# SAFETY AND FERMENTABILITY OF DAIRY PRODUCTS

Jožef Božo[1*], Svetozar Anđel[2]

[1]Institute for Food Technology, Novi Sad, Serbia
[2]Technical College Zrenjanin

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**Abstract:** *With the growth of life standard, the consumption of quality fermented foodstuff has also increased. Normally, it is supposed that successfully enforced technological fermentation process provides health safety of these products. It starts from the fact that microorganisms are sensitive to some food contaminants such are antibiotics, heavy metals, pesticides, etc. In presence of traces of pollutants with antimicrobial effect hinders the physiological processes of starter cultures, technological process of production of fermented products are disturbed. For a consumer, a successfully completed product might be safe. The purpose of this work is to check this presupposition on the example of fermented foodstuff. In different yoghurt specimens, the sensitivity of present cultures towards penicillin has been examined. Obtained values have been compared with the data from the literature and relevant standards. This proves that successfully produced, correctly fermented dairy products does not necessarily have to be safe when residues of beta-lactam antibiotics are observed.*

**Key words:** *yoghurt starters, penicillin residues, minimal inhibitory concentration (MIC)*

## INTRODUCTION

With the pollution of the environment and intensive use of chemical materials in food production, the presence of residues of toxic, mutagen, carcinogenic and other hazardous and noxious substances in foodstuff is not a rare case. The most common pollutants are heavy metals, residues of antibiotics, pesticides, hormones etc. The presence of these substances in milk, the raw material for production of sour-milk products, besides negative influence on the medical product safety, it hinders the technological process of production of the final product.

The bacteria used in dairy production as starter cultures, are very sensitive to antibiotics. The presence of antibiotics in milk can cause undesirable effects - retardation or even complete impossibility of fermentation process. According to various sources, up to 60% of milk which reaches the stage of processing contains penicillin in amount 0,005 IU/mL. This amount usually does not slow down the production of cheese and butter, but it can slow down the process of producing yoghurt. There are numerous data from the literature about different specific sensitivity of lactobacilli towards antibiotics. Different sensitivity can

*Corresponding author:
e-mail: jozef.bozo@fins.uns.ac.rs
Tel: +381 21 485 3773: Fax: +381 21 450725

selectively repress some strains of complex starters and result in disruption of technological procedure and the quality of final product. The data of different authors on the consequences of presence of betalacam antibiotics in milk during yoghurt production are given in table 1. (Mijačić Z., et al., 2001).

On the basis of specific sensitivity of lactobacilli towards antibiotics, numerous methods of detection of antibiotics in milk have been developed and they all bring to determination of change in sourness of milk and morphology of cells, colour indication caused by change of redox potential by enzyme activity, the inhibition zone of growth is measured on hard nutritive beds in the environment of milk specimens application etc. Since microbiological methods detect the action of naturally present thermolabile inhibitors of microorganisms in milk as well, like immune cells, immune-globulins and bacteriophages, for excluding the effects of these factors on results, the boiling of milk for 2-10 minutes is carried out. When testing the presence of penicillin in milk, enzyme preparation of penicillinase is used, which dissolves penicillin. If the growth inhibition of test microorganism in milk with penicillinase terminate - it is concluded that penicillin is present in milk, (Koroleva N.S., 1966).

**Table 1.**
The effect of beta-lactam antibiotics on the activity of yoghurt culture

| Authors | Concentration (µg/kg) | Result |
|---|---|---|
| Holec J., Kimes B. (1964) | 3 | Difference in pH, change in consistency and taste |
| Kondratenko M, Shiskova A.I. (1978) | 4 | Morphology change of lactobacilli, 20-30 minute prolongation of coagulation period |
| Waes G., Naudts M. (1974) | 5-50 | 50-80% inhibition of acidification |
| Jakimov N. (1970) | 63 | Morphology change of lactobacilli |
| Cox W.A., Stanley G., Levis J.E. (1978) | 10-200 | Product disadvantages |
| Mayra-Makinen A. (1993) | 150 | Inhibition of acidification and synthesis of acetaldehydes |

## MATERIALS AND METHODS

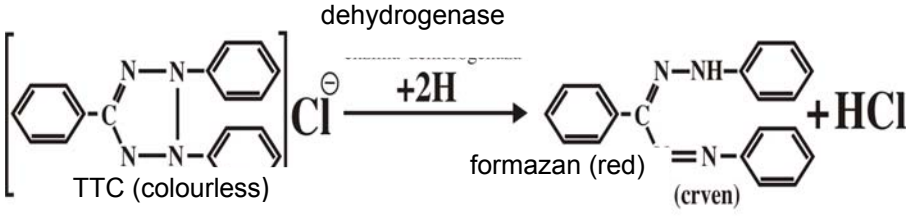
### Indicators

1.Methylene blue - 2.5 mL of cold saturated solution of methylene blue in 96% alcohol is filled to 20 ml of sterile distilled water.

The method of reduction of methylene blue is used for indirect determination of increased number of microorganisms in milk. Bacteria, growing in milk, reduces the redox potential in the bed, methylene blue receives the hydrogen and reduces itself to colourless form. Because of oxidation of the oxygen from the air the surface of the milk in test tube becomes blue, so while observing just decolouring of deeper layers of milk are considered, (Sall A.J., 1974).

2.Triphenyl tetrazolium chloride (TTC) - 4% water solution kept in dark bottle away from light.

Dehydrogenase test with TTC is used for measuring the activity of dehydrogenases. TTC is colourless mixture which after reception of hydrogen is reduced to red colour formazan



In milk in which microorganisms have been developed, colourless tetrazolium salt turns into red formazan. With the growth of microorganism inhibition in milk, there will not be any changes in colour.

**Antibiotic solutions:**

1. Basic - ampoule JUGOCILIN *ad usum veterinum* (Galenika A.D. Beograd),
   800000 IU (procaine-benzilpenicillin 600000 IU **+** benzylpenicillin-potassium 200000 IU).
2. Operational - made by dissolving the basic into distilled water:

   PC1 – 8 IU/mL
   PC2 – 4 IU/mL
   PC3 – 2 IU/mL

**Inoculum**

In experimental work, yoghurt cultures from commercial products by six manufacturers from different geographical locations of Serbia was used as inoculum.

As a media for development of yoghurt culture, a specially reconstituted milk 'Impamil' ('Impaz' Zaječar). The identical procedure was applied for each of six examined yoghurt cultures. Aiming to determine minimal inhibitory concentration (MIC) of antibiotics on bacteria of yoghurt culture, a series of double consequent disolutions of antibiotics in milk have been prepared. Test tubes with corks were used, previously sterilized in a dry sterilizator. In eight test tubes (three control probes and five test tubes of operational series) 10 mL of pasteurized milk was measured out. In each test tube in the series 0.1 mL of indicator was added (methylen blue) and 1 mL of inoculum (yoghurt culture). The concentration of antibiotics was measured at 11 mL of bed (10 ml of milk + 1ml of inoculum). A series of eight test tubes with 11 mL of milk and double consequent concentration of antibiotics is obtained: 0.018 ij/mL – 0.036 ij/mL – 0.073 ij/mL – 0.182 ij/mL – 0.364 ij/mL. The effect of dissolution of other additives (0.1 mL of indicator and the volume of antibiotic solution) is ignored, since there is some evaporation during incubation period, (Uhlik B., 1972).

Test tube with 10 mL milk is pasteurized at 80-85% °C for 20-25 minutes, cooled off at 43 (±1)°C, seeded with 1 mL of yoghurt culture and thermostated at that temperature for 2.5 h. After that time, according to the change of colour of methylen blue, the MIC of antibiotics for examined yoghurt culture are determined. The first test tube in the series in which blue colour remained is marked as the test tube with the minimal inhibitory concentration of antibiotics in milk.

For check and confirmation of the results, in the parallel series of test tubes another indicator (TTC) was added. The red colour of the indicator means that that there was no inhibition of growth of microorganisms in test tube.The rack with test tubes was left on room temperature to stand aside for another 60 minutes, after which the results were readout again. The first test tube in series in which was no change in colour is considered as the test tube with the MIC of antibiotics in milk.

As a means of control, milk without antibiotics or reconstituted milk of strictly controlled origin, meant for nutrition of infants, was used.

Detection of changes in starter culture after incubation, the contents of test tubes of examined sample and control were observed through the microscope. In the specimens with antibiotic decreased number of cells, involutive forms was noticed, in relation to control without antibiotics.

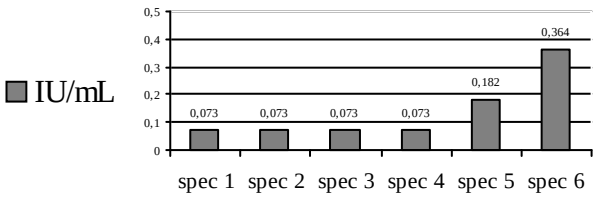
## RESULTS AND DISCUSSION

The purpose of the experiment was determination of penicilline susceptibility of yoghurt cultures used in dairy production. We used the standard definition of MIC according to (Karakašević B., 1987), as the smallest concentration in the series of conescutive double dissolutions of antibacterial materials, which prevents the occurrence of growth of present microorganisms perceived by bare eye.

As an example of antibiotic which residues can be found in milk, we chose an antibiotic based on penicillin mostly used in veterinary medicine and represents the most significant milk contaminant. During the experiment, as a starting concentration of antibiotics for the series of consecutive double dissolutions (0.01IU/mL), a value from the literature has been taken (Sall A.J., 1974). The concentration of the antibiotics was double increased in order to include into the scope MIC values of all

examined specimens of yoghurt cultures. Obtained results are presented in graphic 1.

Comparing the results from the chart with those from the literature (0,0017-0,1 IU/mL, (Carić M. et al., 1988), it can be see from that the penicillin resistance of bacteria from tested yoghurt samples has been increased significantly in last 30 years. During the experiment, it was noticed that the age of culture, presence of reductasas in inoculum, as well as the duration of incubation, can affect the accuracy of results. Above mentioned experimental circumstances were considered when evaluating the results.




Graphic 1. MIC of JUGOCILIN on the tested bacteria of yoghurt cultures

## CONCLUSIONS

Intensive use of antibiotics in animal husbandry in recent years has resulted in frequent occurrence of antibiotics in milk and in greater amounts than earlier. It can be assumed that due to that practice the minimal inhibitory concentration of antibiotics in yoghurt cultures has been increased. The presence of antibiotics in milk represents technological problem as well because of interfering with the process of fermentation using starter cultures. Technological operations (eg. thermic processing) during milk processing don´t bring to inactivation of antibiotic residues in milk. Due to increased resistance to antibiotics, bacteria of yoghurt cultures do not represent a barrier any more for antibiotics in milk, as the raw material for manufacture of sour-milk products. Thus the potential danger for people of presence of antibiotics in sour-milk products is increased, whereby their attribute of safe food is undermined. To this fact indicates a data from the literature as well that minimal inhibitory concentrations of antibiotics for examined yoghurt cultures are greater than the maximal allowed amount. The maximal allowed amounts of beta-lactam residues in milk according to EU regulations is 0,067 IU/mL, (1). A successful run of fermentation process during production operation does not represent assurance for product safety. In order to prevent the problem during milk production and processing, it is necessary to obey the deadlines of waiting period of used antibiotics in the system of integral safety control of raw materials and products.

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