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Original Article

Kinetics of lactose fermentation in milk with kombucha starter

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ABSTRACT

The aim of this research was to investigate the effect of new, non-conventional starter culture on the kinetics of the lactose transformation during milk fermentation by kombucha, at pH 5.8; 5.4; 5.1; 4.8; and 4.6, at two different temperatures 37 °C and 42 °C. Milk fermentation at 42 °C lasted significantly shorter (about 5 h, 30 min) compared to the fermentation at 37 °C. Changes of lactose concentration at the both temperatures are consisting of two retaining stages and very steep decline in-between. The analysis of the rate curves showed that the reaction rate passes through the maximum after 9 h, 30 min at 37 °C and after 4 h at 42 °C. The sigmoidal saturation curve indicates a complex kinetics of lactose fermentation by kombucha starter.

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Q1 1. Introduction

Kombucha is known as a symbiotic association of yeasts and acetic acid bacteria whose metabolic activities on sweetened tea produce a pleasant slightly sweet, slightly acidic refreshing beverage consumed world wide. The microbial composition of kombucha cannot be given because it depends on the culture origin. Novel research [1] has showed significant presence of lactic acid bacteria in kombucha. The major bacterial genus in 5 kombucha samples was *Gluconacetobacter* (over 85% in most samples) and *Lactobacillus* (up to 30%). Only trace populations of *Acetobacter* were detected (lower than 2%). Radulović et al.

[2] also isolated and identified lactic acid bacteria from kombucha tea.

Traditional substrate for the kombucha cultivation is black or green tea extract sweetened with 5%–8% sucrose. Besides traditional substrates, the possibilities of use of alternative substrates have been established in various studies [3–11]. Reiss [12] found that kombucha fermentation on other sugars (lactose, glucose or fructose) produced beverages slightly different in flavour but significantly different in ethanol and lactic acid quantity, compared to sucrose's sweetened tea. For example, fermentation on lactose gave extremely low quantities of ethanol in comparison to the fermentation on sucrose. Bellosso Morales and Hernández-

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Sánchez [13] successfully cultivated kombucha on cheese whey. Malbaša et al. [14] investigated the manufacture of milk-based beverages by application of kombucha starters cultivated on sweetened black and green tea, and top-inambur. Three different inoculums of kombucha starter: cultivated on black tea with addition of sucrose, concentrated by microfiltration and concentrated by evaporation were applied in functional fermented dairy beverages manufacture [15]. Ilić et al. [16] investigated fermentation of lactose from two types of milk, low fat and reduced fat, inoculated with kombucha starter. Milanović et al. [17] revealed that kombucha inoculums cultivated on different tea types could be used in combination with probiotic starter culture in fermented dairy products technology. Vukić et al. [18] also investigated the effect of kombucha starter culture on the rheological properties, texture and microstructure as well as on the protein fractions in different phases of milk fermentation.

Lactose is the main carbohydrate in dairy products. It is a unique disaccharide, composed of glucose and galactose, in the sense that it occurs exclusively in the milk of mammals [19]. Lactose plays an important role in the formation of the neural system and the growth of skin (texture), bone skeleton and cartilage in infants. It also prevents rickets and spondylitis [20]. Lactose fermentation by lactic acid bacteria is widespread in the production of fermented dairy products. In particular, lactose is the principal energy source for the bacteria in the starter culture, while casein, together with calcium and phosphorus gives rise to the basic structure of a gel structure [21]. In general, dairy starter cultures metabolize lactose either through the homo- or hetero-fermentative metabolic pathways. Lactose content is reduced during fermentation (by 20–30 g/100 g of the level in the original milk), while the concentration of lactic acid and some free amino acids increases, for example, proline, serine, alanine, valine, leucine, and histidine [22].

The biochemistry of kombucha fermentation was analysed using green and black teas by Kallel et al. [23]. They followed several biochemical markers of kombucha fermentation for a period of two weeks. The metabolism of carbon was targeted using sucrose, glucose, fructose, cellulose, ethanol and total acetic acid equivalents. Green and black teas exhibited similar kombucha fermentation profiles, but specific biochemical behaviours were observed as well. Lončar et al. [24] defined, by processing the experimental results, two mathematical models for the kinetics of sucrose fermentation by kombucha. It has been shown that both empirical models – one for the change of saccharose concentration during its fermentation, and the other for the rate of the mentioned fermentation, enable better insight into sucrose transformation.

There is no data in available literature about kinetics of lactose conversion during milk fermentation by kombucha starter. Therefore, the aim of this research was to determine the effect of non-conventional starter culture on the kinetics of the lactose transformation during milk fermentation by kombucha, at pH 5.8; 5.4; 5.1; 4.8; and 4.6, at two different temperatures 37 °C and 42 °C.

2. Experimental

2.1. Milk

Homogenized and pasteurized cow's milk from AD Imlek, Division Novi Sad Dairy, was used for the production of kombucha fermented milk products. The composition of milk was as follows: fat content – 2.0 g/100 g, total solids – 10.59 g/100 g, total proteins – 3.30 g/100 g and lactose – 4.60 g/100 g, ash 0.69 g/100 g.

2.2. Kombucha inoculum

Kombucha inoculum was prepared according to procedure published by Milanović et al. [25] (2002). Kombucha is cultivated on a black tea (*Camellia sinensis* – oxidized, 1.5 g/L) with sucrose concentration of 70 g/L. The tea was cooled to room temperature, after which inoculum from a previous fermentation was added in concentration of 10% (v/v). Incubation was performed at 25 °C for 7 days. Inoculum in concentration of 10% (v/v) was used for milk fermentation.

Local domestic Kombucha, used for the fermentation, contains at least five yeast strains (*Saccharomyces ludwigii*, *Saccharomyces cerevisiae*, *Saccharomyces bisporus*, *Torulopsis* sp. and *Zygosaccharomyces* sp.) as determined by Markov et al. [26]. Total number of viable cells was as follows: approximately 5×10^4 of yeast cells per mL of the reaction mixture and approximately 2×10^5 of bacteria cells per mL of the mentioned mixture [24].

2.3. Samples production

Samples were obtained by addition of 30 mL of kombucha inoculum in 300 mL of milk at 42 °C and 37 °C simultaneously. Samples were taken at pH values: 5.8; 5.4; 5.1; 4.8 and 4.6.

2.4. Methods

2.4.1. pH values

The pH values were determined using a pH-metre (pH Spear, Eutech Instruments Oakton, UK).

2.4.2. Sugar content

The content of lactose and galactose at two temperatures were detected in all samples using specific enzymatic tests K-LACGAR 12/05 (lactose and D-galactose) purchased by Megazyme, Ireland. Products of the reactions were monitored using spectrophotometer (T80 + UV/VIS Spectrometer PG Instruments Ltd, UK).

2.4.3. HPLC analysis of lactic acid

Lactic acid content was determined according to the modified method of Jayabalan et al. [27]. Four grams of samples were transferred into a 25 mL volumetric flask, 5 mL of bidistilled water was added and filled up with acetonitrile (Mallinckrodt Baker, Inc., Netherlands). The obtained solution was mixed for 5 min and then was filtered through membrane syringe filter (with diameter pore of 0.45 µm). Agilent 1100 system (USA)

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