Assessment of Vitamin B2 and B3 Contents of Conventional and Probiotic Yogurt after Refrigeration by High Performance Liquid Chromatography

Haniye Tolouie [1], Seyed Hamid Hashemi Toloun [1], Haniyeh Sadat Ejtahed [2], Hamide Jalaliani [3]

[1]M.Sc of Food Science, Tabriz University of Medical Sciences Students Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[2] M.Sc of Nutrition , Tabriz University of Medical Sciences Students Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[3]M.Sc of Food Technology, Amol Azad University, Amol, Iran

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Correspondence to: Seyed Hamid Hashemi Toloun

Address: Tabriz University of Medical Sciences Students Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

Email: hashemi3x@gmail.com

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ABSTRACT

BACKGROUND: Milk and dairy products are natural sources of B group vitamins in the human diet. This study was carried out to compare the ability of probiotic and conventional microorganisms of yogurt to influence the status of vitamin B2 (riboflavin) and vitamin B3 (niacin) during refrigeration.

METHODS: After preparation of conventional and probiotic yogurt, all samples were stored at 4°C, and the content of B2 and B3 vitamins was assessed on first, third and seventh day of refrigeration. Vitamins B2 and B3 were analyzed in samples using high-performance liquid chromatography followed by fluorescence detection and ultraviolet detection, respectively. Data was analyzed using SPSS version 18 (SPSS Inc., Chicago, USA).

RESULTS: Vitamin B2 content of probiotic yogurt samples was significantly higher than the control samples on all experiment days ($P < 0.05$). Significant differences between content of vitamin B3 in conventional and probiotic yogurt were found only on the seventh day of refrigeration ($P < 0.05$).

CONCLUSION: This study showed that using probiotic strains from yogurt manufacturers might provide higher B2 and B3 vitamin content provided yogurt is refrigerated for storage.

Key Words: Probiotic yogurt; Vitamin B2; Vitamin B3

INTRODUCTION

In recent decades, there has been a growing interest in using probiotic micro-organisms as dietary adjuncts. This growth is fueled by technological innovations, development of new products and the increasing number of health-conscious consumers who are interested in products that improve quality of life [1, 2]. Probiotic products are usually defined as live microbial food supplements with beneficial effects on the consumers when administered in adequate amounts [3, 4]. Most probiotics fall into the group of organisms known as lactic acid-producing bacteria and are usually consumed in the form of yogurt, fermented milk or other fermented foods. Lactobacillus and bifidobacterium are the most common types of microbes used as probiotics [5]. In vitro studies with probiotic microorganisms indicate that some species are able to generate several water-soluble vitamins, such as from the vitamin B group and therefore, yogurt can be a good source of these micronutrients [6]. However, whether lactobacilli or other viable bacteria release the synthesized vitamins or utilize vitamins from their surroundings during refrigerated storage is a matter of debate.

Vitamin B2 (riboflavin) is an important vitamin which acts as an intermediary in the transfer of electrons in biological redox reactions and has an important function in cell growth [7]. However, vertebrates, including humans, lack this ability and must therefore obtain this vitamin from their diet [8]. Considering the high concentrations of B complex vitamins present in milk and dairy products, these foods are the best natural sources of vitamin B2 in the human diet [9]. Milk is also a good source of vitamin B3 (niacin). Vitamin B3 is used in the body to form nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). Vitamin B3 deficiency results in pellagra which manifests as dermatitis, diarrhea and depression [10]. This study was carried out to investigate whether probiotic microorganisms are able to influence the status of vitamin B2 and B3 in probiotic yogurt as compared with traditional yogurt during refrigerated storage in developing countries [6, 7, 8].

METHODS AND MATERIALS

Yogurt preparation: Conventional yogurt (2.5% fat) was prepared with *Streptococcus thermophilus* and *Lactobacillus Delbruki*. Yogurt was prepared by preheating milk up to 55˚C while stirring with a mixer. Milk was pasteurized in a stainless steel container at 90˚C for 10 min, with constant stirring. The samples were cooled to 42˚C before inoculation. Conventional yogurt starter culture (Hansen, Denmark) was added to milk (2% v/v), and fermented until a pH of 4.6 was achieved. Probiotic starter culture also included *Lactobacillus acidophilus* and *Bifidobacterium lactis*.

Analysis of B2 and B3 content in yogurt: Vitamin B2 and B3 content of samples was determined during 7 days of refrigerated storage (on days 1, 3 and 7). Vitamin B2 levels were analyzed by high-performance liquid chromatography after acid hydrolysis to precipitate proteins followed by centrifugation and decantation [11]. Homogenised yogurt was poured in a test tube and then acetic acid was added. The sample was mixed vigorously and was incubated at room temperature for 30 min. After acid digestion, the mixture was centrifuged twice. Then, centrifuged solution was diluted to suitable a mobile phase. Vitamin B2 content was determined according to HPLC method reported by Gatti and Gioia (2005) [12] followed by using fluorometric detection (λem = 524 nm with λex = 370 nm). All the reagents including buffers and solvents were HPLC grade. Vitamin B3 content was determined by first hydrolyzing the samples with 0.2M sulfuric acid to liberate nicotinic acid from its combined form. Food samples were digested in duplicate fashion with sulfuric acid followed by enzymatic digestion according to the method of Nobile et al. (1972) and Geoffrey and Skurray (1981) with slight modification [13, 14]. An aliquot of the digest was mixed with 15% NaOH solution and one drop of 1% potassium ferricyanide solution (freshly prepared). The samples were filtered through a 0.25 mm filter. The aliquot of the sample was applied to a C18 column (Perfectsil Target ODS-3 C18, 250*4.6mm, 5μm) and eluted with degassed 0.2M acetate buffer containing 0.005M heptane sulphonic acid. Recovery samples were prepared by adding known amounts of vitamin B3 and vitamin B2, prior to extraction of the sample. A flow rate of 1 ml/min was obtained using a high-performance liquid chromatograph with a 262 nm ultraviolet detector (WellChrom K-120, Germany).

Statistical analysis: Data was analyzed using SPSS 18.0 (SPSS Inc., Chicago, IL, USA). Independent sample t- test was used to compare vitamin B2 and B3 in conventional and probiotic yogurts. Analysis of variance (ANOVA) was performed to determine significant differences between day groups with post hoc test. $P< 0.05$ was considered as significant. On each day we took seven samples from each lot and measured the content of the vitamins.

RESULTS

The average content of vitamin B3 of the two types of yogurt was similar on first day of fermentation (1.90 μg/g and 2.03 μg/g). We found significant differences in vitamin B3 between conventional and probiotic yogurt on 7th day of refrigeration *($P< 0.05$)* (Table 1).
Vitamin B2 content of the probiotic yogurt samples was significantly higher than the conventional yogurt samples on all experimental days *($P< 0.05$)* (Table 1).

DISCUSSION

The present study showed that vitamin B2 and B3 content of probiotic yogurt was higher than conventional yogurt, but the differences for vitamin B3 content were present only at the 7th day of refrigeration. The nutritional effects of probiotics such as increasing levels of some vitamins by fermentation have been shown previously, specially using the lactic acid-producing bacteria like: *Lactobacillus acidophilus* and *bifidbacteria* [15-17]. Similar to our study, increased levels of vitamin B2 and B3 in conventional yogurt by fermentation was reported by Alm 1982 [16] and Parvez et al.,

Table 1: Vitamin B2 and B3 contents of conventional and probiotic yogurts during refrigerated storage

Yogurt types a	Pro	Con	Pro	Con	Pro	Con
	1st Day	1st Day	3rd Day	3rd Day	7th Day	7th Day
Vitamin B2 (µg/g)	2.73 ± 0.08 b*	2.51 ± 0.07	3.36 ± 0.09 *	2.73 ± 0.16	3.42 ± 0.09*	2.58 ± 0.19
Vitamin B3 (µg/g)	2.03 ± 0.03	1.90 ± 0.09	2.36 ± 0.13	2.15 ± 0.08	2.48 ± 0.08*	2.08 ± 0.10

a Pro and Con are probiotic and conventional yogurts, respectively.
b Values presented as means ± SD.
* Significant difference between two groups on the same day (P< 0.05).
** On each day, seven samples were taken from each lot.

2006 [2]. The probable underlying mechanism is that plain yogurt and sour milk contain some derivatives of B2 which are identified as riboflavin-B-D-galactoside [18]. Riboflavin-B-D-galactoside in plain yogurt is a product of the action of specific yogurt microorganisms: *Lactobacillus delbrueckii*, subspecies *Bulgaricus* and *Streptococcus salivarius*, subspecies *Thermophilus*. Glycosides of vitamin B2 are formed by incubation of B2 and appropriate sugar donor with cultures or enzymes from different microorganisms [11].
Fabian et al. (2001) reported that daily consumption of both probiotic and conventional yogurt for 2 weeks increased plasma concentrations of vitamin B1 (thiamine) and free B2 vitamin in healthy women [19].
We observed that content of B3 and B2 vitamins in probiotic yogurt was higher than conventional yogurt. Accordingly, LeBlanc *et al*. reported that using lactic acid-producing bacteria, one of the most important groups of microorganisms used in the food industry, could be useful in the production of novel vitamin-enriched products [20]. Exposure to light, especially to wavelengths below 500 nm, as well as the presence of oxygen can destroy vitamin B2 in dairy products [21, 22]. Saidi and warthesen observed greater vitamin B2 photostability in fermented-milk than unfermented milk [23]. Moreover, Rekha and Vijayalakshmi reported an increase in B2 and B3 vitamin content in fermented soymilk by probiotic microorganisms [24]. They have also suggested that the growth of probiotic yeast in association with probiotic bacteria can enhance the viability of probiotic lactic acid-producing bacteria strains. Improvement in vitamin B2 content of fermented soymilk was also reported by Hou et al. [25]. Hailong and Liang reported that the content of B1, B2 and B3 vitamins was increased when soymilk was fermented with the *basidiomycete Ganoderma lucidum* [26].
The combination of high-performance chromatography, ultraviolet and fluorescent detection has the advantage of speed, sensitivity and selectivity in determining vitamin B2 and vitamin B3 levels in food and this was our study's major strength [14]. However, the major limitation of this study was lack of control on the internal factors of yogurt samples such as dissolved oxygen and hydrogen peroxide content. As previously reported by Dave and Shah, the viability of *Lactobacillus* acidophilus was affected by the presence of *Lactobacillus delbrueckii*, subspecies *bulgaricus*, whereas *bifidobacteria* exhibited better stability in the yogurt prepared from cultures that contained *Lactobacillus delbrueckii*, subspecies *Bulgaricus* [27]. Future studies should focus on research around B2 and B3 vitamin production by using different strains for fermentation and changing fermentation conditions.

CONCLUSION

In summary, we show that using probiotic strains for yogurt preparation is associated with higher amounts of B2 and B3 vitamins in the yogurt. In general, probiotic yogurt is a better source of these vitamins than conventional yogurt.

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