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SLOVENIAN FERMENTED MILK WITH PROBIOTICS

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ABSTRACT

The concept of functional foods and probiotics is a novelty in former Eastern block. The Croatian and Slovenian dairy industries are the first that launched probiotic. In this work, samples of probiotic fermented milks purchased from Slovenian market were stored within 25 days at three different temperatures (4° C, 20° C, 37° C), and changes in acetaldehyde, ethanol and diacetyl were determined. Above chemical compounds have a high impact on the desired product flavour. At the same time sensory evaluation was carried out. Acetaldehyde and ethanol concentration were determined by aldehyde dehydrogenase and alcohol dehydrogenase methods. Diacetyl was measured by modification of the Hill's colorimetric method. Sensory analysis was conducted by five member panel, using a scoring system with weighted factors in the 20-point scale. The concentration of these flavour compounds changed during storage depending on duration and temperature in depot. Acetaldehyde content decreased at each temperature level during 25-days storage. Diacetyl and ethanol content increased with duration at all temperature levels. Sensoric quality decreased with duration and was closely related to changes in the content of aroma compounds. Changes of all investigated parameters were pronounced on higher temperature levels. Thus, there were no significant changes during the refrigerated storage at 4° C while at the other two temperature levels results pointed out significant differences between the values before and after storage.

Key words: milk / milk products / probiotics / yoghurt / probiotic yoghurt / sensory properties / aroma compounds / Slovenia

SLOVENSKI JOGURTI S PROBIOTIKI

IZVLEČEK

Zamisel funkcionalne hrane in probiotikov je novost v bivšem vzhodnem bloku. Hrvaška in slovenska mlekarska industrija sta prvi v teh državah tržišču ponudili probiotični jogurt. V raziskavi smo vzorce navadnega jogurta s probiotiki slovenskih proizvajalcev skladiščili 21 dni pri treh različnih temperaturah in ugotavljali spremembe v količini acetaldehida, etanola in diacetila. Naštete snovi močno vplivajo na želeno aromo izdelkov. Obenem smo opravili tudi senzorične analize. Koncentracije acetaldehida in etanola smo določili z aldehid dehidrogenazno in alkohol dehidrogenazno metodo, diacetil pa smo merili po modificirani Hillovi kolorimetrični metodi. Senzorično analizo je opravila 5 članska komisija ocenjevalcev po točkovnem sistemu z dejavniki tehtanja in uporabo 20-točkovne skale. Koncentracija aromatičnih snovi se je med skladiščenjem spreminjala odvisno od dolžine in temperature skladišča. Med 21-dnevnim skladiščenjem je pri vseh testiranih temperaturah količina acetaldehida padala, količini diacetila in etanola pa sta naraščali. Senzorična kakovost je med skladiščenjem padala in je bila v tesni povezavi s spremembami v vsebnosti aromatičnih snovi. Spremembe vseh proučevanih

parametrov so bile izrazitejše pri višjih temperaturah. Med skladiščenjem pri 4° C nismo ugotovili značilnih sprememb, medtem ko so bile razlike pred in po skladiščenju pri ostalih dveh temperaturah značilne.

Ključne besede: mleko / mlečni izdelki / probiotiki / jogurti / probiotični jogurti / senzorične lastnosti / aromatske snovi / Slovenija

INTRODUCTION

The newest, and perhaps the most important group of foods in the category of functional foods are probiotic foods. The term probiotics is used to describe the "friendly" bacteria that normally live in the intestinal tract and which contribute to good health. Probiotic food products contain health-promoting bacteria primarily lactic-acid bacteria that have a positive influence on the metabolic activity of the intestinal flora. Today, the best studied and the most extensively documented probiotics are lactic-acid bacteria, a large group of bacteria with the common characteristic of producing lactic acid as the principal end product of metabolism, found in milk and other natural environments (Schaafsma, 1996; Scheinbach, 1998).

The most widely used and recognised functional food is yogurt, a coagulated milk product obtained by specific lactic acid fermentation, through the action of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*.

Except the nutritional and health-promoting aspects, the flavour properties of yogurt are appreciated by consumers. Generally lactic acid gives fermented milks their slightly tart taste. Other typical flavours and aromas are additional results of LAB metabolism. Thus, more than 100 chemical compounds have been isolated from yogurt and other fermented milks but only a few (acetaldehyde, ethanol, diacetyl, acetone and butanone-2) have a high impact on the desired product flavour (Ulberth, 1991; Kneifel *et al.*, 1992; Ulberth and Kneifel, 1992).

Acetaldehyde provides the typical aroma of yogurt. The *Streptococcus* may form acetaldehyde from lactose via pyruvate, but only trace amounts are formed by this way and by *Lactobacillus delbrueckii* subsp. *bulgaricus*. Optimum flavour and aroma are obtained between 23 and 41 ppm acetaldehyde. Diacetyl and acetoin result from metabolic activity of *Streptococcus thermophilus* and are very low, only 0.5 ppm. Diacetyl may be produced also by *Lactococcus lactis* subsp. *lactis biovar.* diacetylactis and *Lactococcus lactis* subsp. *cremoris*. The presence of diacetyl contributes to the delicate, full flavour and aroma of yogurt and is especially important if acetaldehyde is low because it can enhance yogurt flavour. Many starter organisms metabolise acetaldehyde to ethanol. The lack of alcohol dehydrogenase, the enzyme catalysing this reaction, in both *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* makes these starters incapable of metabolising acetaldehyde to ethanol, resulting in the accumulation of acetaldehyde as an end product. Acetoin, acetone, lactones and volatile acids are other important flavours that may be present in certain fermented milks as by-products of metabolism.

Lactobacillus acidophilus, *Bifidobacteria* and *Lactobacillus casei* are considered to be probiotics because their consumption in certain numbers may exert various health benefits beyond inherent basic nutrition. They can be used alone or in association with other lactic acid bacteria for organoleptic or technological reasons. In certain countries, they are included in the starter because of their possible beneficial effects in gastrointestinal tract. *Lactobacillus acidophilus* are obligatory homofermentative facultatively anaerobic rods whose major end-product is lactic acid. The flavour and consistency of milk fermented with this organism are often poor. Therefore, it has been incorporated into mixed starters used for yogurt manufacture. *Lactobacillus acidophilus* normally metabolizes acetaldehyde to alcohol and also utilizes pyruvate in the presence of glucose and produces diacetyl. *Bifidobacteria* differ from lactic acid bacteria in that they produce not only lactic acid but also acetic acid as major fermentation

products. *Lactobacillus casei* are facultatively heterofermentative, and mesophilic. They can ferment a wider range of carbohydrates than most lactobacilli found in fermented milks (Davies and Law, 1984).

The aim of this study was to show changes in concentration of some typical flavour compounds (acetaldehyde, ethanol, diacetyl) and changes in sensory value occuring during storage as a function of duration and temperature in depot.

MATERIAL AND METHODS

In this article samples of a probiotic fermented milks (fermented milk product from pasteurized and homogenized milk with 1.6% milk fat and addition of *Lactobacillus acidophilus*, *Bifidobacteria* and *Lactobacillus casei*) purchased from Slovenian market were stored for up to 25 days at three different temperatures (4° C, 20° C, and 37° C). Samples were analysed every 5 days and changes in acetaldehyde, ethanol and diacetyl content were determined. At the same time sensory evaluation was carried out.

Acetaldehyde and ethanol content were determined by aldehide dehydrogenase and alcohol dehydrogenase method, respectively (Boehringer, 1989). Diacetyl was measured by modification of the Hill's colorimetric method (Hill *et al.*, 1954). Sensory analysis (appearance, consistency, colour, odour, flavour) was conducted by five panel members, using a scoring system with weighted factors in the 20-point scale (ISO, 1985).

RESULTS AND DISCUSSION

The obtained results are summarized in figures 1-4.

Changes in aroma compounds at all temperature levels in depot were shown in figures 1-3. Results of sensory evaluation the same samples were shown in figure 4.

The acetaldehyde content decreased during the storage at all temperature levels as a result of methabolic pathway (citric-acid fermentation) in which diacetyl is formed from it. At 4° C, the decrease of the acetaldehyde concentration was less pronounced as at higher temperatures (Fig 1).

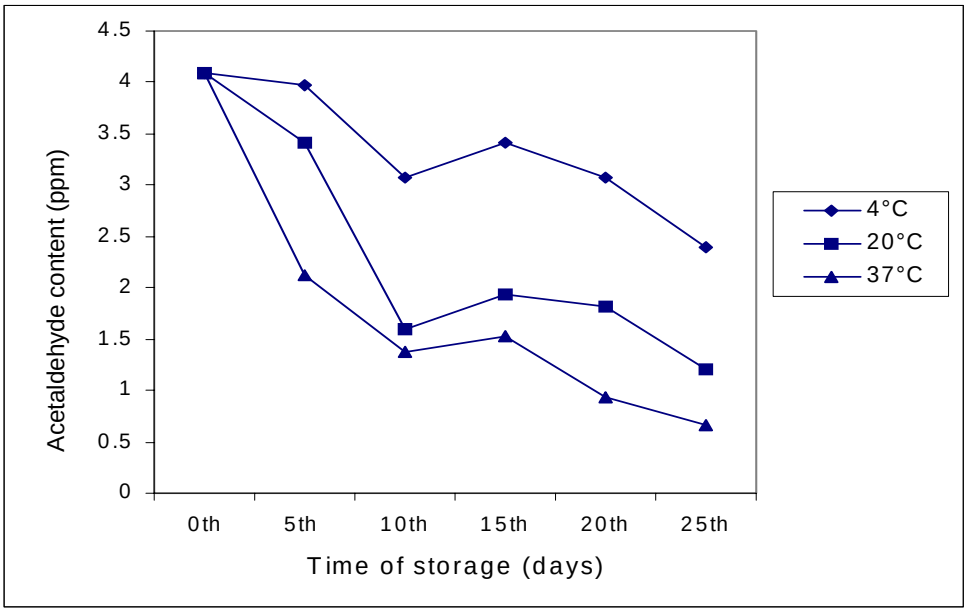

Figure 1. Acetaldehyde content (ppm) in samples during storage at different temperatures.

The results showed the increase in concentration of diacetyl during storage, slightly almost lineary on the lowest temperature (4° C), but significantly on higher temperature levels in depot (Fig 2).

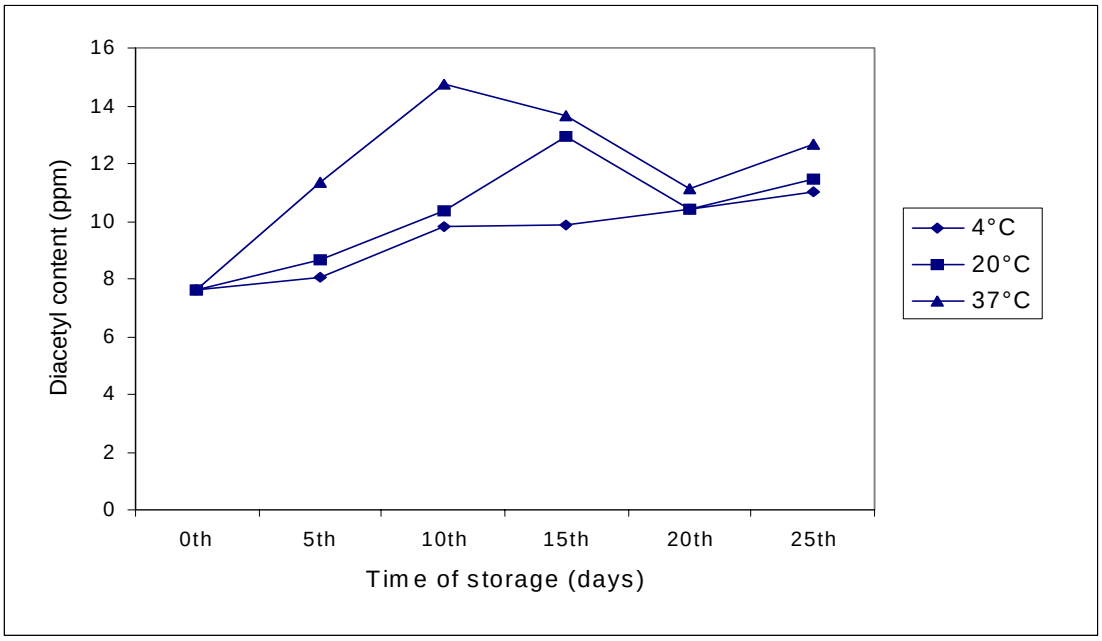

Figure 2. Diacetyl content (ppm) in samples during storage at different temperatures.

The temperature significantly influenced the increase of ethanol concentration and changes were more pronounced during the storage at 20° C and 37° C. After 5, 10 and 15 days of storage, the concentration of ethanol decreased with regard to the storage temperatures as a result of other metabolic processes in which ethanol is a source of carbohydrates (Fig 3).

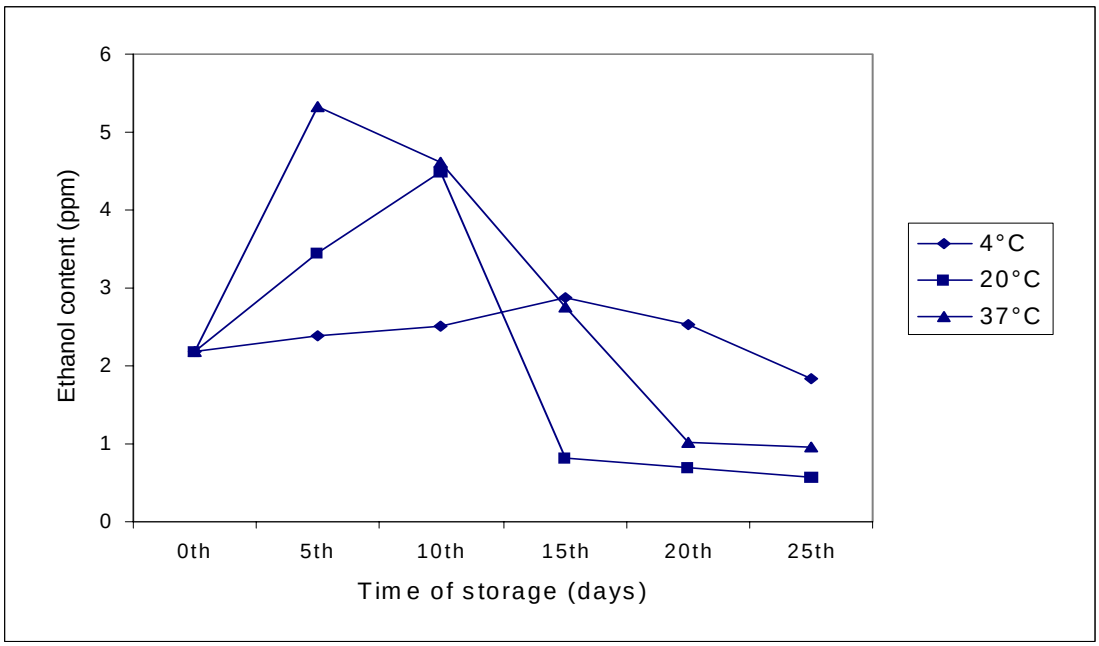

Figure 3. Ethanol content (ppm) in samples during storage at different temperatures.

The sensory scoring pointed out significant changes in quality of fermented milks with probiotics during storage at all temperature levels (Fig 4). These changes were pronounced on higher temperature levels in depot. Thus, samples stored on 4° C and on 20° C were of lower quality after 25 days of storage but still suitable for consuming, while samples stored on 37° C were sensory unsatisfactory after 15 days of storage.

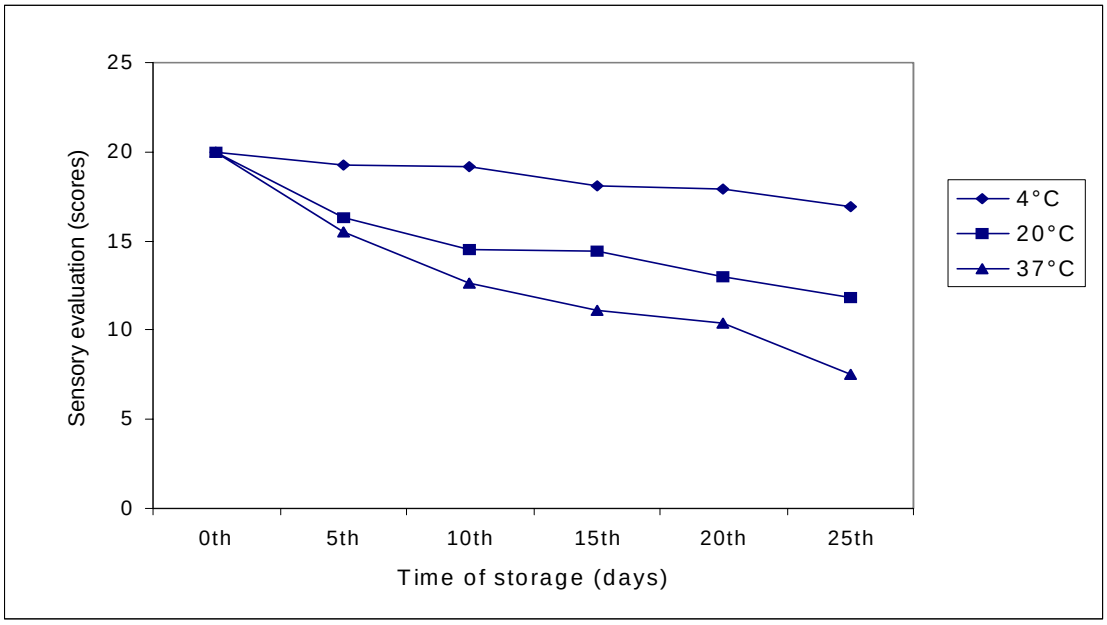

Figure 4. Sensory evaluation (scores) of samples during storage at different temperatures.

It is well known that different starter microorganisms influence the flavour as well as the texture of the final product. Inter relationships between multi-species are not easily studied as numerous researchers pointed out (Tamime and Robinson, 1985). Different acetaldehyde concentrations of yogurt and fermented milks can be found in the literature. Data reported rang from less than 10 ppm up to more than 40 ppm (Kneifel *et al.*, 1992; Schmidt *et al.*, 1983). Although acetaldehyde was the main aroma compound samples of fermented milks with probiotics with the alcetaldehyde concentration about 4.1 ppm at the begining of the storage was sensory described as exellent. Diacetyl and ethanol is normally produced in small amounts (Ulberth, 1991; McGregor and White, 1987). The relatively high diacetyl content, from 7.61 ppm at the beginning to 12.65 ppm at the end of the storage of investigated samples, may have substituted in part for the lacking acetaldehyde.

CONCLUSIONS

Based on the obtained results the following conclusions could be made.

Concentration of acetaldehyde decreased, while concentrations of diacetyl and ethanol increased in samples of fermented milk with probiotics during the storage at three temperature levels (4° C, 20° C, 37° C).

Variations in the concentration of the measured compounds (acetaldehyde, diacetyl and ethanol) were mainly a result of the relevant catabolic pathways of lactose breakdown. It is important to consider that not all of the pathways are common to all of the microorganisms involved in the fermentation of milk.

Temperature level in depot significantly influenced the decrease of acetaldehyde content and increase of concentration of ethanol and diacetyl especially at higher temperature levels (20° C, 37° C).

Sensory evaluation pointed out significant changes in the quality. Changes were pronounced during the storage at higher temperature levels. Thus, samples were not suitable for consuming after 15 days of storage at 37° C.

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