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ORIGINAL ARTICLE

EFFECT OF PREBIOTIC AND STORAGE TIME ON THIAMINE AND RIBOFLAVIN CONTENT IN THE MILK DRINKS FERMENTED BY *LACTOBACILLUS CASEI* KNE-1

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ABSTRACT

Background. Fermented milk drinks are unique products due to content of *Lactobacillus* and *Bifidobacterium* that are recognized as probiotics. They are a natural component of the colon microbiota as well as commonly used probiotics in functional food.

Objectives. The effects of the storage time and prebiotic type (inuline or oligofructose) were studied in banana-milk drink after fermentation by *Lactobacillus casei* KNE-1 on the thiamine and riboflavin concentrations.

Material and methods. The material for the study was fermented fruit milk drinks: banana-milk prepared in laboratory conditions and fruit milk drinks purchased in a local shop, as a comparative material. The thiamine was determined by thiochrome method and the riboflavin was determined by fluorometric method.

Results. The storage time after the end of the fermentation process did not increase the content of thiamine and riboflavin in fermented banana-milk drink more than the output level. The addition of oligofructose significantly affected the synthesis of thiamine by *Lactobacillus casei* KNE-1 irrespectively of the storage time. The storage time but not the type of prebiotic affected the riboflavin concentration. Taking into account the highest content of both vitamins, the banana-milk drink fermented by *Lactobacillus casei* KNE-1 should be consumed immediately or 24 days after fermentation.

Conclusions. This information could be used by manufacturers for the planning of technological process. The content of thiamine and riboflavin in the fermented milk drinks is the result of the type of prebiotic, the individual bacterial strain properties as well as the storage time. These factors should be investigated to optimize the content of B vitamins in fermented milk drinks in the future.

Key words: *fermented drinks, Lactobacillus casei KNE-1, riboflavin, thiamine*

STRESZCZENIE

Wprowadzenie. Mleczne napoje fermentowane są produktami unikalnymi ze względu na zawartość bakterii probiotycznych rodzaju *Lactobacillus* i *Bifidobacterium*. Są one naturalnym składnikiem mikroflory okrężnicy, jak również powszechnie stosowane w żywności funkcjonalnej.

Cel badań. Celem badania była analiza wpływu czasu przechowywania i rodzaju dodanego prebiotyku (inuliny i oligofruktozy) na zawartość tiaminy i ryboflawiny w mlecznym napoju o smaku bananowym fermentowanym *Lactobacillus casei* KNE-1.

Materiał i metoda. Materiałem do badań były mleczne napoje fermentowana o smaku bananowym wytworzone w warunkach laboratoryjnych oraz fermentowane napoje mleczno-owocowe zakupione w handlu detalicznym, jako materiał porównawczy. Tiamina została oznaczona metodą tiochromową, a ryboflawina metodą fluorymetryczną.

Wyniki. Czas przechowywania po zakończeniu procesu fermentacji nie wpłynął na zwiększenie zawartość tiaminy i ryboflawiny w fermentowanych napojach o smaku bananowym w stosunku do poziomu wyjściowego. Dodanie oligofruktozy miało istotny wpływ na syntezę tiaminy przez *Lactobacillus casei* KNE-1, bez względu na czas przechowywania. Czas przechowywania, ale nie typ dodanego prebiotyku wpływał na koncentrację ryboflawiny. Biorąc pod uwagę najwyższą zawartość obu witamin w mlecznym napoju fermentowany przez *Lactobacillus casei* KNE-1, produkt ten powinien być spożywany natychmiast lub po 24 dniach fermentacji.

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Wnioski. Wyniki naszych badań mogą być wykorzystywane przez producentów do planowania procesu technologicznego. Zawartość tiaminy i ryboflawiny w mlecznych napojach fermentowanych jest skutkiem właściwości poszczególnych szczepów bakteryjnych, jak i przechowywania. Czynniki te powinny być poddane dalszym badaniom w celu ich optymalizacji na zawartość witamin z grupy B w mlecznych napojach fermentowanych.

Słowa kluczowe: *napoje fermentowane, Lactobacillus casei KNE-1, ryboflawina, tiamina*

INTRODUCTION

In a typical human diet milk and milk products are one of the major sources of protein, calcium, B vitamins, vitamin A and D. Fermented milk drinks are unique products due to content of *Lactobacillus* and *Bifidobacterium* that are recognized as probiotics. They are a natural component of the colon microbiota as well as commonly used probiotics in functional food [11].The most important benefits of *Bifidobacterium* and *Lactobacillus* are the inhibition of pathogenic microorganisms, reduction of serum cholesterol level, prevention of cancer, enhancement of the immune system, improvement of lactose digestion as well as the synthesis of B vitamins (thiamine, riboflavin, niacin and folate) [7, 13, 16]. Food-grade bacteria make it possible to fortify some raw food products with B vitamins by natural process. On the other hand the levels of some vitamins can be reduced during fermentation as they are absorbed by lactic acid bacteria. This process concerns thiamine, riboflavin, pyridoxine, pantothenic acid and cobalamin, and its rate is dependent on the type of bacterial strain and the conditions of fermentation [15]. It has been shown that the simultaneous application of pre- and probiotics can efficiently enhance these effects [3]. Additionally prebiotics as dietary supplements increase the content and proportion of bifidobacteria and improve an absorption of nutrients, synthesis of vitamins, prevent constipation, colon cancer and exert positive effect on blood sugar and lipid profile [8, 10]. Combination of pre- and probiotic as a synbiotic can improve the survival of the probiotic strains and prolong their beneficial effect in human gut [4]. The probiotic bacteria (*Lactobacillus and Bifidobacterium*) are the producers of B vitamins so that food fermented by them can be the additional source of these vitamins. Moreover, prebiotic supplements intensify bacterial growth and thus vitamin synthesis in the gut as well as in foods. The species of *L. casei* has a good stability in the conditions of the human gastrointestinal tract and its probiotic properties may be intensively used in the dairy industry [12]. To meet the expectations of consumers, manufacturers offer dairy products of different flavours as well as functional additives, including pro- and prebiotics, whose primary role is beneficial effect on human health [5]. Prebiotics are the selectively fermented ingredients that allow specific changes, both in the composition and/or activity of the microflora of the gastrointestinal tract, thereby improving the health and well-being of the organism [9]. The best documented prebiotic properties have inulin and oligofructose, what was shown in human and animal studies [6, 17]. These compounds are used as functional additives, stimulate the growth of probiotics and may contribute to increase of the content of B vitamins in fermented milk drinks. Therefore the aim of this study was to investigate the effect of various types of prebiotics on the contents of thiamine and riboflavin during storage of the banana-milk drink fermented by *Lactobacillus casei* KNE-1 strain.

MATERIAL AND METHODS

The material for the study was fermented fruit milk drinks: banana-milk prepared in laboratory conditions and fruit milk drinks purchased in a local shop, as a comparative material.

Experiment 1 – Fermented banana-milk drink was prepared in Department of Food Gastronomy and Food Hygiene, WULS, Poland [14]. The following materials were used in the process: banana nectar (pasteurized, prepared on the basis of the concentrate, sweetened); 0.5% fat UHT milk, the probiotic strain *Lactobacillus casei* KNE-1 (Institute of Fermentation Technology and Microbiology, Lodz University of Technology, Poland). Oligofructose and inulin with sucrose added were used as prebiotics. Oligofructose (5% added) RAFTILOSE P95 (ORAFTI, Belgium) and inulin with sucrose (5% added) FRUTOFIT (Sensus, the Netherlands). Sucrose was added for taste enhancement [14]. After the fermentation process (12 hours, 37^0C) products were stored for 32 days in 10^0C. The samples were collected immediately after fermentation (day 0) and then after 1, 8, 16, 24 and 32 days of storing to determine the content of thiamine and riboflavin.

Experiment 2 – Commercially available fruit milk drinks fermented by *L.casei* and "yoghurt bacteria" (as named by manufacturer) were used to compare contents of thiamine and riboflavin. These were drinks produced by one company in five different flavours: natural, strawberry, raspberry and cranberry, multi fruit, peach and honey.

The thiamine was determined by thiochrome method with the modification of Soliman [19]. The procedure involved release of thiamine from phosphate binding protein by the enzymatic hydrolysis using Taka-Diastase (Sigma 86247). Next step was thiochrome formation in the reaction with potassium hexacyanoferrate (II) (Chempur, Poland) under alkaline conditions. The procedure was carried out in two parallel samples, in one of which thiochrome formation was inhibited by the addition of benzenesulfonyl chloride (Aldrich 108138) to eliminate column purification [1]. The intensity of the fluorescence of isobutyl alcohol solutions was measured at the maximum excitation and emission wavelengths of 365 nm and 435 nm, respectively. The riboflavin was determined by fluorometric method [2]. After the extraction of vitamin and the oxidation of the interfering substances the fluorescence of riboflavin was measured at the maximum excitation and emission wavelengths of 365 nm and 435 nm, respectively. The fluorescence measurements were carried out using photofluorometer (KONTRON INSTRUMENTS) connected to computer with SFM 25 software. Analysis of the vitamins was repeated 3 times: in experiment 1 – every day of the fermentation; in experiment 2 – for every flavour of the commercial drink.

Data was analyzed using Statistica software version 10 (StatSoft, Poland). The results were presented as the mean ± standard deviation (SD). Data with a normal distribution were subjected to one-way analysis of variance (ANOVA) to assess the potential statistical significance. In addition, two-way ANOVA analysis was done in which the classification factors included time of storage and prebiotic. The Fishers LSD test was used to compare the means between groups. The results with p-values ≤ 0.05 were considered as statistically significant.

RESULTS

The thiamine and riboflavin contents in the banana-milk drink have changed significantly during the storage (Table 1). The length of the storage time significantly influenced the content of vitamins in drink with inulin added as well as with oligofructose added ($p < 0.001$). The type of prebiotic affected only the content of thiamine ($p < 0.05$), which was higher in the drink with oligofructose added. The growth of lactic acid bacteria should result in an increase vitamin production during fermentation. However, the nutrients present in the environment are used by bacteria for growth, therefore nutrient content may vary.

Based on the results of the study of fruit milk drinks available on the Polish market, obtained in the second experiment, there was the highest amount of (17.4 ± 0.98 µg/100 g) and riboflavin (306 ± 6.34 µg/100 g) in a fermented beverage of natural flavour (Table 2). In contrast, the least amount of thiamine and riboflavin were found in fermented multi fruit milk drink (13.3 ± 1.02 µg/100 g; 262 ± 2.99 µg/100 g, respectively). Commercial drinks contained similar amounts of riboflavin, but much more thiamine than tested banana-milk drink.

Table 2. The content of thiamine and riboflavin in the commercial fermented fruit milk drinks

Flavour	Thiamine (µg/100 g)	Riboflavin (µg/100 g)
Natural (n=6)	17.4 ± 0.98	306 ± 6.34
Strawberry (n=6)	17.3 ± 1.10	290 ± 2.97
Raspberry and cranberry (n=6)	14.4 ± 0.81	286 ± 3.05
Peach and honey (n=6)	13.9 ± 0.78	276 ± 4.04
Multi fruit (n=6)	13.3 ± 1.02	262 ± 2.99

DISCUSSION

The length of the storage time significantly influenced the content of vitamins in drink with inulin added as well as with oligofructose added. The type of prebiotic affected only the content of thiamine, which was higher in the drink with oligofructose. Previous results showed

Table 1. The content of thiamine and riboflavin in a banana-milk fermented drink depending on the type of prebiotic and storage time

Storage time (days)	Thiamine (µg/100 g)		Riboflavin (µg/100 g)	
	Inulin	Oligofructose	Inulin	Oligofructose
0	10.9 ± 0.03$^{a*(a)**}$	9.82 ± 0.05$^{a(b)}$	321 ± 1.80^{A*}	303 ± 0.63^{E}
1	8.61 ± 0.02$^{a(a)}$	12.8 ± 0.01$^{a(b)}$	279 ± 4.71^{B}	308 ± 0.75^{B}
8	6.53 ± 0.02$^{c(a)}$	6.43 ± 0.02$^{c(b)}$	233 ± 0.59^{C}	231 ± 1.20^{C}
16	4.62 ± 0.04$^{c(a)}$	6.81 ± 0.03$^{c(b)}$	305 ± 3.10^{D}	248 ± 0.80^{D}
24	9.71 ± 0.07$^{a(a)}$	10.5 ± 0.02$^{a(b)}$	272 ± 6.1^{E}	311 ± 0.73^{E}
32	7.23 ± 0.08$^{b(a)}$	9.51 ± 0.04$^{b(b)}$	263 ± 0.68^{F}	229 ± 3.72^{F}
Two-way ANOVA				
Storage time	$p < 0.001$		$p < 0.001$	
Prebiotic	$p < 0.05$		NS	

Thiamine: $^{*a-c}$ - statistically significant differences between means marked with different letters in the columns ($p \geq 0.05$); $^{**(a-b)}$ – statistically significant differences between means marked with different letters in rows ($p \leq 0.05$).
Riboflavin: $^{A-F*}$ - statistically significant differences between means marked with different letters in the columns and rows ($p \leq 0.05$); NS – no statistical differences ($p > 0.05$).

that some starter cultures that are able to produce B vitamin, most probiotic strains of lactobacilli, consume these vitamins and thus decrease their contents [15].

Studies of the same banana-milk drink with oligofructose, *Jalosińska* have shown that the number of bacteria kept increasing until day 16 of storage (after fermentation) and stay stable on the similar level until day 24 [14]. In the presence of inulin, such differences were not observed. However, there was a similar number of *Lactobacillus casei* KNE-1 in the drinks between day 16 and 24 of storage regardless of the type of prebiotic. There was an increase of thiamine in the beverages with inulin or oligofructose at that time, but the riboflavin - only in product with oligofructose. On day 24 of storage at 10°C synthesis of both vitamins reached optimum level. On that day a high content of thiamine and riboflavin was observed for both prebiotics, which even exceeded the output level in beverage with oligofructose. This proves that fermented banana-milk drink was the best source of thiamine and riboflavin after about 3 weeks of storage. In similar studies *Beitane* and *Ciprovica* [3], using a *Bifidobacterium lactis* (Bb-12) and inulin, have shown a greater amount of thiamine and smaller amount of riboflavin than shown in this study in the same day (day 0), immediately after 16 hours of fermentation. This may suggest better efficiency of the *Bifidobacterium* strain than *Lactobacillus* in the production of thiamine, and the inverse relationship for the riboflavin. Although *Bifidobacterium* and *Lactobacillus genera* are both considered to be efficient producers of B vitamins, it is specific characteristics of individual strains that have recently become a subject of interest [20]. What is more the complete pathway for thiamine production has been predicted only in *L. reuteri* ATCC 55730 and ATCC PTA 6475 [18]. Depending on the type of prebiotic, fermentation time and storage time the amount of thiamine and riboflavin in fermented milk beverages can vary significantly.

Commercial drinks contained similar amounts of riboflavin, but much more thiamine than tested banana-milk drink. Differences could arise primarily from the presence of other bacterial species than *L. casei*, time since the end of fermentation and storage conditions that were not precisely known here. Based on the survey, it was found that the fermented beverage with natural flavor can be the best source of thiamine and riboflavin, among the analyzed commercial beverages.

CONCLUSIONS

Storage time after the end of the fermentation process had no effect on increasing the final amount of thiamine and riboflavin in fermented fruit milk drinks over the output level.

Oligofructose, more than inulin, stimulated the synthesis of thiamine by *Lactobacillus casei* KNE-1, in most days of storage. The type of prebiotic was not significant for the synthesis of riboflavin. The banana-milk drink fermented by *Lactobacillus casei* KNE-1 should be consumed right after fermentation process has been completed, or about 24 days later due to the highest content of thiamine and riboflavin at this time. This seems to be important information for manufacturers and can be used in food labeling. The content of thiamine and riboflavin in milk fermented beverages is the result of the type of prebiotic, the individual characteristics of the bacterial strain and the storage time. These factors should be tested in the future to optimize B vitamin content in this type of drinks.

Conflict of interest

The authors declare no conflict of interest.

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