PAPER • **OPEN ACCESS**

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To cite this article: M I Shramko *et al* 2020 *IOP Conf. Ser.: Earth Environ. Sci.* **548** 082066

Influence of oligochitosans and highly molecular chitosan on *Lactobacillus Bulgaricus* cultivation

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Abstract. It was established that decrease of oligochitosans with molecular masses 7.0, 25.4, 45.3 kDa concentration in the process of *Lactobacillus bulgaricus* cultivation leads to fermented dairy product pH reduction and titratable acidity increase. Further increase in titratable acidity and decrease of lactic acid microorganisms amount was determined during the fermented dairy product storage process. Oligochitosans with molecular masses 7.0, 25.4, 45.3 kDa in concentrations interval from 0.0025 to 0.01 per cent did not exhibit prebiotic properties. Active acidity elevation and titratable acidity depression was observed at the chitosan with molecular mass 350 kDa concentration rises. Also increase of highly molecular chitosan concentration leads to elevation of lactic acid microorganisms total amount, which was more than three degree as many as total count of lactic acid bacteria in control sample.

1. Introduction

Starters of the *Lactobacillus bulgaricus* species pure cultures are widely used for manufacturing of functional fermented dairy products with dietary and health-promoting properties. The prospective way of fermented milks production technological development is enrichment with chitosan [1-3]. Chitosan is a biogenic heteropolymer consists of N-acetylglucosaminaine and glucosamin residues [2, 4]. Chitosan has high molecular mass and soluble in organic acids [5, 6]. Low-molecular derivatives of chitosan are represented by oligochitosans with a molecular mass from 2 to 50 kDa, which are well soluble in water. Chitosan and oligochitosans are able to interact with Lactobacillus bulgaricus cells by a different mechanisms depending of their molecular mass [7-9]. Teichoic acid negatively charged molecules of lactic acid bacteria cells are capable to multi-point ion binding with positively charged high-molecular chitosan, whereas their cytoplasmic membrane proteins interact with oligochitosans [4, 9]. The consequence of this process may be a change in metabolic processes in lactic acid bacteria cells.

The goal of research was to study the effect of different concentrations of highly molecular chitosan and oligochitosans with varying molecular mass on lactic acid fermentation process driven by *Lactobacillus bulgaricus*.

2. Materials and methods

Targets of research were skim milk, starter culture of lactic acid bacteria *Lactobacillus bulgaricus* (producer: Dairy Plant “Stavropolsky”, Russia), chitosan with a molecular mass of 350 kDa and a 95 per cent degree of deacetylation (manufacturer: “Bioprogress LLC”, Russia).

Oligochitosans with molecular masses of 7.0, 25.4, 45.3 kDa and 96 per cent degree of deacetyration was prepared by the previously described technique [5].

Dry skim milk was reconstituted to a dry mass concentration of (10 ± 0.2) % by dissolving in distilled water at temperature 30 to 35 °C. Reconstituted skim milk after recombination was characterized by the following parameters: mass concentration of fat 0.15 per cent, mass concentration of protein 3.2 per cent, mass concentration of lactose 5 per cent.

The solution of chitosan with molecular mass 350 kDa in 2 per cent concentration lactic acid aqueous solution with mass concentration 1 per cent was added into skim milk experimental samples for preparation of mixture with final concentration of chitosan 0.0025, 0.005, 0.0075 and 0.01 per cent respectively. Similar experiments were carried out using oligochitosans with molecular masses of 7.0, 25.4, 45.3 kDa in above mentioned concentrations.

The starter culture of *Lactobacillus bulgaricus* was inoculated in the amount of 3 per cent of the total samples volume after pasteurization of the mixture and cooling to the fermentation temperature of (43 – 45) °C.

The end of the fermentation process was determined by organoleptic curd density, as well as by titratable and active acidity.

Experimental and control samples were stored during 17 days at 4 ± 2 °C after completion of fermentation process. Following parameters were tested in triplicate during storage of control and experimental samples: pH by potentiometry, titratable acidity by titrimetric analysis and total count of lactic acid bacteria (CFU per gram).

3. Results and discussions

3.1. The effect of highly molecular chitosan and oligochitosans on fermented dairy products physical and chemical properties

The effect of highly molecular chitosan and oligochitosans with a molecular weight of 7.0, 25.4, 45.3 kDa various concentrations on fermented dairy products physical and chemical properties during the cultivation of Lactobacillus bulgaricus and long-term storage process was studied.

As shown in tables 1 and 2, decrease in the concentration of oligochitosans leads to significant decrease in pH and increase of titratable acidity after 20 hours of cultivation.

This is explained by the fact that oligochitosans with molecular masses of 7.0, 25.4, 45.3 kDa in concentrations of 0.0025 and 0.005 per cent effectively interact with the proteins of the lactic acid bacteria cytoplasmic membrane. This interaction induces bacterial stress [10]. Consequently lactose enzymatic hydrolysis and lactic acid production are accelerated resulting in titratable acidity increase. The elevation of oligochitosans concentration leads to promotion of their interaction with bacterial cells teichoic acid molecules.

Table 1. Effect of chitosans molecular mass on active acidity of fermented skim milk after 20 hours of *Lactobacillus bulgaricus* cultivation process at 45 °C.

Chitosan molecular mass, kDa	pH				
	concentration of chitosan, per cent				
	control	0.0025	0.005	0.0075	0.01
7.0	5.00 ± 0.1	3.77 ± 0.1	4.77 ± 0.2	5.00 ± 0.1	5.02 ± 0.1
25.4	4.98 ± 0.2	3.65 ± 0.1	3.82 ± 0.1	4.29 ± 0.1	4.56 ± 0.2
45.3	5.00 ± 0.2	3.78 ± 0.1	3.87 ± 0.1	4.70 ± 0.2	4.82 ± 0.1
350	4.93 ± 0.1	4.95 ± 0.2	4.99 ± 0.1	4.98 ± 0.1	5.01 ± 0.2

Table 2. Effect of chitosans molecular mass on titratable acidity of fermented skim milk after 20 hours of Lactobacillus bulgaricus cultivation process at 45 °C.

Chitosan molecular mass, kDa	Titratable acidity, °T				
	concentration of chitosan, per cent				
	Control	0.0025	0.005	0.0075	0.01
7.0	84 ± 1	225 ± 2	115 ± 2	80 ± 1	70 ± 2
25.4	85 ± 2	255 ± 3	209 ± 3	163 ± 2	139 ± 1
45.3	87 ± 2	228 ± 4	202 ± 2	123 ± 3	111 ± 1
350	86 ± 3	89 ± 2	82 ± 2	83 ± 1	75 ± 1

This type of interaction influences on lactic acid bacteria cells cytoplasmic membrane permeability and as a result inhibit rate of lactose metabolism. Highly molecular chitosan concentration variation did not lead to significant changes of pH and titratable acidity of fermented skim milk in comparison with control samples. Chitosan with a molecular mass of 350 kDa puts into effective multi-point ion binding with negatively charged teichoic acid molecules of *Lactobacillus bulgaricus* cells. This is due to the presence into highly molecular chitosan structure of about 1850 amino groups. Lactose assimilation and lactic acid formation rates are changed depending on highly molecular chitosan concentration.

3.2. Physical and chemical properties of fermented dairy products during long-term storage

Physical and chemical properties of fermented dairy products during long-term storage at 4 ± 2 °C were studied after the completion of the Lactobacillus bulgaricus cultivation process. It was established that optimal organoleptic attributes (taste and odor) of fermented product control sample are achieved after 5 days of storage at pH 4.2 – 4.5 and titratable acidity 70 – 140 °T. Organoleptic attributes of this product deteriorated during the further storage.

As shown in table 3, optimal titratable acidity of fermented milks experimental samples containing oligochitosans at a concentration of 0.01 per cent persisted for up to 17 days. Further increase of titratable acidity of experimental samples containing oligochitosans at a concentrations 0.0025, 0.005 and 0.0075 per cent was observed during the storage after the completion of the fermentation process.

Table 3. Effect of chitosan concentration on titratable acidity of fermented product after 17 days of storage at 4 °C.

Chitosan molecular mass, kDa	Titratable acidity, °T				
	concentration of chitosan, per cent				
	Control	0.0025	0.005	0.0075	0.01
7.0	207 ± 2	266 ± 3	209 ± 2	156 ± 2	124 ± 1
25.4	206 ± 3	292 ±4	226 ± 3	197 ± 2	154 ± 2
45.3	205 ± 2	271 ± 3	250 ± 4	193 ± 3	174 ± 3
350	203 ± 2	191 ± 5	186 ± 2	155 ± 3	138 ± 4

Decrease in titratable acidity of fermented dairy product experimental samples was detected when concentration of chitosan with molecular mass 350 kDa increased in interval from 0.0025 to 0.01 per cent. Therefore high-molecular chitosan concentration elevation reduces the intensity of lactic acid fermentation in experimental samples. The most powerful process of lactose homofermentative fermentation inhibition occurred in a sample containing high-molecular chitosan in concentration of 0.01 per cent. The decrease of lactose assimilation intensity by *Lactobacillus bulgaricus* cells may be propelled by two reasons. The interaction between chitosan molecules and lactic acid bacteria cells cytoplasmic membrane leads to disturbance of membrane permeability for β-galactosidase enzyme, which catalases the reaction of lactose into glucose and galactose hydrolysis. At the same time structural changes in cell cytoplasmic membrane cause retardation of lactose hydrolysis products active transport into bacterial cells.

Thus, there is an inhibition of lactic acid formation in the process of fermented dairy product containing high-molecular chitosan storage, which stimulates the preservation of a large number of lactic acid bacteria. This is confirmed by the data of lactic acid microorganisms' quantitative accounting in control and experimental samples after 17 days of storage, as shown in table 4.

Table 4. Count of lactic acid microorganisms in fermented dairy products after 17 days of storage, depending on the concentration and molecular mass of chitosan.

Chitosan molecular mass, kDa	CFU per cm^3				
	dosage of chitosan and oligohitosans, pre cent				
	Control	0.0025	0.005	0.0075	0.01
7.0	$3.7*10^3$	$2.5*10^2$	$2.6*10^2$	$3.4*10^3$	$3.6*10^3$
25.4	$3.7*10^3$	$2.3*10^2$	$2.5*10^2$	$3.9*10^3$	$4.7*10^4$
45.3	$3.7*10^3$	$2.6*10^2$	$2.6*10^2$	$3.5*10^3$	$4.9*10^4$
350	$3.7*10^3$	$3.6*10^3$	$3.6*10^3$	$5.3*10^5$	$6.4*10^6$

The data presented in table 4 indicates that oligochitosans with molecular masses of 7.0, 25.4, 45.3 kDa did not affect significantly on *Lactobacillus bulgaricus* grows rates during fermented dairy products storage process. Addition of highly molecular chitosan in concentrations of 0.0075 and 0.01 per cent in fermented milks increased the content of lactic acid microorganisms, which was more than three degree as many as total count of lactic acid bacteria in control sample.

Thus, tested samples of oligochitosans with varying degrees of polymerization did not exhibit prebiotic properties and did not prolong the shelf life of fermented dairy products.

4. Conclusions

Analysis of experimental data allows establishing that decrease of oligochitosans with molecular masses 7.0, 25.4, 45.3 kDa concentration in the process of *Lactobacillus bulgaricus* cultivation leads to fermented dairy product pH reduction and titratable acidity increase.

Oligochitosans did not inhibit residual lactose fermentation and lactic acid bacteria grow during the fermented dairy product storage process. Oligochitosans with molecular masses 7.0, 25.4, 45.3 kDa in concentrations interval from 0.0025 to 0.01 per cent did not exhibit prebiotic properties.

Increase of chitosan with molecular mass 350 kDa concentration leads to depression of lactose into lactic acid conversion during fermentation of skim milk by lactic acid microorganisms. Also increase of highly molecular chitosan concentration leads to elevation of lactic acid microorganisms' total amount in comparison with control sample.

High-molecular chitosan in a concentration of 0.01 per cent can be recommended as a prebiotic, prolonging the shelf life of fermented milks, manufactured with application of *Lactobacillus bulgaricus* starter cultures.

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