Actalia, Cecalait, Poligny Cedex, France.

# 2.3. Detection of Changes in the Selected Physicochemical Parameters Depending on the Amount of NaCl Added

In this partial task, the effect of artificially added, controlled amounts of NaCl on selected physical and chemical properties of raw cow's milk was analysed under laboratory conditions. For the analysis, 10 samples (the sample volume was 1000 mL) were taken from one particular milk vending machine in Nitra at different times. The samples were taken at relatively short intervals to eliminate the influence of the season on the monitored milk properties as much as possible (collected from 30 September to 28 October). The final number of analysed variants (subsamples) was 80 (10 samples  $\times$  4 variants according to the amount of the added NaCl  $\times$  2 parallel measurements).

The 1000 mL sample was divided into four equal portions of 250 mL each (variants 1, 2, 3, and 4). Each variant was treated with a different amount of chemically pure NaCl to prepare samples with a linear NaCl gradient: variant no. 1: 0 g NaCl, variant no. 2: 0.0253 g NaCl, variant no. 3: 0.0506 g NaCl, variant no. 4: 0.0759 g NaCl (marked as MFT1, MFT2, MFT3 and MFT4). The basic amount of NaCl was determined empirically so that we could prepare samples with NaCl addition in the physiological range of milk freezing temperatures. The procedures for the determination of selected physicochemical parameters of the amended milk were the same as those mentioned in the section 'Determination of other physical and chemical characteristics of milk'. After the addition of NaCl, each sample was analysed for MFT, Cl<sup>-</sup>, total minerals, fats, proteins, lactose, solids-not-fat, °SH and pH.

# 2.4. Statistical Evaluation of the Results, Verification of the Selection Conformity, and the Principal Component Analysis (PCA)

To determine the statistical significance of the differences between the different variants, we calculated the arithmetic mean, the variance and the standard deviation. To confirm whether there was a statistically significant difference between the raw cow's milk samples with added NaCl and the unmodified milk sample, we used a non-parametric method that applies to an unknown distribution, the Wilcoxon test for two samples. We determined comparisons in ten samples from farms in SW Slovakia, while each basic sample had 4 variants (see above).

The principal components analysis (PCA) of the relationships between chlorides and other selected chemical and physical characteristics of raw cow's milk was performed in Canoco (version 4.5, Microcomputer Power, Ithaca, NY, USA, © 1988–2002 Biometris—quantitative methods in the life and earth sciences, Wageningen University and Research Center, NL, USA) and the calculated values (eigenvalues) were visualized in CanoDraw (version 4.0, copyright as above). We used the studies of ter Braak and Šmilauer [49] and Lepš and Šmilauer [50] to interpret the outputs.

The correlation (regression line, correlation equation) of the relationships between the NaCl content in milk and the milk freezing point was calculated and visualized in Microsoft Excel 2010 (Microsoft, Redmond, WA, USA). The degree of adjustment was defined by the determination coefficient  $R^2$  that measures the strength of the relationship between the actual values of the dependent variable and the values lying on the regression line.

The results of laboratory analyses were validated following the document "Validation of test methods (MSA 0111-98, 1998 SNAS)" and Slovak Republic Government Regulation No. 320/2003 Coll. on the monitoring of certain substances and their residues in live animals and products of animal origin (Annex 8 Analytical methods and interpretation of the results of analyses of official samples).

#### 3. Results

#### 3.1. Chemical, Physical and Biological Composition of Milk in Relation to Chlorides

The selected chemical, physical and biological properties of raw cow's milk were determined in pooled milk samples. Basic (untreated) samples were obtained from 17 farms in SW Slovakia. Watered milk treated by the addition of NaCl was examined from 10 samples from one holding. From the chemical characteristics, we determined the total mineral content, content of fat, protein, lactose, solid-non-fat and acidity expressed in pH and milk acidity according to Soxhlet-Henkel (°SH); from the physical properties the freezing point of the milk and from the biological characteristics the somatic cell count (Table 1).

**Table 1.** Chemical, physical, and biological properties of basic samples of raw cow's milk from 17 farms in SW Slovakia.

n	Milk Freezing Point (°C)	Minerals (g·100 g <sup>-1</sup> )	Chlorides (mg·100 g <sup>-1</sup> )	Fats (g·100 g <sup>-1</sup> )	Protein (g·100 g <sup>-1</sup> )	Lactose (g·100 g <sup>-1</sup> )	Solids-Not- Fat (g∙100 g <sup>-1</sup> )	Somatic Cell Count (Thousands mL <sup>-1</sup> )	°SH	pН
1	-0.5280	0.65	47.87	4.07	3.38	4.78	8.81	459	7.20	6.65
2	-0.5300	0.61	59.37	3.26	3.18	4.90	8.69	1012.5	7.40	6.60
3	-0.5340	0.66	46.09	4.37	4.08	5.00	9.74	131.5	7.20	6.50
4	-0.5190	0.79	81.55	4.64	3.87	4.21	8.86	2426.5	7.55	6.57
5	-0.5345	0.72	74.46	4.18	3.49	4.42	8.63	390.5	6.95	6.39
6	-0.4440	0.71	39.00	2.74	2.72	4.12	7.55	248	7.35	6.60
7	-0.5350	0.65	74.41	4.10	3.34	4.73	8.72	365	7.25	6.36
8	-0.5190	0.61	78.89	3.82	3.03	4.84	8.48	627	7.45	6.67
9	-0.5225	0.75	86.84	3.65	3.29	4.68	8.72	186.5	7.00	6.53
10	-0.5335	0.76	74.43	4.41	3.40	4.67	8.83	680.5	7.15	6.47
11	-0.5330	0.79	86.83	3.91	3.64	4.58	9.00	158	7.30	6.62
12	-0.5290	0.77	85.08	3.70	3.18	4.57	8.52	226	7.30	6.65
13	-0.5300	0.77	88.59	3.77	3.42	4.61	8.79	188	7.25	6.67
14	-0.5290	0.76	81.49	4.25	3.52	4.69	8.96	314.5	7.20	6.44
15	-0.5320	0.77	88.62	3.52	3.59	4.61	8.97	3416.5	7.40	6.23
16	-0.5895	0.84	92.16	6.20	4.36	4.53	9.73	298.5	7.10	5.41
17	-0.6075	0.9	76.23	4.29	3.83	3.95	8.68	1175.5	7.15	5.37

#### 3.2. Validation of Laboratory Analysis Results

The calculated standard uncertainty of the Soxhlet–Henkel titration acid (°SH) type A measurement was uA = 0.033; converted to a relative measurement uncertainty of 0.004022151 at cv (in %) = 0.4022. The resulting uncertainty B was found to be uB = 0.033955854. In the

next part of the analysis, we calculated the combined standard uncertainty of type C, uC = 0.047113339. The resulting expanded measurement uncertainty was U = 0.094226677 °SH; in % it was 1.160427057.

The calculated standard uncertainty of measuring the chloride content in type A milk samples was uA = 0.827, converted to relative uncertainty cv (in %) = 0.97. The resulting expanded measurement uncertainty was U = 1.95%.

When we compared the baseline samples (untreated with NaCl) with the samples with a small amount of added NaCl, we found a minimal increase in the absolute average values of the milk freezing point, while the statistical variance of the values was low (Table 2).

	Basic Samples (without Added NaCl)	Samples with Added 0.0253 NaCl g∙250 mL <sup>-1</sup> H <sub>2</sub> O	Samples with Added 0.0506 NaCl g∙250 mL <sup>-1</sup> H <sub>2</sub> O	Samples with Added 0.0506 NaCl g·250 mL <sup>-1</sup> H <sub>2</sub> O
Arithmetic mean Standard deviation	−0.4964 °C ±0.01 °C	$-0.5026\ ^{\circ}{ m C}\ \pm 0.01\ ^{\circ}{ m C}$	−0.5083 °C ±0.01 °C	−0.5157 °C ±0.01 °C
Number of samples	10	10	10	10
Degree of freedom	9	9	9	9

Table 2. Analysis of variance of freezing temperature (° C) of raw cow's milk.

The individual values of variance and the basic parameters for the U (W) value of the Wilcoxon test were determined by inserting the values of the milk freezing point into the standardized statistical formulas (cf. Methods). We had two choices and tested the hypothesis that their distribution functions were equal. If min (U1, U2)  $\leq$  wm, n ( $\alpha$ ) we rejected the null hypothesis about the equality of distribution functions. The method we used distinguished the values of *Z* (with a minimum number of measurements of 20 and more) and W for a number of 10 and less. As the number of our observations was 10 in each variant, we compared the calculated value W with the critical value (Table 3). We tested the results at a significance level of  $\alpha = 0.05$  and subsequently also at the level of  $\alpha = 0.01$ . After comparing the basic 10 samples without added NaCl (MFT1) with samples with different content of added NaCl (MFT2, MFT3, MFT4) in each case, we found that min (U1, U2)  $\leq$  wm, n ( $\alpha$ ), so we rejected the zero hypothesis on the equality of distribution functions and we accepted an alternative hypothesis (Table 4). The alternative hypothesis stated that there was a statistically significant difference between the untreated samples and the samples with added NaCl at a significance level  $\alpha = 0.05$  and  $\alpha = 0.01$ .

**Table 3.** Wilcoxon test values for two selections (MFT1, MFT2, MFT3, MFT4 = experimental variants; milk freezing temperature is in  $^{\circ}C$ ; Abs, *R* and *S* = statistical quantities of order and pair differences).

Sample	MFT1	MFT2	MFT3	MFT4	1 Abs	1 R/S	2 Abs	2 R/S	3 Abs	3 R/S
1	-0.511	-0.518	-0.523	-0.530	0.007	8	0.012	4.5	0.019	5
2	-0.488	-0.493	-0.501	-0.507	0.005	2	0.013	7	0.019	5
3	-0.500	-0.504	-0.51	-0.518	0.004	1	0.01	2.5	0.018	2
4	-0.498	-0.505	-0.505	-0.519	0.007	8	0.007	1	0.021	9.5
5	-0.499	-0.506	-0.513	-0.519	0.007	8	0.014	9	0.02	7.5
6	-0.502	-0.509	-0.517	-0.52	0.007	8	0.015	10	0.018	2
7	-0.476	-0.482	-0.489	-0.496	0.006	4	0.013	7	0.02	7.5
8	-0.480	-0.486	-0.492	-0.499	0.006	4	0.012	4.5	0.019	5
9	-0.505	-0.512	-0.518	-0.526	0.007	8	0.013	7	0.021	9.5
10	-0.505	-0.511	-0.515	-0.523	0.006	4	0.01	2.5	0.018	2

**Table 4.** Calculated statistical values of Z and W, standard deviation (SD) from W, value of *p* and critical value of W at the level of significance  $\alpha = 0.05$  and  $\alpha = 0.01$  (MFT1, MFT2, MFT3, MFT4—experimental variants).

Compared Variants	Z	W	W <sub>mean</sub>	SD from W	Value of <i>p</i>	Critical Value of W at $n = 10$ and $\alpha = 0.05$	Critical Value of W at $n = 10$ and $\alpha = 0.01$
MFT1:MFT2, MFT1:MFT3, MFT1:MFT4	-2.8031	0	27.5	9.81	0.00512	8	3

#### 3.3. Relationship between Chlorides and Other Selected Milk Characteristics

In the second part of the study, we analysed the relationship between the natural content of NaCl and other characteristics of raw unprocessed cow's milk from farms in SW Slovakia. Principal components analysis (PCA, Figure 1) showed that there was a negative relationship between the chlorides on one side and the acidity (pH) and freezing point of the milk on the other side.



**Figure 1.** Principal component analysis (PCA) biplot of the relationships between chlorides and other selected chemical and physical characteristics of raw cow's milk. Abbreviations: Cl-chlorides, Miner-minerals, Fat-fats, Prot-proteins, SNF-solids-not-fat, Lact-lactose, pH-milk reaction based on pH value, SH-milk acidity according to Soxhlet–Henkel (°SH) and FP-milk freezing point.

Another aim was to find out how the artificial addition of NaCl affected the milk freezing temperature and other selected chemical properties of the milk. We wanted to find out if the added NaCl could make it impossible to detect the intentionally added water to the milk. Along the chemical gradients (four milk variants amended with different amounts of chemically pure NaCl), the influence of chloride(s) in the amended milk was significantly (p < 0.005) strengthened and became the most important factor. Total minerals had less strength, but due to the increased content of cations along with pH, they correlated the most positively with the milk freezing temperature. Chlorides had a negative relationship with the milk freezing temperature again (Figure 2).

The working hypothesis was that the effects of water added to milk (in adulteration) can be eliminated partially or completely by adding NaCl, which has the opposite effect on the milk freezing temperature than water. When we correlated all measurements of relationships between Cl<sup>-</sup> content and freezing temperature of raw cow's milk in one regression curve (Figure 3), the individual reference points of the measurements were highly scattered, and the coefficient of determination  $R^2$  was very low (y = -0.0004x - 0.4678,

 $R^2 = 0.3022$ ). This high diffusion of the values was probably generated by the different chemical composition of the ten basic samples (other factors related to the milk freezing temperature, such as lactose content, pH, etc., entered into the correlation relationships). The results indicate that it is not possible to define limit values for Cl<sup>-</sup> (e.g., reference values) to detect milk adulteration by water and added NaCl at this stage of the research. We believe that such values could only be determined in a constant composition of raw cow's milk (however, milk has a variable composition).



**Figure 2.** Principal component analysis (PCA) biplot of the relationships between chlorides in the milk and other selected chemical and physical characteristics of raw cow's milk from 10 samples (80 variants) with the added NaCl (evaluated similarly to Figure 1).





# 4. Discussion

Milk is being adulterated in sophisticated ways that demands for cutting edge research for the detection of the adulterants [21]. In our case, the chemical composition of raw milk

from 17 farms was in line with the literature, the fat content was lower and in some cases, exceptionally low (once less than 3%), and in general the mineral content did not reach the average of milk samples. Based on the chemical composition of milk, the values of salt content were average and some minerals could be associated with the casein micelle according to [30,51–53]. Raw cow's milk samples were taken in the summer (30.7., 15.8., 20.8., 21.8., 28.8.), when the highest freezing temperature of milk is (max. -0.4440 °C).

Our analyses showed that the relationships between chlorides and other studied chemical and physical parameters were very diverse. We found a negative relation between the chloride content and the milk acidity, based on Soxhlet–Henkel (°SH). According to some authors, the titratable acidity is mainly influenced by other factors (e.g., higher incidence of microorganisms and older age of milk, [54]). However, some published data indicate that NaCl lowers the milk pH, and thus the milk freezing temperature rises [25,26,29–32]. The added NaCl also affects the pH of concentrated milk, including the charge of casein micelles [34]. According to the Soxhlet–Henkel value, milk acidity depends significantly on proteins, and changes in protein consistency may be crucial. Casein occurs in milk, either as a micelle or in solution, and its condition is also affected by the addition of salts, including NaCl [30]. The final amount of chlorides and MFT in raw cow's milk is influenced by a number of factors, such as feeding, drinking water, dairy breed, methods and time of milking, milk storage, amount of milk produced by the dairy cow, cleaning of equipment, etc. e.g., [55].

Near Infrared (NIR) spectroscopy [56] has also used to detect water as well as whey in milk. Later, this method was improved and a Medium Infrared (MIR) spectrometer was used, which appeared to be better for detecting tap water, whey, hydrogen peroxide, synthetic urea and urine [57]. A chemical analysis based on the addition of 0.1 N silver nitrate solution and 0.5 mL of 10% potassium chromate solution is used to detect the added salt in the milk. Proof of added salt is the appearance of a yellow colour, and a red colour indicates that the milk is free from added salt (limit of detection 0.02% w/v [58]).

Our findings apply only to raw cow's milk, so we must be careful with the generalization of the results. We have not investigated the relationships between the micellar structure of casein and its state dissolved in water. The micellar state of casein may be disrupted by various factors, e.g., dissociation of casein and salt [59], acidification, high or low temperature, the addition of chelates, the addition of divalent cations, and, importantly for us, the addition of NaCl (NaCl lowers the pH and increases the Ca content in solution [30]).

We can add that milk adulteration with water is still an existing problem, especially because of its simplicity and minimal costs. The risk of detection is low, especially if NaCl is also added to adjust the milk freezing temperature. The current available scientific literature hardly discusses this issue, as there are no relevant statistics on the use of NaCl and almost the only source of information is data from farm workers, who are reluctant to cooperate due to job loss concerns. The method proposed by us failed because the NaCl content in raw cow's milk appeared to be very variable, and may be above average, due to either natural or management factors (e.g., it comes from feeding). It would help to distinguish natural NaCl from artificially added NaCl, but so far it is only hypothetical. Iodine seemed to be a good candidate for table salt detection (in many countries, table salt is iodized for health reasons), but it can also come from pre-milking teat disinfection. However, the determination of added table salt in milk could be triggered by another way of milk adulteration with cheap raw sea salt (we already have internal information on the use of sanding salt that was intended for winter road maintenance). Future studies could focus on other properties of NaCl and on concomitant elements and compounds occurring together with NaCl.

# 5. Conclusions

Sustainable food security does not tolerate the adulteration of food resources and, therefore, food safety should be of first priority. Milk can be adulterated by water already on the farm. The determination of milk freezing point has been used for more than half

a century to detect the adulteration of milk with water. Our results clearly show that if the watered milk is intentionally amended with NaCl, the current reference method based on the cryoscopic determination of the milk freezing temperature does not reveal such intentional adulteration if it had been done reasonably. The determination of chlorides as a marker has also proved unsuitable, as it is not possible to determine critical amounts of chlorides originating mainly from the added NaCl. When adulterating milk, different amounts of NaCl must be used in different milk samples to adjust the freezing point of the watered milk, as milk of different origins has different natural physicochemical properties influencing the freezing point. Limit values for NaCl or chlorides could be set for the needs of food control and safety authorities only for a constant composition of raw cow's milk, which is not realistic. We also state that the determination of the NaCl content in milk is not sufficient to detect the milk watering as the milk freezing temperature is affected by many other properties of each milk sample and, therefore, sample generalization and quantification of the bonding NaCl—water content added to milk is not possible.

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