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Obtaining functional fermented beverages by using the kefir grains

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Abstract

In this experimental work were developed beverages from products obtained after processing of milk. There have been opportunities for functional beverages through the use of probiotic microorganisms contained in kefir grains and whey from the manufacture of Bulgarian brine cheese and ultrafiltrate from milk or whey. Observed and analyzed is the rate of lactic acid fermentation and established appropriate conditions for development of kefir grains in obtaining fermented beverages. Applied is non-contacts ultrasound method for diagnosis of beverages. Determined is the technological sequence of production stages, physicochemical and microbiological characteristics during manufacture and storage of functional product.

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Keywords: kefir grains; fermentation ;milk beverage; non-contact ultrasound method

1. Introduction

The increased interest to industrial dairy manufacture bring to full use of milk component, a fuller and more rational use of separate secondary raw materials from processing. This rationalization correlated from one side to ensure sustainability on production and high quality of products, and from other side environmental protection through better utilization of productive resources through the development and implementation of effective technological systems to dairies for manufacturing of secondary raw materials [1]. These products are - whey, milk serum, buttermilk and filtrates. By-products obtained after

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milk processing represented a complex food containing various nutrients to which may be used different functional nutrients in aim to create healthy food [2]. The establishment of functional beverages enriched with various ingredients is a top priority of modern technology.

It is known to obtain fermented by kefir grains milk with high biological value [3, 4, 5, 6, 7, 8, 9, and 10]. The kefir contains a variety of useful lactic acid bacteria and yeasts differentiation symbiotic complex matrix of probiotic microorganisms. The combination of these bacteria and yeasts regulate and reduce the development of pathogenic microorganisms, while maintaining beneficial flora in the gastrointestinal tract [5, 8, and 10]. Obtaining large quantities of milk serum from the cheese manufacturing and separately after membrane processing-filtrate is a prerequisite for searching a different ways for their recovery [9].

The aim of this article is to obtain, examine and analyze fermented by kefir grains beverage from whey, produced in the manufacture of Bulgarian brine cheese and ultrafiltrate.

2. Materials & Methods

The raw materials accompanying the experimental part are - low fat milk, whey and ultrafiltrate. Milk is used like a control sample to the analysis, the whey is separated after the production of Bulgarian brine cheese by classic technology, cooled and stored refrigerated at $t = 4-6^{\circ}\text{C}$. Filtrate obtained by using membrane technology-ultrafiltration. Physicochemical parameters of the main raw materials are shown in Table № 1.

Analysis by which are determine the parameters of raw materials and products after fermentation process are calculated by using the standard rules.

The fermentation process for whey and filtrate are lead at $20-22^{\circ}\text{C}$ with inoculums about 10% kefir grains and duration for 360 minutes.

Table 1. Composition of main raw materials

Raw material	Physicochemical parameters						
	Dry material, %	Protein, %	Fat, %	Carbohydrates, %	Solid, %	pH	Lactic acid %
Control sample(milk)	10,3	3,4	1,5	4,7	0,7	6,59	0,0
Whey	6,4	0,4	0,5	4,7	0,8	6,01	0,08
Filtrate	5,3	0	0	4,6	0,7	6,29	0,03

The quantity of residual lactose is defined as:

$$G_{res. lact.} = G_{lac} - G_{degr.lac.} \quad (1)$$

$$G_{degr.lac} = [(T_{beg.} - T_{cur.}) * 0,009 * 342] / 360 \quad (2)$$

Where:

$G_{res. lact.}$ – residual quantity of lactose;

G_{lac} – beginning quantity of lactose;

$G_{degr.lac}$ – degraded quantity of lactose;

$^{\circ}T_{beg.}$ – beginning titratable acidity;

$^{\circ}T_{cur.}$ - current titratable acidity.

Variation of pH from 24 to 168 hours during storage of products at $t = 6-8^{\circ}\text{C}$ are determine by measuring. Parallel are made visual observations of the microscopic preparations by using OLYMPUS BX41 microscope with immersion lens 100x and 200x, eyepiece 10x by enlargement 1000x and 2000x.

Micrograph was taken with the built-in digital camera to a microscope OLYMPUS SC30 3, 3 MP. Macrograph of kefir grains is made using a camera Canon EOS DIGITAL REBELXT, with lens Canon EF-S 18-55, with facilities plug-in micrograph. The experiments are realized in off-line mode with ultrasound sensors of type UST40T/UST40R from Nippon Ceramic Company [13, 14].

3. Results & Discussion

The dynamics of the change in pH during fermentation in raw materials (low-fat milk, whey and filtrate), inoculated with kefir grains.

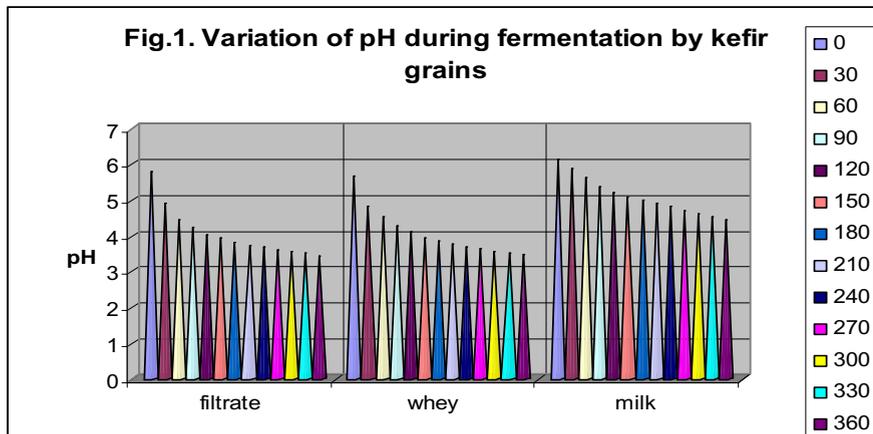


Fig.1. Variation of pH during fermentation by kefir grains

Tables In depending on the qualitative and quantitative composition of raw materials and existing microflora are observe a gradual variation of pH in the process of lactic fermentation in common cultivate of lactic acid bacteria and yeast (Fig.1). The available amount of lactose is the only one substrate for the growth of the microbial population, a source of lactic acid and a factor influencing the decrease of pH in the media. Protein content of the raw materials also reflects on the changes in pH value. The control milk sample is characterized by a higher buffering capacity, due to higher levels of protein substance (3.4%). From the data in Figure 1 is observed gradual decrease in pH during fermentation. Up to 120 minutes to reach pH 5, 18, which coincides with izoyonic point of milk and then are destabilize the casein. The filtrate and whey as opposed to the control sample characterized by low buffering capacity (no protein substances), which allows for a short time (20 minutes) the formation of small amounts of lactic acid, cause lowering the value on pH at 5.00 to 5.20. The duration of the fermentation process of milk is about 4hours, but for whey and filtrate is 45 minutes. Characteristic of the control milk levels at the end of the fermentation process for pH ranging from 4.5 to 4.6 and reached about 300-360 minutes, but with others raw materials that period is five times shorter. At the end of the first hour of the beginning of fermentation the amount of lactose in whey and the filtrate was 0.14 and 0.25% (Table 2), expressed in percentage relative baseline levels are 3.03 and 5.31%.

Table 2. Quantity of ferment lactose (%) during fermentation, min

Raw Material	Lactose, (%)	Quantity of ferment lactose (%) during fermentation, min				
		60	120	180	240	300
Filtrate	4.62	0.14	0.23	0.31	0.37	0.42
Whey	4.7	0.25	0.35	0.43	0.49	0.54

The end of the fermentation process is considered to reach pH values of 3.54 for filtrate and value of pH 3.56 for whey, the quantity of ferment lactose is 0.42 and 0.56%, which corresponds to 9.1 and 11.89% of contained lactose at the beginning of the process. The quantity of residual lactose during fermentation clearly shows proportional change of pH in accordance with the active life activity of microorganisms growing in whey and filtrate (Fig. 2).

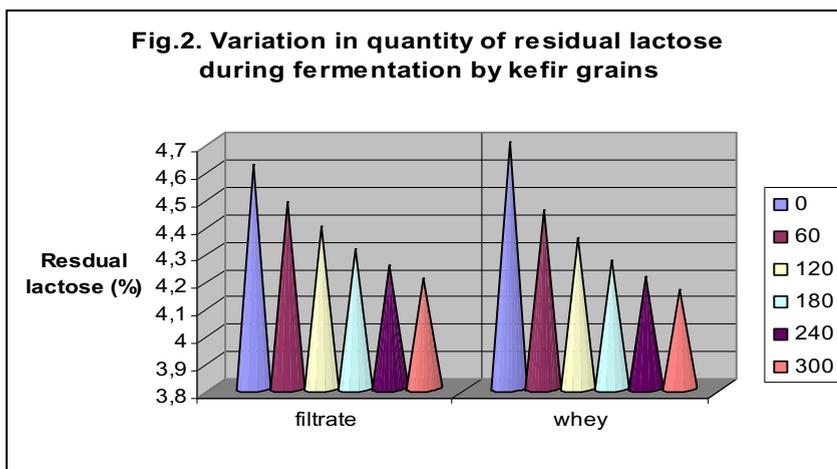


Fig. 2. Variation in quantity of residual lactose during fermentation by kefir grains

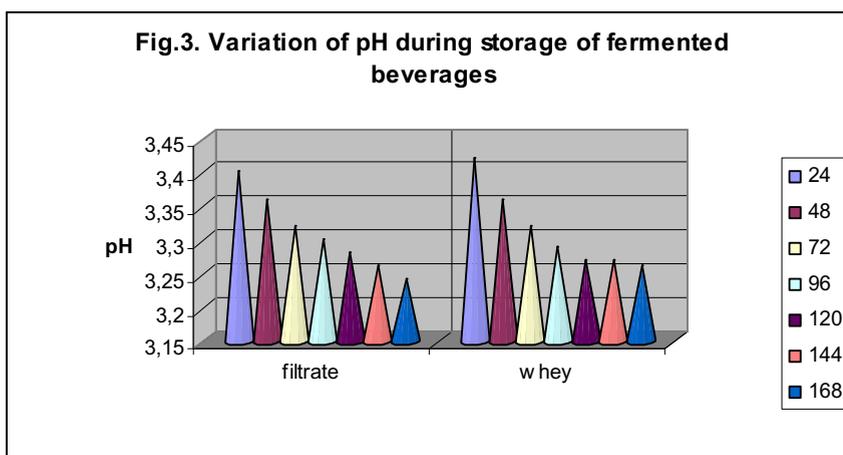


Fig.3. Variation of pH during storage of fermented beverages

The quantity of residual lactose is sufficient for normal fermentation process, to accumulate the required amount of lactic acid and taste-aromatic components. The amount of residual lactose at the end of the fermentation process in the filtrate was 4.2% and 4.17% in the whey. This explains the continued development of lactic acid bacteria and yeasts during the cooling and storage, regardless of lowering the temperature. In the storing process of beverages at a low temperature $t = 6 - 8^{\circ}\text{C}$, is observed variation of pH value.

During the first 24 hours, observed the greatest variation in pH values, respectively 0.04 and 0.06. The next 48 hours pH is amended in the range 0.06 and 0.07, to reaching values up to pH 3.24 and 3.26 at the end of the period. Macrograph shown in Figure 4 indicates that the raw materials used in the analysis are conducive environment for the growth of kefir grains. After fermentation these grains are gelatinous irregular masses white or lightly yellow, the size of the grains is variable but reaches 1.5 – 2cm in diameter. During fermentation the grains increase their weight and this is how new biomass is obtained.

Microscopic picture of Fig. 5 characterized in detail the microbial population in the lactic acid beverage and morphological status of the species of bacteria in it. On the microscopic pattern is observed rich field singles located lactococci and diplococci. Clearly visible as single and short-chains of lactobacilli. In the field observed and yeast cells (right on the picture), some of which are in the process of budding.



Fig.4. Macrograph of kefir grains after fermentation

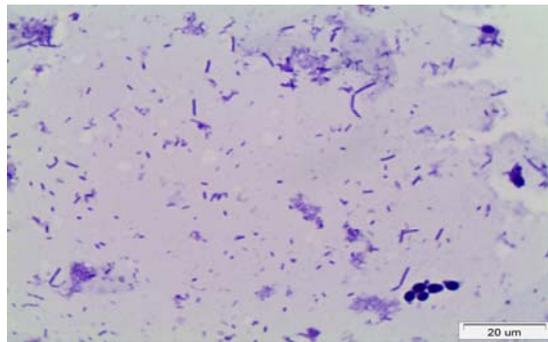
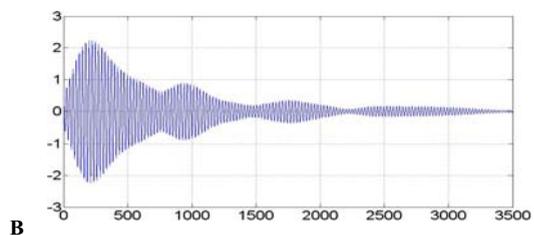
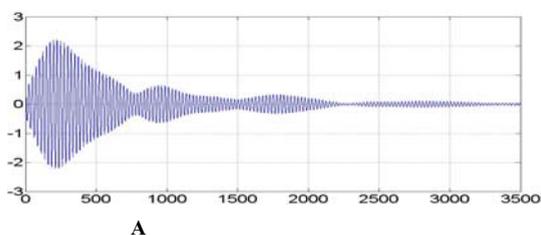


Fig. 5. Microscopic picture of fermented beverage (increase 2000x)

Non-contacts ultrasound method is beside on the effect of reflection. When passing through a medium the ultrasound reduces its intensity, leading to its weakening. The absorption of ultrasound energy in which ultrasound is spread depends on the characteristics of the medium-density and elasticity. Thus, the ultrasonic signal carries information about the characteristics of the medium between the transmitter and the receiver [15].



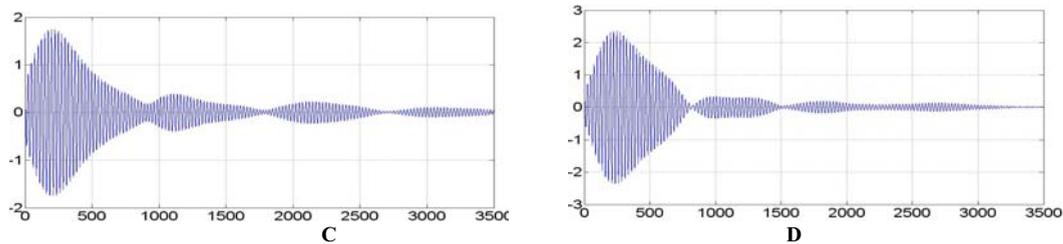


Fig.6. Experimental data from measurements of beverages to control sample-pH 6.59(A), starting point-pH 6.35 (B), beginning coagulation-pH 5.2 (C), end of fermentation-pH 4.5 (D)

4. Conclusions

1. The resulting experimental combinations of beverages enable development and deployment of effective technology-base whey and filtrate, inoculated with kefir grains, in aim rationalization of production processes and expand the range of preventive and medicinal products on market.

2. The dynamics in the decrease of pH value to the filtrate and whey (pH 3.43 - 3.47) is greater than in control milk sample, due to lower buffering capacity.

3. The quantity of residual lactose (4.2 %) allow regulation development of lactic fermentation and in the storage of beverage.

4. A decrease in pH to 168th hour of storage is up to 0.15 units, does not substantially alter the taste characteristics of products.

5. Non-contacts ultrasound method is applied to identification of foodstuff with aim to determine a medium, objects and their production stage in real time.

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