STUDY REGARDING THE CHANGES OF COW MILK REDOX POTENTIAL AND *p*H FOLLOW THE ADDITION OF SPECIFIC MICRORGANISMS

P. Savescu, A. Dunoiu, I. Stan

Universitatea din Craiova, Romania
e-mail: psavescu@gmail.com

Abstract

In this paper to pursue the monitoring of the changes that the milk of cows suffering during processing at acidic dairy products - means using electrochemistry combined mathematics and statistical analysis.

Have been pursued thus correlations are established between the redox potential, pH of cow's milk during fermentation and after sowing to determine the optimum fermentation time for each milk product lines.

Study variation redox potential and pH of cow's milk after sowing some microorganisms can provide specific information - the costs - about the conduct of fermentation processes.

Key words: the redox potential, pH, cow milk, microorganisms

INTRODUCTION

Cow's milk has a *p*H of 6.5 and an r_H of 12 - 18 (field work favorably for the activity of optional anaerobic microorganisms). These organisms have an optimal activity in a lower redox potential.

If the cow's milk to store a certain period of time, due to the difference in weight between the emulsions compounds, at the surface can be accumulated a milk fat, which prevents access of air. Is thus decreasing redox potential and anaerobic bacteria can grow. With a varied chemical composition, milk is an excellent environment for developing microorganisms, especially for the development of lactic bacteria.

From the multitude of lactic bacteria used in dairy industry are selected the genus of Lactococcus bacteria (constantly present in milk) and bacteria genus of Lactobacillus (rarely seen).

The number of microorganisms in milk arriving from internal sources may vary between 1000 and 3000 cells/cm^3 [1].

Very important for the dairy industry are the A Class bacteria (Bacteria CLASS), II-EUBACTERIALES Order, Family Lactobacillaceae-unsporulated lactic bacteria , anaerobic optionally, in the form of bacillus (thin rod) or in a coccus (small spheres called cocs) . They have as common feature the ability to ferment lactose with the formation of lactic acid. [5]

The lactic fermentation is the most important process - used in the manufacture of dairy acid to find reliable methods for monitoring this process to constitute a challenge for the technologist.

It is knowning the importance of these microorganisms in controlling redox reactions in milk subjected to processing and their influence on the development of flavors in dairy products [2].

Therefore, we proposed to study the variation of redox potential, the rate of reduction, the *p*H and the rate of acidification in time after the action of microorganisms in milk.

E_h changes occurring in the cheese sulphhidril activation clusters, the responsible groups for production of specific aromas expressing the relationship between variation of E_h and form the flavors[3]. The dysulphuric's bridges are reduced to clusters sulphidrilyc by lactic bacteria [5], participated in the development of flavors in cheese [6].

MATERIAL AND METHOD

For study the redox potential change in time was used fresh cow's milk (at a maximum of 2 hours of mechanical milking), normalized (homogenized and brought to the same percentage of fat 3.5%).

For this study the variation of E_h after the inoculation of cultures of starter lactic bacteria were used the microorganisms of *Lactococcus lactis* type (with optimal temperature at 26°C), *Helveticus lactobacillus* and *Streptococcus thermophillus* (both with the optimal temperature 42°C). The microorganisms originated from the EZAL company and were MA011 type (mesophylic) for *Lacococcus lactis* subspecies, TA 061 type for *Streptococcus thermophillus* and the LH100 for *Lacobacillus helveticus*. All cultures of microorganisms were as "Direct Vat Inoculates" (set to direct inoculation). Bacteria were inoculated into nutrient medium of lactose MRS type (lactobacills and lactococcus) and nutrient medium M17 type (streptococcus). The preparation for inoculation and the inoculation of microorganisms in milk under study are described under 'technology standards in force [7].

For determinations of *p*H and E_h, used a cell measuring analyzer Multitester Consort C535 type which have an measure electrode like as the glass electrode with the outer Platinum ring SP60X type and the reference electrode is made according to the normal hydrogen electrode. The compensation of temperature it is realized in automatly mode. For calibrate of *p*H it is used the standard solutions (4.0 and 7.0 value of *p*H). For the purpose of cleaning *p*H electrode, after use, it is used a solution of pepsin / HCl. The redox electrodes were clean with fine powdered aluminum oxide to recover the platinum.

The samples of sowned milk with pure cultures of microorganisms (at an level of 1.4 mL of inoculation medium containing lactic bacteria for 250 mL milk) were kept in specific sterile conditions. The samples have met all the conditions for repeatability were met even sowing microbiological technique for milk with starter cultures. Each analysis was repeated 5 times. In terms of repeatability of results it were defined as differences between them in just over 0.01 *p*H units and 1 mV from E_h. It was taken into account the influence of temperature, for the fermentation and the *p*H and E_h measurements.

The measurements of *p*H and E*h* were made during a period of 14 hours after the start of fermentation. Moment of time "0" was the moment of starting lactic fermentation (at 2 hours after inoculation).

The rate of acidification (V_a) of environment has been given to changes in *p*H difference, the reported difference in time (d_PH/dt) and expressed in *p*H units / hour. The lowest curve of acidification can be obtained at the time that will be the maximum (ta).

The rate of reduction-V_r-environment (for the inoculated milk with starter bacteria) was determined from the variation of the difference in E_h reported time difference (dE_h/dt) and expressed in mV / h. From the minimal of reduction curve results the time at which the environment is the lowest (tr).

After the rate of acidification V_a and the rate of reduce V_r of the environment, the maximum period shall be determined in each fermentation product lactate. For each type of milk product (more or less acidophil, more or less fermented) it corresponds to a time of acidification or reduction, time may be determined by calculation, by the technologist before the inoculation of starter cultures.

RESULTS AND DISCUSSION

The results from this study are presented in Tables 1-4 and graphs in Figures 1 a, b, 2 a, b.

Table 1
Changes in pH and Eh's time to milk inoculated with microorganisms like *Streptococcus thermophyllus*

TIME (h)	*p*H	E_h (mV)
0,00	6,70	235
1,00	6,67	225
2,00	6,50	208
3,00	6,40	188
3,45*	5,89	148
4,00	5,90	148
4,37**	5,50	141
5,00	5,60	135
6,00	5,35	130
7,00	5,25	132
8,00	5,00	133
9,00	4,90	135
10,00	4,87	137
11,00	4,78	142
12,00	4,66	145
13,00	4,62	144
14,00	4,61	145

* - t_a, t_r ** - (analysis of graphics)

Table 2
Changes in pH and Eh's time-at milk inoculated with microorganisms like *Lactobacillus helveticus*

TIME (h)	*p*H	E_h (mV)
0,00	6,70	235
1,00	6,50	232
2,00	6,40	231
3,00	6,30	228
4,00	6,20	220
5,00	5,80	210
6,00	5,50	195
7,00	5,30	186
7,56*	4,66	166
8,00	4,50	162
9,00	4,50	161
9,58**	4,18	149
10,00	3,90	147
11,00	3,60	149
12,00	3,48	154
13,00	3,47	156
14,00	3,46	154

* - t_a, t_r ** - (analysis of graphics)

Table 3
Changes in pH and Eh's time-at milk inoculated with microorganisms like *Lactococcus lactis*

TIME (h)	*p*H	E_h (mV)
0,00	6,70	235
1,00	6,62	233
2,00	6,50	225
3,00	6,50	185
3,22*	6,17	0
4,00	6,15	-150
5,00	6,05	-180
6,00	5,85	-160
7,00	5,50	-155
8,15**	5,14	-148
8,00	5,12	-145
9,00	4,75	-130
10,00	4,50	-120
11,00	4,47	-117
12,00	4,43	-108
13,00	4,42	-106
14,00	4,41	-101

* - t_a, t_r ** - (analysis of graphics)

Table 4
Changes in pH and Eh's time to raw milk *

TIMPUL (h)	*p*H	E_h (mV)
0,00	6,70	286
1,00	6,62	291
2,00	6,54	296
3,00	6,51	299
4,00	6,41	315
5,00	6,25	322
6,00	5,85	317
7,00	5,50	310
8,00	5,12	321
9,00	4,74	332
10,00	4,51	341
11,00	4,47	351
12,00	4,43	368
13,00	4,42	371
14,00	3,42	385

- to decrease the pH solution was used for lactic acid 5% and to increase the pH used 0.1 N NaOH solution.

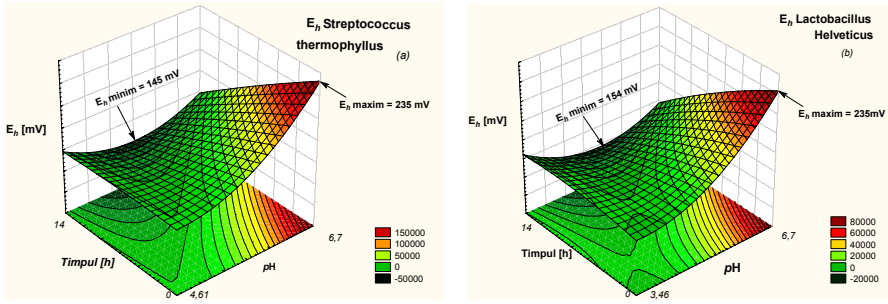

Fig 1 a, b-Calculus Eh depending on pH and time for the milk attacked by *Streptococus thermophillus* (a) and *Lactobacillus helveticus* (b)

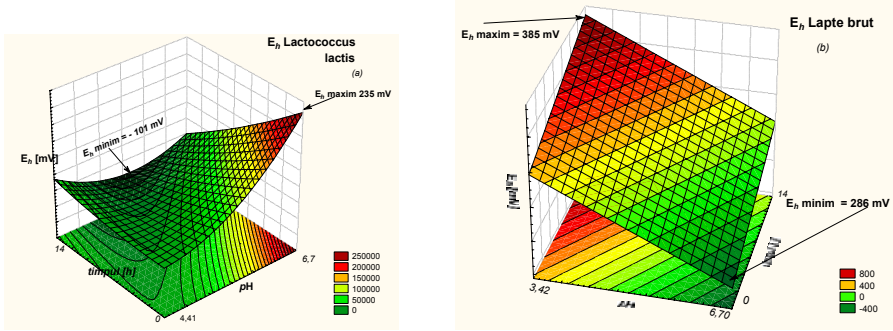

Fig 2 a, b - The Eh calculation based on pH and time for the milk attacked by *Lactococcus lactis* (a) and raw milk-witness variant (b)

From those presented in tables and graphs above it can be seen that each microorganism used in lactic and propionic fermentation it corresponds to a rate of acidification of the environment and a specific rate of reduction of the environment. The used microorganisms are indispensable for achievement of certain milk products obtained by lactic and propionic fermentation (acidophile milk, fermented cream, cheese Schweizer type).

From the graphs of the *p*H/E_h and E_h /time can be obtained - by interpolation, at normal temperature and pressure-time at which the rate of acidification and the rate of reduction is maximum. These t_a, t_r times can be very important in the used technologies for the fermentation of milk. Knowing the time of acidification and reduction, an technologist can eliminate a number of other physical-chemical and sensorial analysis that is used today for determining the end of fermentation. This is very important for the reduce costs and production time.

Following the analysis of experimental variants was found that variants that have used *Streptococcus thermophyllus* recorded a time of acidification (t_a) of 3.45 hours and a reduction time (t_r) of 4.37 hours. The variants that have used *Lactobacillus helveticus* had time to reduce acidification and higher (t_a = 7.56 hours and t_r = 9.58 hours) while variants that use *Lactococcus lactis* had the higher capacity to reduce environmental at least for acidification (t_a = 3.22 hours and t_r = 8.15 hours). The stronger decreasing (and faster) to use environment for microorganisms of the *Streptococcus thermophyllus* genus is specify for the increased capacity to liberation of SH groups in the fermentation media.

The achieving of 4.65-4.75 units (optimum *p*H) it is made with *Streptococcus thermophyllus* in 11 hours and 30 minutes (E_h = 143 mV) and using the microorganisms of the *Lactococcus lactis* genus in the 9 hours (very low E_h = -130mV). The best time of fermentation was when it is used microorganisms of the *Lactobacillus helveticus* genus in 7 hours and 30 minutes (E_h = 175mV).

If you would use as a fermentation media the pasteurized milk, that surely this time would

be reduced substantially. This is corect because the pasteurization of milk improves the hygienic quality of milk used as a medium for the development of lactic bacteria. By pasteurization process, the peroxidase was inactivated, is eliminated the oxygen and is favored the formation of compounds with reducing action (in particular forms of enzymes oxidoreducteses).

For a more detailed analysis of the variation in the E_h function of *p*H and time we used three-dimensional graphics surface. Three-dimensional surface graphs were constructed using Statistica 6.0 software and equipment they used to level the corresponding data series values of axes O_x, O_y, O_z. Thus, three-dimensional response surface in each case resulting from inoculation with bacteria was corresponding E_h values represented on the axis O_z. Depending on the scope of changing values and the number of levels used to define the contours of each 3D graphic surface were established specifications surface. The "Three-dimensional surface" graphs were like "square" (Quadratic)-in order to highlight the points that make up the polynomial of 2degree $E_h = f$ (time, *p*H).

Follow the analyze of "3D surface" graphs from the figures 1 a, b and 2a was observed that where we have used the lactic micro flora currently used in production of dairy acid, the redox potential E_h has decreased, the time was increased and the *p*H has decreasing .

From figure 2b can to see that the redox potential E_h for the raw milk has increased, while *p*H of the environment has decreased. All this occurred over time and were due to increased concentrations of oxidized forms. Over time, the raw milk to ferment a part of lactose to lactic acid, and for this reason increasing the concentration of oxidized forms and the environmental acidity.

From the analysis of figures 1a,b and 2 a,b could see that there were different levels of the E_h depending on *p*H and time. These levels E_h ranged depending on the nature of used micoorganisms: 7 levels of E_h - if it was used *Streptococcus thermophillus*, 6 levels E_h - where we have used *Lactobacillus helveticus* or *Lactococcus lactis* and only 4 E_h-levels in raw milk.

For a more detailed analysis of data, to appeal to the study of correlations between E_h of the raw milk (EHLB) and E_h's milk has been added *Streptococcus thermophyllus* (EHST), E_h of the milk has been added *Lactococcus lactis* (EHLL), E_h of the milk has been added *Lactobacillus helveticus* (EHLH). For a better analysis were established the correlations of E_h map depending on *p*H and time.

For this calculation was used the statistical software SPSS 11.5 for Windows and some acronyms:

PHLB = the *p*H for raw milk;

PHST = the *p*H for inoculated milk with *Streptococcus thermophillus*;

PHLL = the *p*H for inoculated milk with *Lactococcus lactis*;

PHLH = the *p*H for inoculated milk with *Lactobacillus helveticus*;

TLB = time in which the measured variations of E_h and *p*H in raw milk;

TST = time of measured variations of E_h and *p*H of milk with *Streptococcus thermophillus*;

TLL = time of measured variations of E_h and *p*H of milk with *Lactococcus lactis*;

TLH = time of measured variations of E_h and *p*H of milk with *Lactobacillus helveticus*.

Using statistical software SPSS 11.5 for Windows has facilitated the calculation and selection of only the actual nonparametric correlations to be set between these sizes.

Results of parametric correlations Pearson type and non-parametric correlations τ (Kendall type) and ρ (Spearman type) they try more differences are summarized in the conclusions[8].

CONCLUSIONS

Between the pasteurization of milk, the reduction degree of sowing environmental to attack of various microorganisms used in the manufacture of milk and the acidification of the environment is a direct link;

The E_h-can be treated as a polynomial function of *p*H and time where it uses statistical analysis of data from monitoring sowing with different microorganisms (especially the statistical graphs of the "three areas of response");

Under the three-dimensional surface analysis to experimental variations taken into account (fig. 1a, b and 2 a, b) the potential of small changes in E_h and variable *p*H for the recorded version which use in cultures *Streptococus thermophyllus*;

The largest variations of the E_h depending on the time and *p*H at seeding with microorganisms has been registered to the variant that uses *Lactococcus lactis*, where the issue of SH groups in the environment is very strong - under normal temperature (25-28^{o}C);

The best time of fermentation was registered when it used microorganisms of the genus *Lactobacillus helveticus* in 7 hours and 30 minutes (E_h of 175mV);

Both *Lactococcus lactis* and *Streptococcus thermophyllus* can produce significant quantities of hydrogen peroxide in the media, peroxide which at one time is an inhibitor of their development;

From the analysis of reduction time (average, in the case of *Lactococcus lactis* cultures), appear on the knowledge of variation in redox potential (ranging from + 235 mV when the milk is used as a culture media to -150 mV for yoghurt - when the small groups of -SH being in the environment towards the end of bulk fermentation) can be detached easily conclude that measurement of these redox parameters (E_h, t_r) is extremely important in the technology of yoghurt and other dairy products;

Follow the knowing the acidification and reduction time, an technologist can eliminate a number of other physical-chemical and sensorial analysis that is used today for determining the end of fermentation, a study of this kind can be a model for calculating the times for milk 3.5% beef fat and organisms of this kind;

The Pearson more negative correlation, was established between E_h of the raw milk and the E_h of milk that was inoculated with *Lactobacillus helveticus*;

The largest positive correlation (0.962) was recorded between the E_h and *p*H of milk that was inoculated with *Lactobacillus helveticus*, this microorganism was the best protector for the environment in the oxidation conditions and the decreased *p*H;

The Pearson lowest negative correlation it has been established between E_h of the raw milk and the E_h of milk that was inoculated with *Lactococcus lactis*;

The lowest positive correlation (0.624) was recorded between the E_h and *p*H of milk that was inoculated with *Lactococcus lactis*, thus explaining the lowest recorded in acidification in the inoculation conditions with the microorganism and the much greater reduction time;

The largest negative correlation (-0.944) was among the E_h of the milk inoculated with *Lactobacillus helveticus* and time of analysis for this type of milk, which shows a reduction for the environment than in the other experimental variants;

The lowest negative correlation (-0.683) for E_h and time was in the case of milk inoculated with *Lactococcus lactis* (milk that had the greatest decrease of E_h because reducing free SH groups released by the bacteria in the environment);

The Kendall and Spearman coefficients of correlation were established between EHLB and EHLH are negative and the largest Kendall and Spearman coefficients of correlation were established between EHLB and EHLL and are negative and smaller.

From all these considerations result the importance of knowledge the electrochemical potential E_h, the *p*H, the acidification time (t_a) and time reduction (t_r).

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