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ORIGINAL RESEARCH PAPER

STUDY OF GUSTATORY AND STRUCTURAL PROPERTIES OF KEFIR PRODUCT

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Abstract: The subject of the study is the improvement of Kefir culture. For this investigation it was used Kefir culture and two series of experiments were carried out. Yeasts from probiotic strain *Saccharomyces boulardii* were added in the first series and the lactic acid bacteria (LAB) of *Streptococcus thermophilus* strain, with a polysaccharide activity were added to Kefir culture in the second series. The fermentation conditions were 30°C, duration 16 hours and cooling 4 hours up to 4°C. The characteristics of Kefir were analyzed by determination of: pH, acidity, qualitative reaction for existence of diacetyl, cell number of LAB and yeasts. The structural properties of Kefir were evaluated by microscopic study.

Keywords: *fermentation, kefir, lactic acid bacteria, Saccharomyces boulardii, Streptococcus thermophilus.*

INTRODUCTION

Saccharomyces boulardii is a tropical strain of yeast first isolated from lychee and mangosteen fruit in 1923 by French scientist Henri Boulard. *S. boulardii* has been shown to be non-pathogenic, non-systemic (it remains in the gastrointestinal tract rather than spreading elsewhere in the body), and grows at the unusually high temperature of 37°C [1]. *S. boulardii* is often marketed as a probiotic in a lyophilized form and is therefore often referred to as *Saccharomyces boulardii* lyo. It is a bio therapeutic agent, used primarily for antagonism against pathogens. Some of the benefits of *S. boulardii* are antitoxin effects. They are expressed at interaction between *S. boulardii, E. coli* and *Salmonella typhimurium*. Last two are pathogenic bacteria often associated with acute infectious diarrhea, were shown to strongly adhere to mannose on the surface of *S. boulardii* via lectin receptors (adhesins). Once the invading microbe is bound to *S. boulardii*, it is prevented from attaching to the brush border; it is then eliminated from the body during the next bowel movement [2]. *Saccharomyces* is not part of the naturally occurring gut flora like some bacterial probiotics. *Saccharomyces* is resistant to stomach acids, bile and pancreatic juices, as it is can tolerate varying pH levels, so its survival through the gut is greater than that of bacteria probiotics. There are also no concerns with the impact of antibiotics on *Saccharomyces* as there are with probiotic bacteria. Yeasts are naturally resistant to antibiotics and are completely safe when taken during antibiotic treatment. Additionally, unlike some bacteria probiotics, *Saccharomyces* does not colonize the intestine; it is a transient. After 3 days of supplementation, it will reach a maximum steady state.

In particular, in the experiment was used *Streptococcus thermophilus* strain. *Streptococcus salivarius* subsp. *thermophilus* (common name *Streptococcus thermophilus*) is a Gram-positive bacteria and a homofermentative facultative anaerobe, of the viridans group [3]. *Streptococcus* derives from a Greek term meaning "twisted berry" and refers to the way the bacterium is grouped in chains that resemble a string of beads [4]. Thermophilus derives from the Greek term thermotita meaning "heat". It refers to an organism's ability to thrive at high temperatures [5]. It tests negative for cytochrome oxidase and catalase, and positive for alpha-hemolytic activity [3]. It is non-motile and does not form endospores [3]. It is also classified as a lactic acid bacterium (LAB) [6]. *S. thermophilus* is found in fermented milk products. It is not a probiotic (it does not survive the stomach in healthy humans) and is generally used in the production of yogurt [7], alongside *Lactobacillus bulgaricus*. The two species are synergistic, and *S. thermophilus* probably provides *L. bulgaricus* with folic acid and formic acid which it uses for purine synthesis [8]. *S. thermophilus* has a thick cell wall that allows it to survive high temperatures. This is useful for the process of dairy production where bacteria are heated to temperatures of about 100°C. *S. thermophilus* are less pathogenic than other *Streptococcus* species because it lacks some key surface proteins, cell structures that can allow viruses to replicate. *S. thermophilus* is a facultative anaerobe, which is an organism that is capable of generating ATP through both aerobic respiration and fermentation (depending on the presence or absence of oxygen).

Kefir is a fermented milk drink that originated with shepherds of the North Caucasus region, who discovered that fresh milk carried in leather pouches would occasionally ferment into an effervescent beverage. It is prepared by inoculating cow, goat, or sheep's milk with kefir grains. Kefir grains are a combination of bacteria and yeasts in a

matrix of proteins, lipids, and sugars, and this symbiotic matrix forms "grains" that resemble cauliflower. For this reason, a complex and highly variable community of lactic acid bacteria and yeasts can be found in these grains. Kefir grains contain a water soluble polysaccharide known as kefiran, which that imparts a rope-like texture and feeling in the mouth, appear in hues ranging from white to yellow, and usually grow to the size of walnuts. Traditional kefir is fermented at ambient temperatures, generally overnight. Fermentation of the lactose yields a sour, carbonated, slightly alcoholic beverage, with a consistency similar to thin yoghurt [9]. Kefir fermented by small-scale dairies early in the 20th century achieved alcohol levels between 1 and 2 percent, but kefir made commercially with modern methods of production has less than 1% alcohol, possibly due to reduced fermentation time [10]. Variations that thrive in various other liquids exist, and they vary markedly from kefir in both appearance and microbial composition. Kefir contains compounds that have antimutagenic and antioxidant properties in vitro, although it is not established that these compounds have any physiological properties when kefir is consumed [11]. Like Yogurt, it introduces good bacteria into human's body. But it must be eaten regularly to keep good bacteria in intestine system. Kefir's beneficial or friendly bacteria come from kefir grains and actually resides in the intestines when consumed, where they can continue to aid in digestion and repel harmful bacteria. There are also studies and proof that it cures constipation, ulcers and makes immune system better.
The aim of present work is to study the influence of both strains – *Saccharomyces boulardii* and *Streptococcus thermophilus* over a natural product Kefir.

MATERIALS AND METHODS

Materials

For experiments Kefir culture was used in 7 fermentations:
- Control - Kefir grains 8 g in 0.400 L sterilized skimmed milk;
- Sample 1 - Kefir grains 8 g in 0.400 L sterilized skimmed milk + 0.400 g freeze-dried *Streptococcus thermophilus* culture;
- Sample 2 - Kefir grains 6 g in 0.400 L sterilized skimmed milk + 0.080 g freeze-dried *Saccharomyces boulardii.*

Fermentation conditions

The fermentation conditions were 30°C, duration 16 hours and cooling 4 hours up to 4°C.

Measurement of acidity and pH

In the glass with pipette were measured 10 cm^3 of the sample, 20 cm^3 distilled water, 3 drops of phenolphthalein and the mixture was shaked. The sample was measured with titration with 0.1 N NaOH until appearance of slight pink color. This color must keep 1 min. A pH meter was used to measure pH.

Qualitative reaction for existence of diacetyl

In a small tube were putted 2 mL of the sample, 2 mL 0.1 N NaOH and 0.2 g creatine. The sample must be well mixted. The acetoine was oxidized to diacetyl. It was obtained colored by positive reaction.

Counting the number of LAB and yeasts

Number of LAB was determinated in MRS agar, M17 agar and PCA agar according to IDF qualities and Bulgarian standards. Number of yeasts is determinate in YGC agar according to IDF qualities and Bulgarian standards [12 – 14]. For microscope observations, samples were stained with 2% solution of methylene blue for 2 minutes at room temperature, washed with distilled water and dried for 24 hours at 37°C. Further, the samples were analyzed by using bright field microscope Olympus BX53, Camera SC30 (Japan).

RESULTS AND DISCUSSION

The pH of the samples was measured during all fermentations. Results are shown in Figure 1. The normal value of Kefir's pH is about 4.40. At the beginning pH started to go down and that is the reason some cultures with aromatic properties to be added after 3rd fermentation. And after 5th and 7th fermentations it was added the same culture of *Streptococcus thermophilus* for Sample 1, respectively of *Saccharomyces boulardii* for Sample 2, strain, too. It was made to keep the pH at standard values.

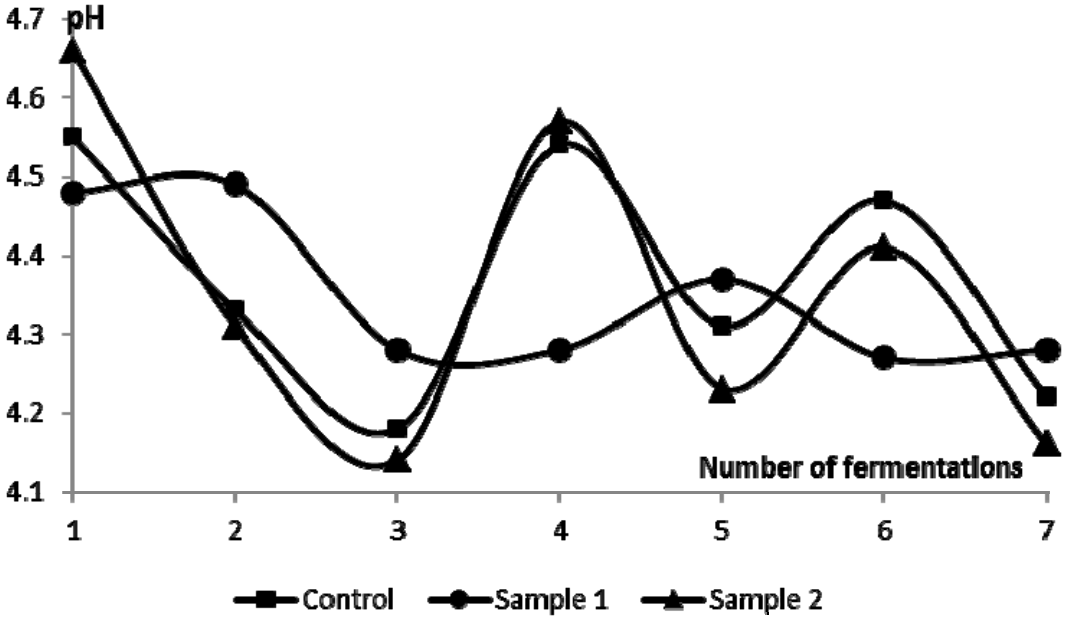

Figure 1. *Change of pH during fermentations*

The acidity of the samples was measured after all fermentations, too. Results are shown in Figure 2. The acidity varies after 3rd fermentation in the range from 140°T to 103°T due to addition of cultures with aromatic properties.

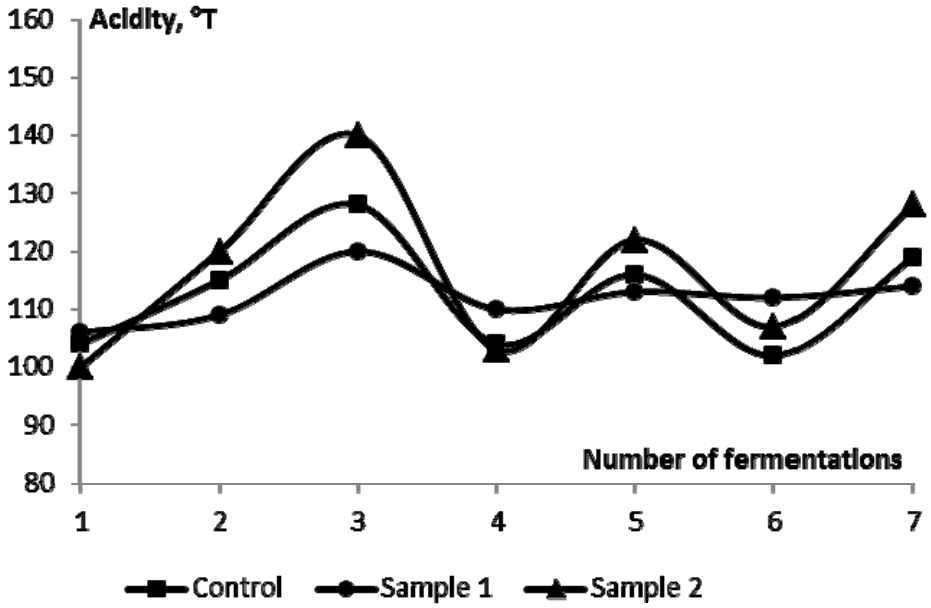

Figure 2. *Change of acidity during fermentations*

Table 1. *Results of qualitative reaction for existence of diacetyl*

Culture	Time of appearance for each of 7th fermentation [min]						
	1	2	3	4	5	6	7
Control	3:30	3:00	2:00	2:30	4:00	3:00	4:00
Sample 1	4:30	3:00	4:30	5:00	3:00	3:00	4:00
Sample 2	3:30	4:00	2:00	3:00	4:30	3:30	4:30

Qualitative reaction for existence of diacetyl was done for all fermentations. The obtained results are shown in Table 1.
This qualitative reaction shows aromatic properties of Control, Sample 1 and Sample 2. The best results belong to Control and Sample 2.
Cell number of LAB and yeasts was monitored at the beginning and in the end of the experiments. Results of LAB and yeasts count are presented in Table 2.

Table 2. *Cell number of LAB and yeasts*

Culture	Cell number [CFU/mg]			
	Cell number of LAB		Cell number of yeasts	
	After 1st fermentation	After 7th fermentation	After 1st fermentation	After 7th fermentation
Control	2.5×10^8	2.5×10^9	5.5×10^5	1.1×10^5
Sample 1	2.5×10^8	2.5×10^9	1.19×10^6	3.3×10^5
Sample 2	2.5×10^8	2.0×10^8	9.90×10^5	14.8×10^6

The microscopic study of the investigated samples is shown in Figures 3 and 4.
By Sample 1 it was observed that after 7th fermentations the number of LAB increased 10 times compared to the 1st fermentation. However the number of yeasts cells was constant during all fermentations (Figure 3).
The addition of *S. boulardii* by Sample 2 lead to double increase the number of yeast at the end of 7th fermentation compared to the 1st fermentation. However the number of LAB was without any changes (Figure 4).

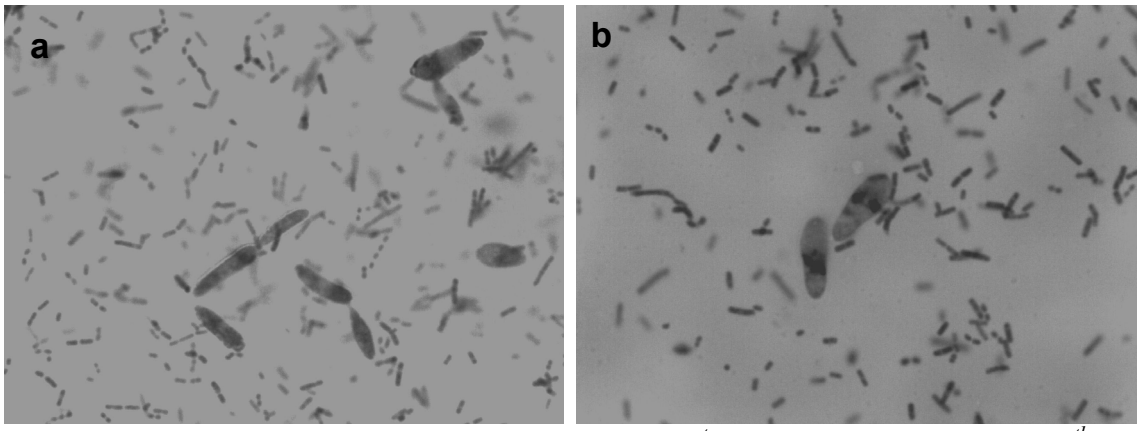

Figure 3. *Microscopic picture of Sample 1 after 1st fermentation (a) and after 7th fermentation (b)*

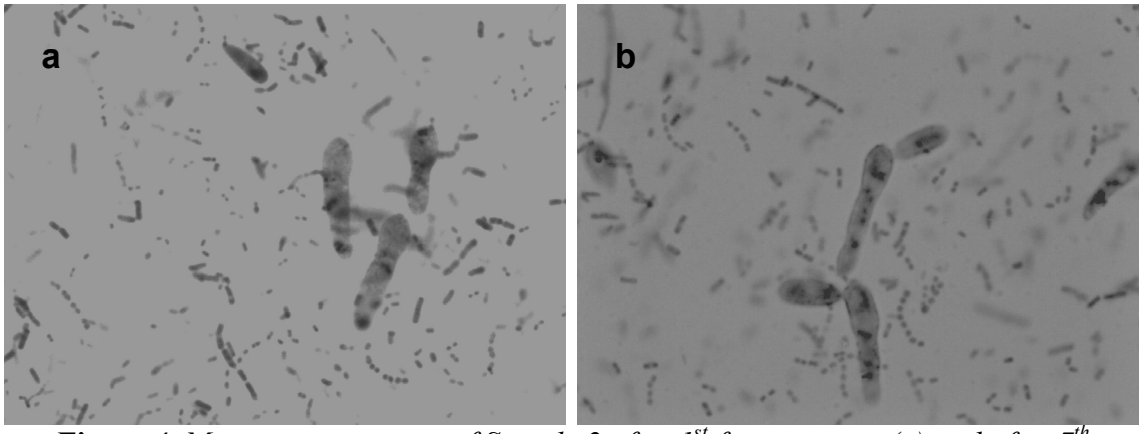

Figure.4. *Microscopic picture of Sample 2 after 1st fermentation (a) and after 7th fermentation (b)*

CONCLUSIONS

Structural properties of Kefir culture were improved by adding of LAB - *Str. thermophilus*. In this case worse taste was achieved. Double increase in the number of yeasts was detected by adding of *Saccharomyces boulardii* strain. It helps to strengthen probiotic resident in Kefir product. This was established in the microscopic picture. The addition of some aromatic cultures in Kefir affects pH and acidity. This may be help in the industry when some changes in pH are necessary.

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LIST OF NOTATIONS AND SYMBOLS

CFU - Colony-forming unit
IDF - International Diabetes Federation
LAB - lactic acid bacteria
MRS - Lactobacillus MRS Agar
M17 - M 17 agar acc. to TERZAGHI for microbioligy
PCA - Plate Count Agar
YGC - Chloramphenicol Yeast Glucose Agar

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