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Microbiological interactions between lactic acid bacteria and saccharomyces cerevisiae brewer's yeast in mixed culture for effective production of a kefir type product

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Abstract

Changes in certain microbiological parametres of a kefir type were studied during the shelf-life of this product. Kefir batches were prepared using mesophilic starter culture, a single strain yeast Debaryomyces hansenii and brewer's yeast, Saccharomyces cerevisiae, which were inoculated in skim milk, incubated at 30ºC and stored at 4ºC. The study was realized during the storage period of product, at the beginning, at the middle and at the end of the validity period. The co-cultivation of these microbiological species caused the inhibition of lactic acid flora by yeast Saccharomyces cerevisiae. The lactic acid flora decreased under the limits which are required by the legislation for kefir while yeasts' numbers increased very much exceeding the limits

Keywords: kefir, microbiological interactions, brewer's yeast, lactococcus

1. Introduction

All In the entire world, the production and consumption of fermented milk products are increasing. Simultaneously, the commercial production and use of lactic acid bacteria (L.A.B.), in the diary-industry, are also increasing. Lactic acid bacteria are now used extensively as starter cultures in the dairy industry and therefore the optimisation of growth conditions appears to be essential for successful industrial applications. Strains belonging to the species *Lactococcus lactis* and *Leuconostoc sp*. are the most important microorganisms in the manufacture of these products at a moderate temperature. Mixed cultures of these bacteria are commonly used as starter in manufacture of cheese and certain fermented milk, like kefir [1].

Kefir is an acid-alcoholic fermented milk; it is traditionally produced by inoculating milk with grains of kefir. Kefir grains are irregularly shaped, gelatinous masses varying in size from 1 to 6 mm diameter. These grains contain lactic acid bacteria (lactobacilli, lactococci, leuconostocs), acetic acid bacteria and yeast mixture coupled together with casein and complex sugars by a matrix of polyssacharide [2]. Yeast is important in kefir fermentation because of the production of ethanol and carbon dioxide. Kefir grains usually contain lactose fermenting yeast (*Kluyveromyces lactis, Kluyveromyces marxianus, Debaryomyces hansenii*) as well as non-lactose-fermenting yeasts (*Saccharomyces cerevisiae*) [3]. But the industrial manufacture of kefir using grains as the starter culture is very difficult due to the complexity of their microbiological composition, which varies widely depending on the origin of the grains and the conditions of storage. Also, is difficult to obtain products with constant characteristics because of the instability of the microbiological composition of the kefir grains during the production of successive batches. The desired characteristics of kefir could be obtained using starter cultures [4]. Several aims of starter cultures' utilisation

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in dairy products are: to produce the lactic acid by fermenting lactose from milk, to prevent the growth of undesirable microorganisms, to provide an acidic aroma and aroma compounds, by proteolitic activity contribute to enhance the flavour and texture of products, to enhance the preservation capacity.

Today's modern technologies have made it possible to produce DVS (Direct Vat Set for direct inoculation of the process milk), cultures consisting of the same strain families as the ones found in some kefir grains and various yeast strains which add specific characteristics to the kefir. The characteristic flavor of kefir products is a result of a complex interaction between the milk matrix and compounds formed during the metabolic activity of the applied bacteria culture and especially the yeast culture.

During the alcoholic fermentation by the yeast CO_2 and ethanol are formed. Both compounds are among others responsible for the characteristic flavor of kefir products. Also, the metabolism in the bacteria culture results in flavor formation. The acidity from lactic acid and the flavor characteristics from diacetyl, acetaldehyde and acetate originate from the metabolism [5]. The bacteria culture converts lactose into lactic acid and into flavor compounds. Diacetyl will be formed from the metabolism of some mesophilic cultures [6]. Furthermore, these mesophilic cultures are able to metabolize citrate. The yeast cultures are responsible for the alcoholic fermentation where ethanol and CO_2 are formed. The lactose fermenting yeasts degrades lactose directly, whereas the non-lactose fermenting yeasts are dependent on lactic acid bacteria to hydrolyze lactose into galactose and/or glucose that is subsequently metabolized by the yeast into ethanol [7]. Debaryomyces hansenii is a yeast strain, which gives a product with a note of butter flavor (diacetyl). The CO_2 content is lowest when choosing this strain compared to the other yeasts.

The aim of this study was to study changes in certain microbiological parametres of a kefir type during the shelf-life of this product. Kefir batches were prepared using mesophilic starter culture, a single strain yeast Debaryomyces hansenii and brewer's yeast, Saccharomyces cerevisiae, which were inoculated in skim milk.

2. Materials and Method

2.1. Manufacturing kefir products

For this study three batches were manufactured in a pilot plant. Skimmed milk with 1.8% fat content was heated at 85-90°C for 30 min in a flash pasteurizer (plates heat exchanger), than was cooled to inoculation temperature 30°C and freeze-dried comercial starter cultures were added. The DVS starter culture contained the following microbial species in unkown proportions:*Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris, Lactococcus lactis* subsp. *lactis,*biovar *diacetylactis, Leuconostoc mesenteroides* subsp.*cremoris*.The second starter culture contained a single yeast strain of *Debaryomyces hansenii.* The yeast culture may be used to provide a balanced flavor and medium to high levels of CO_2 formation in kefir type products. In two batches was added brewer's yeast, *Saccharomyces cerevisiae*, which was drawn from beer's second fermentation process and the yeast's sludge was filtrated and centrifuged. The yeast's inoculation level was 0.8-1% up to the milk quantity. After this, cultures were added, the milk was agitated for 15 min and then distributed in packaging plastic glasses, each of which was sealed with a metal screw lid and then incubated in a thermostatically controlled chamber at 29-30°C for 12 hours. The acidification and coagulation process took place in the packaging cups and then these cups were cooled. Cooling is used to control the metabolic activity of the starter cultures. Cooling of the kefir commences directly. The primary objective of the initial cooling step, when the product is cooled as quickly as possible from incubation to below 25°C, is to control the final acidity of the product. Finally the product is cooled

slowly in the refrigerated storage room at 4~6°C.

2.2. Microbiological analysis

Sterile peptone water at a concentration of 1g/l was used to prepare the dilutions for microbiological analyses. Surface seeding was used in all cases. Lactococci counts were carried out on MRS medium (pH 5.5+_0.2) at an incubation temperature of 30 °C under anaerobic conditions (5% CO_2) for 2 days. Yeasts *Saccharomyces cerevisiae* were grown on WLN medium (pH 5.5+_0.2), a selective medium for this microorganism, at 25°C under aerobic conditions for maximum 5 days. Also, total yeasts and moulds were enumerated in Sabourand dextrose+ chloramphenicol agar medium, after incubation at 22°C for 5 days. The presence of yeasts on yeast-mould specific medium was examined through colony morphology. Two plates were inoculated from each dilution for each sampling point. Samples were taken into propylene boats and were analysed for 24h following inoculation and after storage at 4-5°C for 1,7 and 14 days. After the end of incubation period, only plates containing between 10-300 colonies were randomly taken.

3. Results and Discussion

Counts of lactococci increased considerably during the first 24h of fermentation reaching their maximum values; similar situation was reported by [8]. This situation was correlated with initial pH value, 4.62, which is the optimal value for the casein curdling and with the coagulum aspect. It was used a pH-meter from Hanna Instruments. From then onwards, there was a progressive and marked decrease until the end of storage period. At the last sampling point, the lactococci counts were close to zero in all batches studied.This situation is due probably to the hight concentration of yeast's suspension which was added. Figure1 shows the changes in the microorganism populations during storage period of the kefir type product. Lactococci levels in the first 24h were 108 cfu/ml, which agreed with the legislation and with certain dates from literature. After 48h the lactococci level decreased to 106 and after 7 day to 102. The total yeast population level was 105 cfu/ml in line with other reports. But the Saccharomyces cerevisiae levels, counted on the selective medium, were 104 and progressively increased to 105 after 48h and to 106 after seven day. Mould population levels were 102 cfu/ml on day 14 and there-after increased progressively [9].

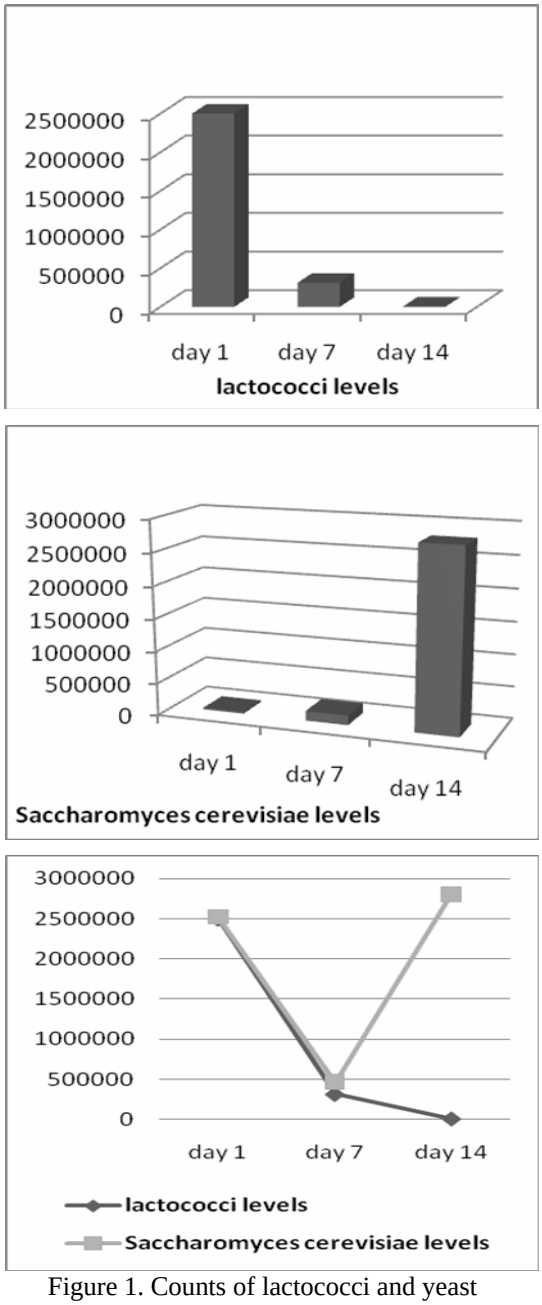

Figure 1. Counts of lactococci and yeast *Saccharomyces cerevisiae* over the storage period

4. Conclusions

Significant variation in quantitative microbial composition of a kefir type product with brewer's yeast occurred in partially controlled cultivation conditions. Variation in microbial abundance was observed for lactococci and yeast. It was evidenced a different growth dynamics for lactococci and yeast during the product's shelf life.

The co-cultivation of these microbial species, at these levels, realised the inhibition of lactococci, probably by the brewer's yeast metabolism. Even if the *Lactococcus* ssp. predominated during the first 24h, their counts decreased under the limits which is required by the legislation for this product (Codex Standard for Fermented Milks CODEX STAN 243-2003).Yeasts,*Saccharomyces cerevisiae,* developed progressively and constantly during the storage period. Yeast's count increased very much and exceed the limit at the end of shelf-life.

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