Research into Correlation of Milk Electrical Conductivity and Bacterial Contamination

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Introduction

Our previous studies [1] point to existence of direct correlation between specific conductivity in milk and the temperature of the sample, as well as indirect correlation with its compositional parameters (content of fat, protein and lactose). Another relevant factor that has impact on specific electric conductivity of milk, on its compositional parameters and freezing point depression is bacterial contamination of the sample.

Electric conductivity of milk as a relevant parameter can be used to evaluate expiry date of dairy products, to determine microorganisms in milk [2]. Few studies have been carried out to define modelling of bacterial growth by means of electrical parameters. As a function of specific electrical conductivity only modelling of growth of yeast and *Yersinia enterocolitica* has been described [3], however these studies are neither milk nor this culture analysis in milk related.

In order to evaluate possible effect of bacterial contamination of milk on variation of specific electric conductivity an experimental analysis has been carried out. More than 30 different milk samples were taken and stored at a constant temperature with their electric conductivity being recorded with their total bacterial count being simultaneously determined. The scheme of the experimental analysis outlined in Fig. 1.

Experiment and result analysis

Right after milking until the beginning of the experiment each milk sample was stored at +2...+6°C temperature in order to prevent bacteria from growing. During the experiment the sample was stored at +15°C temperature. Selection of this temperature was determined by the fact that such a temperature is favourable to bacterial growth; besides, there is high probability that the average temperature of the milk sample since sampling until analysis in the laboratory will not exceed this temperature.
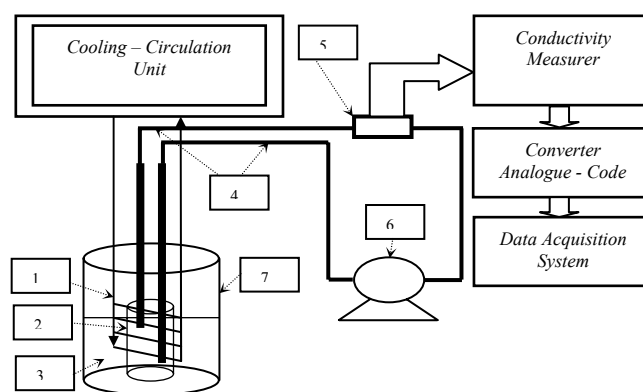

Fig. 1. Structural scheme of correlation between specific electric conductivity and bacterial contamination determination

The structural scheme of the experiment in Fig.1 consists of the following:
 1 – cooling coil;
 2 – glass test tube with a sample;
 3 – water;
 4 – sample circulation tubing;
 5 – electrode for specific electric conductivity measurement;
 6 – peristaltic sample circulation pump;
 7 – thermos container.

During the experiment milk sample is poured into a glass test tube *2*, which is placed into thermos container *7*. The test tube containing milk sample *2* is immersed in water in its full height in thermos container, to maintain constant temperature of the sample. The temperature of water *3* is maintained at (+15±0.5)°C by means of a cooling-circulating device, which cools water through the coil *1*. Milk sample is delivered to the electrode for measurement of specific electric conductivity *5* by the peristaltic pump *6* through the autoclaved tubing. Flow speed is about 1ml/min. Sample circulation through the measurement electrode is constant; the sample is being constantly mixed throughout the experiment so as to avoid contamination of the measurement electrode. To determine

bacterial contamination a portion of the sample is taken every 4 hours. Total bacteria count is determined by the standard method i.e. standard plate count [4].

During the experiment specific electric conductivity is measured by an analogical measurer, which feeds data through converter analogue – code (Omega DAQ-802, USA) to a data processor, a PC in our case. Electrical conductivity is recorded at 10-minute intervals, with a few measurements made and their mean value recorded. Duration of one sample analysis is about 30 hours.

When analysing change of electrical conductivity as a function indicative of bacterial contamination, the first electrical conductivity measurement value (time moment=0) is subtracted from the obtained measurement data in order to determine the correlation of these changes.

Table 1. Results of analysis of specific electric conductivity change due to bacterial contamination

No.	Time t, hours	Change in specific electric conductivity ΔC, mS/cm	Change in bacterial contamination ΔTBC, CFU/ml*10^3
1	2	3	4
1	4	0.00	0
2	8	0.00	0
3	12	0.00	0
4	16	0.00	0
5	20	0.00	3
6	24	0.02	79
7	28	0.04	1944

Table 1 demonstrates change of bacterial contamination (column 2) and specific electrical conductivity in time t. Columns 3 and 4 respectively present change (means of all changes of analysed samples) of specific electrical conductivity and bacterial count.

As we can see from Table 1 change of specific electric conductivity of milk throughout the whole duration of the experiment (28 hours) consisted in 0.04 mS/cm. During the first 20 hours of the analysis no change was observed. Speed of variation reached its maximum at 25...30 hours of the experiment. The selection of the experiment duration of 28 hours was determined by the fact that the increase in bacterial count by 2 million CFU/ml leads to such acidification of the sample that it becomes no longer suitable for spectrometric analysis (pH<6.0 pH), therefore, further analysis (t>28 hours) was terminated. The data presented in Table 1 is graphically represented in Fig. 2 and Fig. 3.

To relate data obtained from referential analysis (total bacteria count determined by the standard plate count) and the results of specific electric conductivity determination (obtained in the experiment) to time t, the following exponential mathematical model (1), optimal for result approximation, was used.

$$\Delta Y_i(t) = a_i * e^{b_i(t-c_i)};\qquad(1)$$

where $\Delta Y_i(t)$ – value of ΔY at moment of time t; a_i, b_i, c_i – model (equation) coefficients; i – index of unknown value (bacteria count or change of specific electric conductivity).

Coefficients a, b, c were calculated using the least squares regression. Then having estimated the mathematical model of equation (1) and the results of the experiment, change of electric conductivity in time (t=0…28 hours) can be defined by equation (2):

$$\Delta C = 0.95 * e^{0.3(t-38.25)}.\qquad(2)$$

Having likewise found coefficients a, b, c, change of bacterial contamination in time ($t = 0…28$ hours) can be described by equation (3):

$$\Delta TBC = 0.99 * e^{0.805(t-10)}.\qquad(3)$$

Fig. 2 and 3 present graphs of values of change in electrical conductivity and bacterial contamination in time obtained in the experiment and calculated according to formulas (2) and (3).
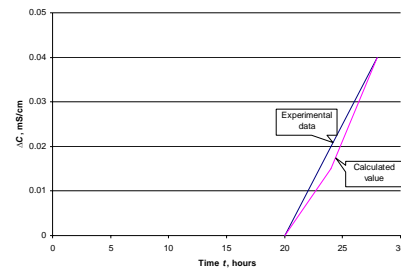

Fig. 2. Change of specific electric conductivity in time t (experimental and calculated)
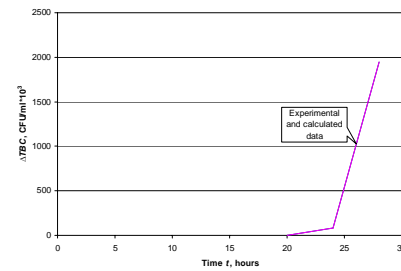

Fig. 3. Change of total bacteria count in time t (experimental and calculated variations superpose)

As we can see from the data presented in Fig. 2 and 3 formulas (2) and (3) precisely define the analysed change. The error of result in determination of change in specific electric conductivity is 0.005 mS/cm, while error of result in determination of bacterial count is 1*10^3 CFU/ml.

On the basis of the experimental data (Table 1) we can determine the mathematical model (4) of interdependence between bacterial contamination and change of specific electric conductivity, which relates the logarithm of bacterial contamination change with specific electric conductivity change:

$$\Delta C = a * \ln(\Delta TBC) + b;\qquad(4)$$

where ΔC – change in specific electric conductivity, mS/cm; ΔTBC – change in total bacteria count, CFU/ml; a,b – coefficients of the model.

Having estimated the results of the experiment and determined coefficients a and b using the least squares regression, we get equation (5), which defines change of bacterial contamination and electric conductivity which is

dependent on the latter in the analysed strips ($\Delta TBC=(0...2000)*10^3$ CFU/ml).

$$\Delta C = 0.00390 * \ln(\Delta TBC) + 0.00543. \qquad (5)$$

The results of values of specific electric conductivity change calculated using model (5) and those obtained during the experiment are presented in Fig. 4.
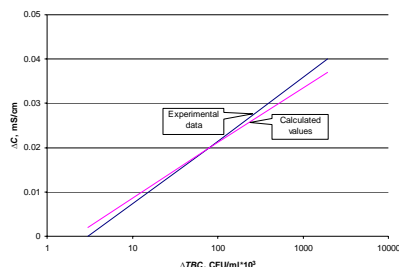

Fig. 4. Interdependency (experimental and calculated) between variations in conductivity and TBC

As it can be seen from Fig. 4, in the analysed strips interdependency between changes in bacterial contamination and specific electric conductivity is precisely approximated by equation (5).

The results of the experiment (Table 1) point out that with increase in bacterial contamination of milk $>10^6$ CFU/ml (milk near to sour or sour i.e. no longer suited for spectrometric analysis), change of specific electric conductivity constituted 0.04 mS/cm. In order to determine the impact that this change of specific electric conductivity can have on milk freezing point depression, we shall model a procedure for milk freezing point determination using the method suggested here [1].

Impact of specific electric conductivity and bacterial contamination on freezing point depression

By means of modelling we have estimated the impact of specific electric conductivity and bacterial contamination on milk freezing point depression. For analysis we used equation (6) and values of its coefficients $a_0...a_4$ [5]. Calculations were performed using a few combinations of values of variables F, P, L, C from an interval of their possible variation. Accepted standards were employed to determine the interval of variation.

$$FPD = a_0 + a_1 * F + a_2 * B + a_3 * L + a_4 * C. \qquad (6)$$

The modelling scheme is presented in Fig. 5, where F, P, L, C – values of variables; $a_0...a_4$ – (9) equation coefficients; $\Delta C=f(TBC)$ – variation in specific electric conductivity due to bacterial contamination of the sample. Values of random combinations are presented in Table 2.

Milk freezing point depression as well as its change was determined for each combination of random values (Table 2) when $\Delta C=0.04$. Results are presented in Table 3.

As we can see, the results of the modelling (Table 3) indicate that when bacteria count in milk increases $>10^6$ CFU/ml, i.e. milk is sour or near sour (cannot be analysed by the spectrometer), change in specific electric conductivity $\Delta C=0.04$ mS/cm increases the error of result

of the forecast milk freezing point depression till 0.001ºC, which in its turn means error of result of 0.2% in determination of extraneous water.
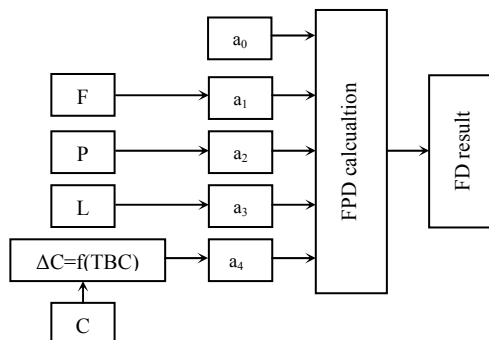

Fig. 5. Analysis of dependency of milk freezing point depression on change in specific electric conductivity due to TBC

Table 2. Combinations of values in modelling of impact of specific electric conductivity variation on freezing point depression determination

Combination No.	F, %	P, %	L, %	C, mS/cm
1	2	5	4	7
2	3	4	5	6.5
3	4.5	3.5	3	6
4	6	3	5	8
5	7	2	6	7.5

Table 3. Results of modelling

Combination No.	FPD in normal conditions, °C	FPD when $\Delta C=0.04$ mS/cm, °C	
		Value	Change
1	-0.423	-0.424	-0.001
2	-0.460	-0.461	-0.001
3	-0.386	-0.387	-0.001
4	-0.539	-0.540	-0.001
5	-0.576	-0.577	-0.001

Therefore, it is expedient to analyse the sample within less than 20 hours since the time of sampling or estimate the error of result due to bacterial contamination.
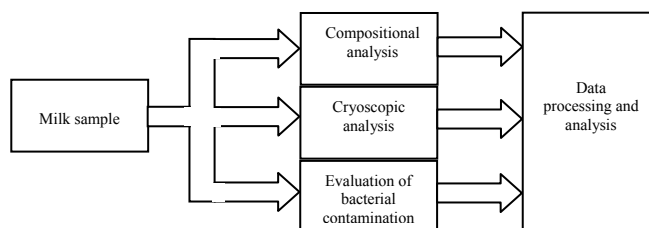

Fig. 6. Structural scheme of verification of results in experimental modelling

To verify theoretical presumption that bacterial contamination influences determination of the freezing point depression we have carried out an experiment the structural scheme of which is presented in Fig. 6.

During the experiment the sample was divided into 3 parts. One of them was intended for compositional analysis (determination of fat, protein, lactose in milk) as well as determination of specific electric conductivity. The second part was meant for determination of the freezing point

depression by the cryoscopic (reference) method. The third part was intended for evaluation of bacterial contamination. More than 300 unpreserved samples were used in the experiment with the sampling time given. The samples were not analysed simultaneously, but within 24 hours following sampling. 4 groups of samples were formed (Table 4). The freezing point depression of each sample was calculated. Mean error or result for each group was determined (I group was analysed first, IV – last) by means of comparison drawn between the calculated freezing point depression with that determined by the cryoscope, and taking into account the time passed after sampling, based on (5), the freezing point depression recalculated for the samples. The mean values of each group's error of result obtained after recalculation are presented in Table 4.

Table 4. Error of result in the freezing point determination of milk

Group	Error, uncorrected, °C	Error, corrected, °C
1	0.004	0.003
2	0.004	0.003
3	0.004	0.003
4	0.005	0.004

As the conducted study demonstrates, having evaluated the bacterial contamination of the sample, the determination of freezing point depression is noted for higher precision.

Conclusions

1. The study proves that specific electric conductivity, bacterial contamination and freezing point depression of milk are interrelated parameters, which have to be evaluated in order to achieve higher precision in determination of the freezing point depression in milk.
2. Evaluation of bacterial contamination is obligatory when a milk sample is analysed after 20 or more hours have passed since sampling.
3. Information system for bacterial contamination analysis is suggested.

References

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There is presented a discussion on change of milk conductivity caused by an increase in bacterial contamination of milk and correction of milk freezing point prediction according to the time passed after milking until analysis. The dependence of conductivity and bacterial contamination is analysed, the experimental schematics, experimental data, mathematical models of dependences and correlation are presented. Change of milk freezing point based on bacterial contamination is simulated and the simulation schematics is presented. The system that estimates the influence of bacterial contamination on freezing point determination is presented together with the experimental data and analysis. Ill.6, bibl.5 (in English, summaries in English, Russian and Lithuanian).

Й. Даунорас, А. Книш. Исследование связи между электрической проводимостью и общим бактериальным загрязнением молока // Электроника и электротехника. – Каунас: Технология, 2006. – № 6(70). – С. 95–98.
Анализируются изменения специфической электрической проводимости молока из-за общего бактериального загрязнения молока и уточнение прогноза температуры замерзания молока с учётом пройденного времени между доением и анализом образца. Анализируется зависимость между электрической проводимостью молока и бактериальным загрязнением, представлены схема анализа, экспериментальные данные, математические модели, описывающие изменения и зависимость между ними. Моделируется изменение результата прогноза температуры замерзания молока из-за изменения его бактериального загрязнения, представлена схема модели. Представлены экспериментальные данные системы, в которой учитывается влияние бактериального загрязнения молока на результат прогноза температуры замерзания образца, их анализ. Ил. 6, библ. 5 (на английском языке; рефераты на английском, русском и литовском яз.).

J. Daunoras, A. Knyš. Pieno elektrinio laidumo ir bakterinio užterštumo ryšio tyrimas // Elektronika ir elektrotechnika. – Kaunas: Technologija, 2006. – Nr. 6(70). – P. 95–98.
Nagrinėjamas pieno specifinio elektrinio laidumo pokytis dėl bendro bakterinio užterštumo bei užšalimo temperatūros prognozės tikslinimas atsižvelgiant į laiką, praėjusį nuo mėginio paėmimo iki tyrimo momento. Analizuojama elektrinio laidumo priklausomybė nuo bakterinio užterštumo, pateikiama tyrimo schema, eksperimentiniai šios priklausomybės parametrai, kitimą ir tarpusavio priklausomybę aprašantys matematiniai modeliai. Modeliuojami užšalimo temperatūros prognozės rezultatų pokyčiai keičiantis mėginio bakteriniam užterštumui, pateikta modelio schema. Pateikiami sistemos, kuri įvertina mėginio bakterinio užterštumo įtaką užšalimo temperatūros nustatymui, eksperimentiniai tyrimo duomenys ir jų analizė. Il.6, bibl.5 (anglų kalba; santraukos anglų, rusų ir lietuvių k.).