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Technological features of production of lactate-containing additives from milk whey fermented with lactic acid bacteria

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Abstract. Milk whey becomes a product of interest to researchers and manufacturers due to stricter environmental protection requirements. This paper discusses bioconversion of whey lactose into lactate-containing additives using microorganisms of *Lactobacillus* genus. The biotransformation of lactose from curd whey and standard solutions of cheese whey into lactic acid derivatives was assessed by the following parameters: the productivity of lactic acid bacteria, the rate of lactose fermentation, the total amount of calcium lactate and its formation rate. Selection of the medium preparation and lactic acid biosynthesis parameters based on these measurements proved to yield optimal results. Lactic acid bacteria from the subgroup of thermophilic bacilli *L. acidophilus*, *L. bulgaricus*, *L. casei*, *L. lactis*, *L. helveticus*, *L. plantarum* were also tested. The optimal synthetic activity in the terms of calcium lactate turnover and formation rate was demonstrated by *L. acidophilus* in a medium based on the concentrated whey with 8% lactose.

Key words: lactate-containing additives, lactic acid bacteria, lactose, whey.

INTRODUCTION

Milk whey processing attracts attention of researchers and manufacturers because of stricter environmental protection requirements and a concept of whey by-products valorization.

According to Mollea et al. (2013) up to 70% of the produced whey is subjected to industrial processing to obtain food and feed products. Other researchers suggest that 3.2 million tons (1.7%) of 187 million tons of curd whey produced worldwide were processed by various industries into added value products such as whey powder, whey protein isolate, whey protein concentrate (Chavan et al., 2015). Increasing the efficiency of manufacturing of products with added value from whey and reducing the unused dairy material's impact on the environment is possible with increasing the recycling capacity and introducing waste-free technologies. Russia, along with other countries, has considerable potential for producing and marketing similar added value products. Whey has unrivaled potential in this regard due to its polymorphism and exceptional properties

of the principal carbohydrate. Ingredients obtained by whey bioconversion may be introduced into many products, including functional and sports nutrition and baby food (Sinel'nikov et al., 2007; Hramtsov, 2011; Nath et al., 2016).

A large number of scientific and technical developments using dairy raw material containing lactose have been produced these days. Based on recent research and patent data, the biotechnological method is one of the promising directions for whey processing (Magalhães et al., 2011; Mikhneva et al., 2013; Ryabtseva et al., 2014; Gavrilov et al., 2015; Rosolen et al., 2015). This is due to the presence of nutrients necessary to grow industrial microorganisms producing a variety of useful metabolites such as lactic acid, ethanol, and acetic acid. Microorganisms for whey processing are representatives of aerobic and anaerobic cultures.

Previously, production of butanol from cheese and curd whey by *Clostridium acetobutylicum* provided productivity of 2.66 g L^{-1}, a final concentration of 4.9 g L^{-1}, a yield of 0.3 g g^{-1}, and a selectivity of 82% $^{W}/_{W}$ (Gonzalez–Siso, 1996; Raganati et al., 2013). Cheese whey was effectively used as a substrate for kefir-like beverages production with a composition similar to traditional milk kefir: 7.8–8.3 g L^{-1} ethanol, 5.0 g L^{-1} lactic acid, and 0.7 g L^{-1} acetic acid (Magalhães et al., 2011; Rosolen et al., 2015). Most often, lactic acid bacteria *Lactobacillus* are chosen as a producer culture. Using pure cultures of lactobacilli *L. acidophilus* and *L. casei*, whey can be transformed into products with improved dietary and functional properties and prolonged shelf life (Mikhneva et al., 2013). Additionally, lactic acid bacteria *L. jugurti* can be used to obtain calcium lactate from whey. Of interest are the data that lactobacilli are able to synthesize, along with lactic acid, various antimicrobial substances which have an inhibitory effect on the growth of microorganisms that cause food products spoilage during storage and⁄or diseases of humans and animals when consumed. This antimicrobial property is particularly valuable for manufacturers of probiotic additives and products (Raskoshnaya et al., 2016).

The metabolites with antimicrobial action derived from whey media can be incorporated into high concentration *Lactobacillus casei* biomass for fodder (Bernardez et al., 2008). In the complex treatment of patients with gastroenterological diseases, a drink based on cheese whey showed effects equal to whole milk dairy products with recognized probiotic action during clinical trials (Irkitova & Vechernina, 2010).

Processing of whey in chemical and biochemical synthesis is known to provide various derivatives. The range of the derivatives is quite wide, with more than 50 products having prospects in various fields (Sinel'nikov et al., 2007). These can be divided into lactose hydrolysates (galactose, tagatose, fucose, lactobionic acid, lactitol, galactooligosaccharides, lactosaccharose, lactosyl urea) and lactose biotransformation products (lactic acid, citric acid, ethanol, acetic acid, propionic acid, bacteriocins, antibiotics, yeast, vitamins, enzymes, proteins, and lipids).

Recent research has shown an increase in the productivity of lactic acid bacteria and subsequent intensification of whey lactic acid fermentation. For instance, Kumar et al. (2014) described a biocatalytic effect of a composite of porous de-lignified cellulose, Ca-alginate and polylactic acid on the biosynthetic activity of *L. bulgaricus* bacteria immobilized on this carrier. In experimental fermentations of cheese whey with *L. acidophilus* it was found that the average duration of the lactose biotransformation process is 48 h with the mass concentration of sodium chloride from 0.1 up to 0.8%, the

percentage of the inoculation material from 3 up to approximately 10% $^{V}/_{V}$, and the lactose mass fraction from 3.8 up to 4.2% (Eveleva et al., 2018).
Castro et al. (2013) showed that the growth of *L. acidophilus* and the biosynthesis of organic acids, lactic acid in particular, depended on the content of lactose in whey.

The main purposes of whey conversion, apart from the benefits for consumers, are commercial benefits for entrepreneurs and environmental protection (Shchetinin & Dorokhova, 2013). Better profit margins and environmental safety can be achieved if whey is concentrated by removing moisture, which helps to prolong its shelf life and reduce production costs. The traditional method of concentration is evaporation, in which moisture is removed by boiling the liquid at low pressure and constant temperature (Kretov et al., 2010). Considering the energy costs, the preferred method is freezing-out, due to the preservation of heat-sensitive whey components and their biological value. Considering the amino acid composition of curd whey and comparing two techniques, concentrating by freezing-out preserves more whey amino acids (Kretov et al., 2010). Lactose-containing raw materials are a source for additional produce and improved efficiency of dairy production facilities.

The purpose of this study was to identify technological features of production of lactate-containing additives from milk whey fermented with lactic acid bacteria.

MATERIALS AND METHODS

Materials used in the study

- solutions of whey subjected to experimental fermentation with lactic acid bacteria (hereinafter 'fermented whey');
- curd whey with lactose mass fraction from 3.8% to 4.2% produced by Losevo dairy factory LLC;
- standardized solutions of cheese whey, obtained by adding edible salt to whey in amount of 0.1% to 2.0%;
- individual strains from the subgroup of thermophilic bacteria of *Lactobacillus* genus: L. *acidophilus*, L. *bulgaricus*, L. *helveticus*, L. *lactis*, L. *plantarum*;
- organic and inorganic growth stimulants, viz.: non-granulated malt sprouts produced by Baltic Malting Company llc; the extract of such sprouts; the enzyme Celloviridin g20h with cellulase activity of 200 units g^{-1} (hereinafter 'celloviridin') obtained from Sibbiofarm; disodium phosphate;
- suspension of chalk in various media: drinking water, curd whey, standardized solutions of cheese whey, solutions obtained by lactic acid fermentation of whey.

Methods of processing and determination

The studies were carried out in the laboratory of the All-Russia Research Institute of Food Additives using physical, chemical and microbiological test methods.

The process of biotransformation of curd whey and cheese whey lactose into lactic acid derivatives was studied with regard to productivity (biosynthetic activity) of lactic acid bacteria, the rate of lactose fermentation, the total amount and the rate of calcium lactate formation.

The controlled parameters included the following: specific gravity, active and titratable acidity, mass fraction of lactose and calcium lactate in the solutions.

The controlled parameters were determined by the common industrial and research methods. Specific gravity was determined with hydrometry and pycnometry; active acidity was determined with potentiometry; titratable acidity was measured with acidic-basic titration; the mass fraction of calcium lactate was measured with trilonometric titration; the mass fraction of lactose was measured with Bertrand's permanganate method.

Taking the assumption that the efficiency of lactose biotransformation by lactic acid bacteria depends on the acidification activity of a microorganism, as well as the culture age, the amount of the inoculation material, method of material preparation to fermentation, the presence of stimulants in the medium, and the neutralizing agent, the experiments were planned so that all these parameters underwent variations.

All measurements were taken in triplicate, and error margins were calculated via analysis of variance to prove statistical significance with $p < 0.05$.

Experimental bioprocessing of whey using lactic acid bacteria comprised the preparation of dairy raw materials and lactic acid bacteria cultures, whey fermentation and product recovery. For whey preparation, fat phase removal, separatory sedimentation of solid particles, whey pasteurization, and addition of organic and inorganic growth stimulants was carried out.

Preparation of lactic acid bacterial culture included culture inoculation from ampoules to tubes containing sterile skim milk; keeping them in a thermostat at a temperature of 40 to 42 °C for 6 to 24 h; re-inoculation to skim milk; pasteurization at 70 °C for 10 minutes and cooling to the cultivation temperature according to the common methods of microbiology. The last generation of lactic acid bacteria was inoculated to whey pasteurized under similar conditions.

Batch fermentations of the curd whey and the standardized solutions of cheese whey were carried out in a thermostat at the temperature of (40 ± 3) °C in 0.5 and 1.0 dm^3 Erlenmeyer flasks for optimal parameters of the preparation of the nutrient medium, biosynthesis and acid neutralization.

RESULTS AND DISCUSSION

Comparative studies of the growth and production of lactic acid by the following strains of lactobacilli: *L. acidophilus*, *L. bulgaricus*, *L. lactis*, *L. helveticus* and *L. plantarum* were carried out. Among these bacteria, the highest values of productivity (up to 0.60 g dm^{-3} h^{-1}) and maximum acidity (up to 370 °T) were registered for *L. acidophilus*. Additionally, *L. acidophilus* had the highest resistance to the presence of salt in the environment. When fermenting standardized solutions of cheese whey with a 2% mass fraction of salt, the growth of acidophilic bacilli was maintained, though there was a decrease in biosynthetic activity.

Having selected *L. acidophilus* for further experiments, the influence of the amount and the age of the inoculation material on the growth of *L. acidophilus* was studied. It was found that the greatest efficiency of the lactose fermentation process was achieved by introducing the inoculation material into the whey at the age of 8 h and a volume fraction of 5%, as evidenced by the changes in the mass fraction of calcium lactate in the fermented solutions (Tables 1, 2).

Table 1. Mass fraction of calcium lactate in fermentation media based on curd whey at variation of the age of the inoculation material of *L. acidophilus* ($p < 0.05$)

Age of the culture, h	Mass fraction of calcium lactate, % at the duration of the fermentation process, h			
	0	24	48	72
4	0.84 ± 0.01	1.78 ± 0.01	3.35 ± 0.03	4.30 ± 0.04
6	1.0 ± 0.01	2.20 ± 0.02	3.35 ± 0.03	4.42 ± 0.04
8	0.84 ± 0.01	1.89 ± 0.02	3.65 ± 0.04	4.61 ± 0.05
12	0.94 ± 0.01	1.78 ± 0.02	3.04 ± 0.03	3.77 ± 0.03
24	0.94 ± 0.01	1.78 ± 0.02	2.72 ± 0.03	3.56 ± 0.03

Some processes involving fermentation of carbohydrates by lactic acid bacteria include the addition of organic growth stimulants to the medium. Thus, the influence of such stimulants as non-sterile and pasteurized malt sprouts, non-sterile malt sprouts with an addition of celloviridin, pasteurized aqueous extract of the malt sprouts on curd whey lactose biotransformation was further studied. Table 3 summarizes the parameters of curd whey fermentation by *L. acidophilus* with different organic growth stimulants. The data show that introducing the tested organic growth stimulants into the whey improved the efficiency of the whey fermentation process, which is reflected in an increase in the mass fraction of calcium lactate and a decrease in the mass fraction of lactose after 72 h of whey fermentation by *L. acidophilus*. However, the use of organic stimulants for this purpose in industry cannot be recommended, since large amounts of processed whey form a significant amount of deposit after fermentation, which in turn requires recycling. It is also worth noting that the introduction of non-sterile malt sprouts into the nutrient medium can produce undesirable contaminations.

Table 2. Mass fraction of calcium lactate in fermentation media based on standardized solutions of cheese whey at variation of volume fraction of *L. acidophilus* inoculation material ($p < 0.05$)

Volume fraction of the inoculant, %	Mass fraction of calcium lactate, % at the duration of the fermentation process, h		
	24	48	54
5.0	1.97 ± 0.02	3.44 ± 0.03	3.93 ± 0.03
7.5	1.84 ± 0.02	3.45 ± 0.03	3.83 ± 0.03
10.0	1.82 ± 0.02	3.35 ± 0.03	3.84 ± 0.03
12.5	1.76 ± 0.01	3.29 ± 0.03	3.67 ± 0.03

Table 3. Curd whey fermentation parameters using *L. acidophilus* with different growth stimulants ($p < 0.05$)

Growth stimulant	Percentage during fermentation	
	start of process	after 72 h
CALCIUM LACTATE		
Control (no stimulant)	1.15 ± 0.01	3.56 ± 0.04
Non-sterile malt sprouts (10% $^{W}/_{W}$)	1.05 ± 0.01	5.24 ± 0.05
Malt sprouts, pasteurized (10% $^{W}/_{W}$)	1.05 ± 0.01	5.24 ± 0.05
Non-sterile malt sprouts (2% $^{W}/_{W}$)	1.05 ± 0.01	4.70 ± 0.04
Non-sterile malt sprouts (2%) with celloviridin (0.25% $^{W}/_{W}$)	1.05 ± 0.01	4.70 ± 0.04
Water extract from malt sprouts, pasteurized (1 : 10 ratio)	1.05 ± 0.01	4.61 ± 0.04

Table 3 (continued)

LACTOSE		
Control (no stimulant)	4.94 ± 0.05	1.30 ± 0.02
Non-sterile malt sprouts (10% $^{W}/_{W}$)	4.78 ± 0.04	0.29 ± 0.01
Malt sprouts, pasteurized (10% $^{W}/_{W}$)	4.78 ± 0.04	0.29 ± 0.01
Non-sterile malt sprouts (2% $^{W}/_{W}$)	4.78 ± 0.04	0.92 ± 0.01
Non-sterile malt sprouts (2%) with celloviridin (0.25% $^{W}/_{W}$)	4.75 ± 0.04	0.79 ± 0.01
Water extract from malt sprouts, pasteurized (1 : 10 ratio)	4.78 ± 0.04	0.55 ± 0.01

Inorganic growth stimulants such as disodium phosphate are used in milk protein production as an effective and safe source of inorganic nutrients for the bacteria *L. acidophilus*. It was found that disodium phosphate stimulates the biosynthetic activity of the microorganism and contributes to a significant increase in productivity of *L. acidophilus* during whey fermentation. The data in Table 4 confirm that the introduction of disodium phosphate in the nutrient medium in the amount of 2% helps to achieve the highest biosynthetic activity of *L. acidophilus* and provides optimal pH of the medium for the development of the bacteria: 5.2 to 5.6.The effect of various neutralizing agents (calcium carbonate, sodium carbonate, potassium carbonate and aqueous ammonia solution) on the fermentation rate of whey lactose by *L. acidophilus* was assessed. The addition of powdered calcium carbonate, sodium carbonate and potassium carbonate did not maintain the pH of the fermented solutions within an acceptably narrow range. The use of calcium carbonate in the form of an aqueous suspension is impractical due to dilution of fermented solutions and sedimentation instability of the suspension. Neutralizing the fermented whey with a 25% ammonia solution provided the highest rate of lactose fermentation (0.53 ± 0.01 g h^{-1}) among the neutralizing agents tested. Even though this value is still not up to the standard (Hramtsov, 2011), a 25% ammonia solution appeared to be the best agent from the list above.

Table 4. Productivity of *L. acidophilus* during fermentation of cheese whey with different concentrations of disodium phosphate in fermentation media ($p < 0.05$)

Mass fraction of disodium phosphate, %	Productivity, g dm^{-3} h^{-1}
0	0.65 ± 0.04
0.5	0.80 ± 0.02
1.0	0.86 ± 0.02
2.0	0.90 ± 0.03
3.0	0.81 ± 0.01

Taking into account the findings by Kumar et al. (2014) that the growth and the biosynthetic activity of *L. acidophilus* depends on the content of lactose in whey, experimental fermentation of curd whey with the mass fraction of lactose from 4% to 10% was done. The rate of formation and and the total amount of calcium lactate was found to correlate with the mass fraction of lactose and the duration of the biosynthesis. The greatest biosynthetic activity was achieved on a whey medium with 8% lactose (Figs 1, 2).

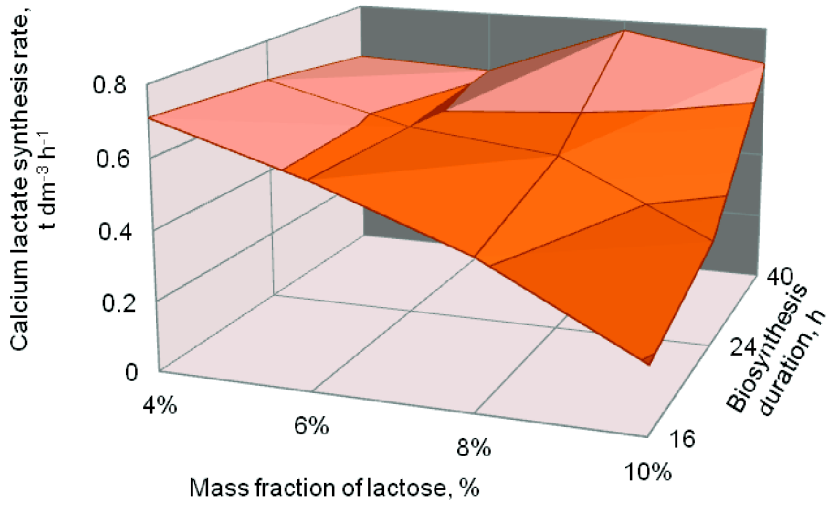

Figure 1. Response surface of the rate of calcium lactate formation to lactose mass percentage in whey media and the duration of biosynthesis.

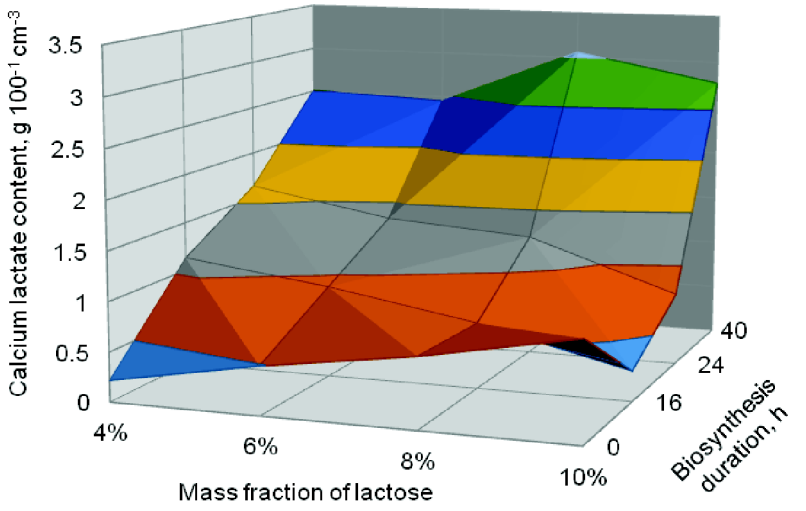

Figure 2. Response surface of the content of calcium lactate to lactose mass percentage in whey media and the duration of biosynthesis.

The observed correlations suggest that in order to improve the efficiency of whey processing, production lines of lactate-containing food and feed additives could benefit from a material concentration step.

CONCLUSIONS

It was established that the greatest efficiency of lactose fermentation can be reached by *L. acidophilus* with introducing the inoculation material into whey at the age of 8 h and a volume fraction of 5%.

It was demonstrated that introducing organic growth stimulants (non-sterile and pasteurized malt sprouts, non-sterile malt sprouts with an addition of celloviridin, pasteurized aqueous extract of the malt sprouts) into whey contributes by increase in the mass fraction of calcium lactate and decrease in the mass fraction of lactose after 72 h of whey fermentation. However, the industrial use of organic stimulants cannot be recommended due to significant amounts of sediments formed.

Regarding inorganic growth stimulants, disodium phosphate proved to stimulate the biosynthetic activity of the bacteria *L. acidophilus* and to contribute to a substantial increase in their productivity during whey fermentation. The introduction of disodium phosphate in the nutrient medium in the amount of 2% helps to achieve the highest biosynthetic activity of the bacteria *L. acidophilus* and provides optimal pH of the medium for the development of *L. acidophilus* (5.2 to 5.6).

Having tested the effect of various neutralizing agents (calcium carbonate, sodium carbonate, potassium carbonate and aqueous ammonia solution) on the rate of fermentation of whey lactose by *L. acidophilus*, it was found that calcium carbonate was the agent of choice from the list above.

Correlations between the formation rate and the total amount of calcium lactate, on one end, and the mass fraction of lactose and the duration of biosynthesis, on the other, were found. In this regard, the greatest biosynthetic activity of the microorganism can be achieved on a medium based on concentrated whey containing 8% lactose.

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