

STUDY OF MILK WITH HIGH PROTEIN CONCENTRATION FERMENTATION BY LACTIC ACID BACTERIA STARTER CULTURES

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Abstract

The beneficial effects of probiotic foods on human health and nutrition are increasingly recognized by health professionals. Yogurts are an assortment group of fermented milk drinks, characterized by high nutritional value. Promising areas of improvement of yogurt technology are the inclusion in the composition of combined starter cultures of pure cultures of probiotic lactic acid microorganisms and bifidobacteria, and enrichment of the product with biologically valuable proteins of animal origin. The aim of research is study of high protein concentrate "Mobilyuks-lite" influence on skim milk fermentation efficiency.

Targets of research were dry skim milk, starter cultures of lactic acid bacteria "Acidolact", "Symbylact", "Yoghurt", and concentrate "Mobilyuks-lite". The research was carried out in the laboratory of food science of the Faculty of science and technology of the Free University of Bolzano - Italy. The influence of "Mobilyuks-lite" concentrate dosage (3, 5 and 7%) on skim milk fermentation by starter cultures (skim milk + "Acidolact", skim milk + "Symbylact", skim milk + "Yoghurt") in comparison with control samples without protein concentrate addition was studied by calorimetric method.

Based on the results of the skim milk fermentation dynamics studies, characteristic curves of the heat flow of normalized samples by weight, on the time of incubation were obtained. The influence of different doses of "Mobilyuks-lite" concentrate (3, 5 and 7%) on pH and titratable acidity of fermented skim milk was studied. The results of the experiments allow establishing that the optimal dosage of "Mobilyuks-lite" concentrate is 5%.

The studies proved that the concentrate "Mobilyuks-lite" make positive impact on starter cultures of lactic acid bacteria growth and skim milk fermentation. The practicability of "Mobilyuks-lite" concentrate as an ingredient for yoghurt and other functional fermented milk products, enriched with biologically high-grade protein of animal origin and essential micronutrients, manufacturing is validated.

Key words: Starter cultures, Skim milk, Concentrate "Mobilyuks-lite", Fermentation, Calorimeter.

1. Introduction

The consumption of food products with functional properties is a keystone of healthy lifestyle in the conditions of modern ecology and external environmental influences. In modern sense, a functional product can be called as a kind of "must have" for active people who care about their health (Pytel *et al.*, [1], Montowska and Fornal, [2]). Fermented milk drinks, being products of daily consumption of the general population, have a direct impact on the functioning of the human body and can be positioned as one of the most important components of healthy nutrition and active longevity (Ganina *et al.*, [3], Gerasimova *et al.*, [4], Tamim and Robinson [5], Tihomirova [6]).

The beneficial effects of probiotic foods on human health and nutrition are increasingly recognized by health professionals. Recent scientific work on the properties and functionality of living micro-organisms in food have suggested that probiotics play an important

role in immunological, digestive and respiratory functions, and that they could have a significant effect on the alleviation of infectious diseases in children and other high-risk groups (Tihomirova [6], Al-Saadi *et al.*, [7]).

Yogurts are wide spread group of fermented milk drinks, characterized by high nutritional value due to the high content of milk solids, primarily protein. In addition to a large amount of nutrients, yogurts provide a high percentage of daily recommended values of essential nutrients, such as calcium and vitamin B₁₂ (Montowska and Fornal, [2], Tamim and Robinson, [5], Gorbatova, [8], Adolphi B. *et al.*, [9]). These components, along with the nutritional functions of yogurt make it a particularly important dairy product, which is widespread throughout the world and is associated with a healthy diet. In addition, growing evidence suggests that yogurt consumption may be inversely related to certain health parameters, especially weight control, blood pressure, and type 2 diabetes (Behare *et al.*, [10], Cheng, [11]). Promising areas of yogurt technology enchainment are (Pytel *et al.*, [1], Tihomirova, [6], Hramtsov *et al.*, [12]):

- Inclusion of pure cultures of probiotic lactic acid microorganisms and *Bifidobacteria* in the composition of combined starter cultures.

- Enrichment of the product with biologically valuable food proteins of animal origin.

The aim of research is study of high protein concentrate "Mobilyuks-lite" influence on skim milk fermentation efficiency.

2. Materials and Methods

Targets of research were:

- Skim milk powder with a mass concentration of fat no more than 1.5 %, acidity from 14 to 21 °T, conforms to requirements of GOST R 52791-2007.

- Protein concentrate "Mobilyuks-lite" conforms to requirements of Regulatory Document 9219-016-35305730-10 (see Table 1).

- Commercial starter cultures Vivo[®]: "Acidolact", "Simbilact", "Yogurt".

The specific composition of lactic acid microorganisms' starter cultures is presented in the Table 2. Samples of skimmed milk powder was reconstituted to a dry mass concentration of (10 ± 0.2%) by dissolving in drinking water at a temperature of (40 - 45) °C. Reconstituted skim milk was subjected to pasteurization at a temperature of (85 - 87) °C with exposure time (2 - 3) minutes and cooled to fermentation temperature (40 - 42) °C.

Starter cultures "Yogurt", "Simbilact" and "Acidolact" were inoculated in pasteurized and cooled skim milk in dosage of 5% to initiate the process of lactic acid fermentation. The influence of different doses of "Mobilyuks-lite" concentrate (3, 5 and 7%) on titratable acidity of fermented skim milk was studied. The research was carried out in the laboratory of food science of the Faculty of science and technology of the Free University of Bolzano - Italy. The influence of "Mobilyuks-lite" concentrate dosage (3, 5 and 7%) on skim milk fermenta-

Table 1. Food concentrate "Mobilyuks-lite" quality and safety attributes

Name of quality indicators	Quality requirements
Mass concentration of protein, %, not less than	50
Mass concentration of moisture, %, no more than	6
Mass concentration of ash, % not less than	8
Mass concentration of dietary fibre, %	20
Calcium content, mg per kg	20,000 ± 2,000
Iron content, mg per kg	120 ± 20
Iodine content, µg per kg	4,000 ± 400
Melamine	Not allowed (< 1 mg/kg)
Quantity of mesophilic aerobic and facultative anaerobic microorganisms, CFU per g, no more than	5 x 10 ⁴
<i>Escherichia coli</i> group bacteria (coli-forms) in 1.0 g	Not allowed
Pathogenic microorganisms, including <i>Salmonella</i> spp., in 25 g	Not allowed
Sulphate-reducing clostridia in 0.01 g	Not allowed

Table 2. Species composition of lactic acid microorganisms' starter cultures

Type of the starter culture	Species composition
Yogurt	<i>Streptococcus thermophilus</i> , <i>Lactobacillus delbrueckii</i> ssp. <i>bulgaricus</i> , <i>Lactobacillus acidophilus</i> , <i>Bifidobacterium lactis</i>
Simbilact	<i>Streptococcus thermophilus</i> , <i>Lactobacillus delbrueckii</i> ssp. <i>bulgaricus</i> , <i>Lactobacillus acidophilus</i> , <i>Bifidobacterium lactis</i> , <i>Lactococcus lactis</i> ssp. <i>cremoris</i> , <i>Lactococcus lactis</i> ssp. <i>lactis</i> , <i>Lactococcus lactis</i> ssp. <i>lactis</i> var. <i>diacetylactis</i>
Acidolact	<i>Streptococcus thermophilus</i> , <i>Lactobacillus delbrueckii</i> ssp. <i>bulgaricus</i> , <i>Bifidobacterium lactis</i> , <i>Lactobacillus acidophilus</i>

tation by starter cultures (skim milk + "Acidolact", skim milk + "Symbilact", skim milk + "Yoghurt") in comparison with control samples without protein concentrate addition was studied by calorimetric method.

The study of the fermentation process of control and experimental samples was carried out with the use of the device "Isothermal calorimeter TAM III, TA Instruments". Isothermal calorimeter TAM III consists of a highly stable oil thermostat, built-in computer and 4 channels for connecting calorimeters. Each of these 4 channels can be expanded to 6 using a multicalorimeter, so the maximum number of calorimeters used at the same time is 24. TAM III is the most flexible and efficient calorimeter, allowing measurements with very high sensitivity and accuracy at the same time. Modes of operation of the device include isothermal, step isothermal and slow scanning [13].

The thermostat of the device is a liquid system that uses mineral oil to absorb excess heat as quickly as possible and minimize temperature gradients in the system. The effective circulation of the liquid also contributes to an accurate temperature change according

to the program. The average temperature fluctuation in the calorimeter does not exceed $10 \mu\text{K}$, in the range from 15 to 150 °C. The temperature drift does not exceed $100 \mu\text{K}$ in 24 hours. Such stability ensures high sensitivity of thermal effects detection in both fast and slow current processes.

3. Results and Discussion

Based on the results of the studies, characteristic curves of skim milk titratable acidity on fermentation duration were obtained (Figures 1 - 3). Addition of "Mobilyuks-lite" concentrate in doses of 3 and 5% leads to increase of lactic acid production rates in comparison with control samples of fermented skim milk. This effect indicates positive influence of proteins and micronutrients, presented in the composition of "Mobilyuks-lite" concentrate, on lactic acid microorganisms of tested starter cultures grows rates. The decrease of titratable acidity of fermented skim milk samples with addition of 7% "Mobilyuks-lite" concentrate can arise from lactic acid bacteria metabolism inhibition due to increase of viscosity and osmotic pressure of culture medium.

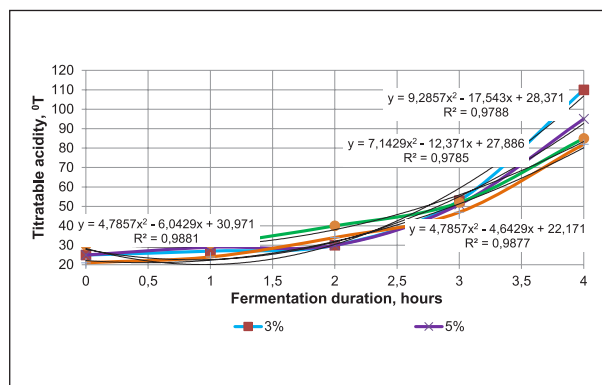


Figure 1. Dynamics pattern of skim milk fermented by "Acidolact" starter culture titratable acidity

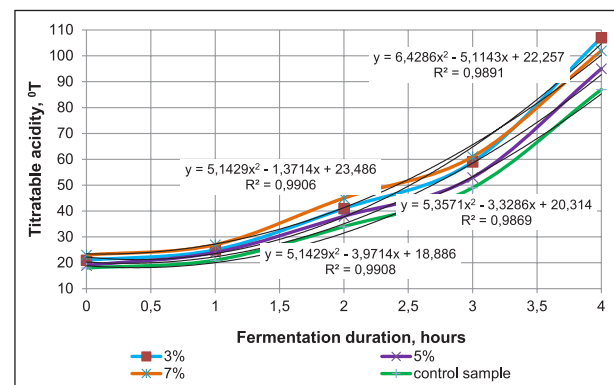


Figure 2. Dynamics pattern of skim milk fermented by "Yoghurt" starter culture titratable acidity

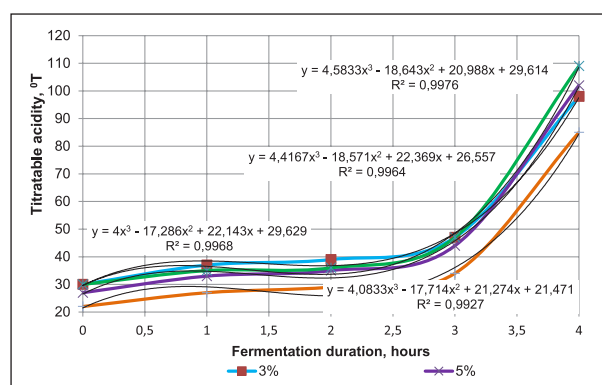


Figure 3. Dynamics pattern of skim milk fermented by "Symbilact" starter culture titratable acidity

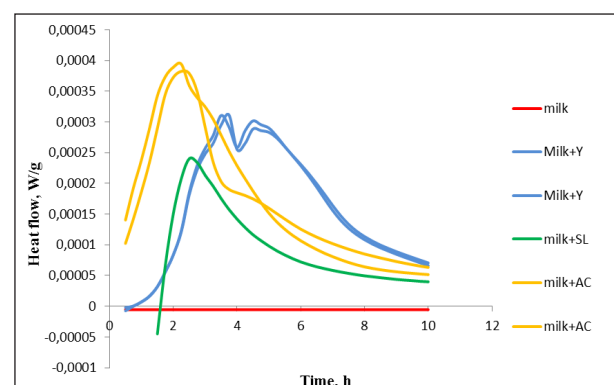


Figure 4. Normalized heat flow produced by milk, milk inoculated with "Yoghurt" (milk + Y), "Symbilact" (milk + SL), and "Acidolact" (milk + AC) starter cultures - without "Mobilyuks-lite" concentrate introduction; W/g

The analysis of fermented skim milk titratable acidity changes regularities and organoleptic characteristics allows recommending for further research a samples with the addition of 5% "Mobilyuks-lite" concentrate. During the fermentation of samples with 3% content of concentrate whey separation occurs. Fermented products with 7% concentrate content are characterized by high viscosity and the presence of insoluble precipitate.

The heat flow of normalized by weight skim milk samples inoculated with "Acidolact" starter cultures, "Simbilact", "Yogurt" at different doses of % "Mobilyuks-lite" concentrate (3, 5 and 7%) in comparison with the control samples without protein enrichment was measured. Data obtained are shown in Figures 4 - 7.

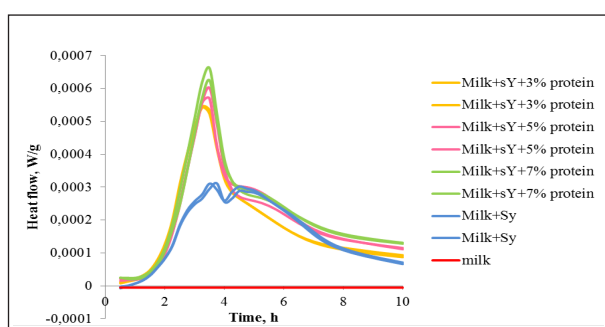


Figure 5. Normalized heat flow produced by milk, milk inoculated with Yoghurt starter and different concentrations of "Mobilyuks-lite" concentrate (3, 5, 7%); W/g

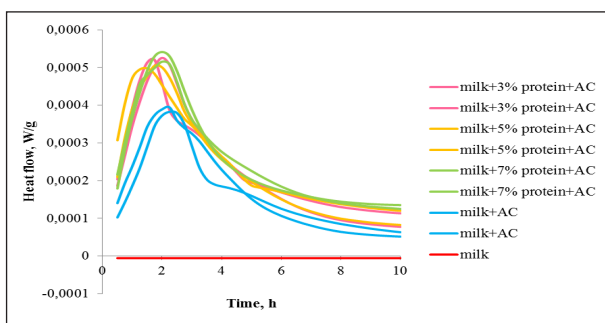


Figure 6. Normalized heat flow produced by milk, milk inoculated with "Acidolact" starter and different concentrations of "Mobilyuks-lite" concentrate (3, 5, and 7%); W/g

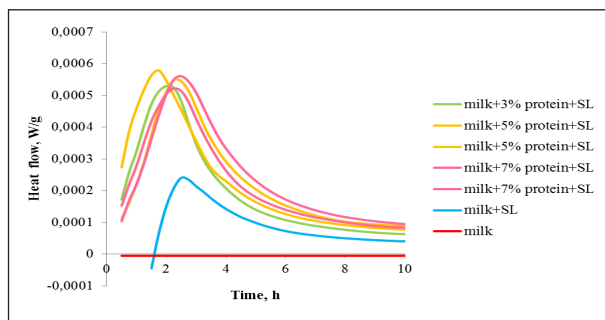


Figure 7. Normalized heat flow produced by milk, milk inoculated with "Simbilact" starter and different concentrations of "Mobilyuks-lite" concentrate (3, 5, and 7%); W/g

The analysis of the experimental data presented in Figures 4 - 7 confirms the applicability of the calorimetric method for the study of the dynamics of milk substrates fermentation by of lactic acid microorganisms' starter cultures. For all studied starter cultures, there is a significant increase in heat flow compared to the control - skimmed milk without the inoculation of starter cultures. The maximum values of heat flow for skim milk fermented with "Simbilact" and "Acidolact" starter cultures is observed after 2 - 3 hours from the moment of inoculation; for samples fermented with "Yogurt" starter culture - after 4 - 6 hours. The most intense heat flow from the growing culture of microorganisms was detected for skim milk samples inoculated with "Acidolact" starter cultures.

There is a significant increase in the heat flow generated during the fermentation of all the studied samples with addition of "Mobilyuks-lite" concentrate (Figures 5 - 7), compared with the control samples (Figure 4). This fact leads to the conclusion about the positive effect of proteins, macro - and microelements included in its composition on the growth of lactic acid microorganisms' cultures.

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4. Conclusions

- The results of the experiments allow establishing that the optimal dosage of "Mobilyuks-lite" concentrate is 5%.
- The studies proved that the "Mobilyuks-lite" concentrate make positive impact on starter cultures of lactic acid bacteria growth and skim milk fermentation.
- The practicability of "Mobilyuks-lite" concentrate as an ingredient for yoghurt and other functional fermented milk products, enriched with biologically high-grade protein of animal origin and essential micronutrients, manufacturing is validated.

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