DEFICIENCY OF VITAMINS IN DAIRY PRODUCTS AND SUPPLEMENTATION IN YOGURT BY ADDING THE BIOACTIVE NATURAL PRODUCTS

Drd.ing. Anca-Maria TIFREA
Faculty of Agricultural Sciences ,Food Industry and Environment Protection , "Lucian Blaga" University of Sibiu

Ph.D.eng. Ovidiu TITA
Faculty of Agricultural Sciences ,Food Industry and Environment Protection
„Lucian Blaga" University of Sibiu

Drd.eng. Alina-Florentina DAN
Faculty of Agricultural Sciences ,Food Industry and Environment Protection , "Lucian Blaga" University of Sibiu

REZUMAT. Iaurtul are un nivel scăzut de vitamine mai ales în comparaţie cu laptele. Conţinutul acestor vitamine se schimbă pe parcursul procesului de fabricaţie din mai multe motive. În acest studiu se va fortifica iaurtul cu compuşi bioactivi din cătină albă. Motivul pentru care nivelul de vitamine scade in iaurt este excesul de oxigen dizolvat şi/sau un tratament termic moderat de lapte poate reduce în mod semnificativ conţinutul de vitamine, cele mai susceptibile vitamine fiind C, B6, B12, PP. Iaurtul cu cătină albă conţine o serie de compuşi care furnizează elemente critice nutriţionale, imunologice de protecţie şi substanţe biologic active, atât nou-născuţilor cât şi adulţilor, prin urmare, atunci când acestea sunt suplimentate cu probiotice, ele intră, în cele din urmă, în domeniul de alimente funcționale. În plus, alimentele lactate fermentate oferă funcţionalitate fiziologică, reprezentată de activitatea biologică intrinsecă a microorganismelor folosite.

Cuvinte cheie: vitamine, iaurt, cătina, lapte, nutriție, produse naturale bioactive.

ABSTRACT. Yogurt has a low level of vitamins and especially in compared with milk. The content of these vitamins changes during manufacture due to several reasons. In this study it will fortify yogurt with bioactive compounds in sea buckthorn. The reason why the level of vitamins decreases in yogurt is an excess of dissolved oxygen and/or a moderate heat treatment of milk can reduce significantly vitamins content and the most susceptible vitamins are C,B6, B12,PP.Yogurt with sea buckthorn contains an array of compounds that provide critical nutritional elements, immunological protection, and biologically active substances to both neonates and adults; therefore, when they are complemented with probiotics, they eventually fall within the realm of functional foods. Furthermore, fermented dairy foods provide physiological functionality, which is accounted for by the intrinsic biological activity of the microorganisms employed.

Keywords: vitamins, yogurt, Sea Buckthorn, milk, nutrition, bioactive natural products.

1. INTRODUCTION

Yogurt has been consumed since recorded time. It is not exactly known how yogurt was discovered, but it is assumed that it was by accident, perhaps by Mesopotamians in about 5000 BC (Kosikowski & Mistry, 1997). These people also eventually realized the health benefits of eating yogurt, and much later, some observers wrote about living a longer and healthier life as a direct result of frequent consumption of the fermented products (Andrews, 2000)

The fortification of yoghurt with vitamins, such as vitamins A or C, is possible

(Anonymous, 1997), and losses over two weeks in storage are unlikely to exceed 50%, since low fat yoghurt is very popular in many countries, fortification with vitamin A should become mandatory in order to maintain the nutritive value of milk.

Mineral and vitamins deficiencies prevent as many as a third of the world's people from reaching their physical and mental potential. Vitamin A deficiency compromises the immune system of approximately 40% of the developing world and leading to the deaths of approximately 1 million young children each year (Beaton, Martorell, L'abbe, Edmonston, & McCabe, 1992).

Vitamins are very interesting compounds, because of their high biological activity abundance in natural and synthetic food matrices and because of the indispensable importance of their properties.

Simultaneous separation of water and fat-soluble vitamins is a difficult problem. High-performance liquid chromatography(HPLC) is the most popular method for determining vitamins (Eitenmiller, Ye, & Landen, 2008). However, this method requires large amounts of sample and mobile phase and expensive stationary phases, and difficulties arise in the simultaneous determination of hydrophilic and hydrophobic compounds.

2. MATERIALS AND METHODS

Yogurt with Sea Buckthorn is prepared in laboratory and the water soluble vitamins and fat vitamins are used for analysis HPLC Knouer which is described below.

I chose more technology schemes to ensure fresh sea buckthorn fruit for yogurt and maintain stability of vitamins in viable state as long is possible.

That scheme involves the preparation of yogurt at the end of thermostatic adding fruit, because the most vitamins are destroyed during pasteurization and thermostatic..

Yogurt was obtained in the laboratory with the thermostat regime more time and temperature ranges under discussion and results, adding fruits at the end to ensure stability of the final product. I made several attempts on the form of addition of sea buckthorn.

Equipment: The RP-HPLC analyses were performed using an HPLC SMB OSMOMETRY KNAUER system (Advanced Scientific Instruments Wissenschaftliche Geratebau GmbH, Berlin, Germany). The HPLC system consisted of a pump (Smartline Pump 1000 series isocratic,LPG,HPG), a sample injector (model 7725 ⁄ 7725i), a UV/VIS detector (Smartline UV S 2550) and colon oven (Smartline S4000),autosampler(Smartline AS 3950).

Preparation of standard solutions for HPLC analyses: Stock solutions of 40 lg mL)1 were prepared in the mobile phase. Working standard solutions were prepared by mixing stock standard solutions in appropriate proportions with the mobile phase. Solutions were kept in dark bottles at 4 ^{0}C to avoid vitamin degradation and filtered through a 0.45 lm nylon membrane filter. In addition, they were stored in dark vials before injecting to HPLC column.

Sample preparation for HPLC analysis: Samples (12 g) of each yogurt were weighed into separate 250 mL conical Erlenmeyer flasks. A solution (80 mL) containing 1% clara-diastase was added to each sample. Then, 0.5 mL of fromaza solution was added. The pH value was adjusted to 6.0 using 2 mol L)1 NaOH. The flasks were heated and shaken for 1 h in a water bath at 25^{0}C and then placed in a temperature controlled oven at 30 ^{0}C for 20 h. Thereafter, the samples were cooled to laboratory temperature.

HPLC analysis: Chromatographic separation and analysis of the vitamins were done using the reversed-phase Supelcosil LC 8 column (150 4.6 x 5l mm). The column temperature was set at 25 ^{0}C. A mobile phase of 90% phosphate buffer (0.1 mol L)1 potassium dehydrogenate phosphate, pH 7.0) and 10% methanol were used in isocratic elution. The flow rate was set at 1.0 mL min)1 and the time of analysis was 30 min. The detector wavelengths were set at 220 nm for B3 and B6 and 204 nm for B5. A sample of 20 µL was injected. Calibration curves were measured under the same condition as the yogurt samples. The linearity of the standard curves was assessed by least squares regression using standards over the range 1–6 lg mL for nicotinamide, d-pantothenic acid and pyridoxine. The target vitamins were identified from the retention time matches against those of the calibration standards. The concentrations of the water-soluble vitamins were calculated from integrated areas (peak area) of the sample and to the corresponding to the standards.

3. RESULTS AND DISCUSSION

The yogurt starter bacteria use some of the vitamins present in milk during the fermentation period to meet their growth requirements. This factor contributes, to some extent, to a reduction in the nutritional properties of the product. Chromatogram shows the addition of sea buckthorn fruits in yogurt after thermosetting determinate an increase in vitamin A up to 400 µg / ml(Fig.1 and Tabel no.2.)Much larger in proportion unlike normal vitamin content of yogurt are the other vitamins that increased B12, B6, D3 and PP.

Stability was very important for sea buckthorn addition in yoghurt and this is clear from the content of vitamins, especially vitamin C, an index of pasteurization and thermostatic.

Some vitamins decrease during the storage of yoghurt with sea buckthorn at 4°C, i.e. vitamin B12 observed losses of folic acid and vitamin B12 of 28.6 and 59.9%, respectively, during the storage of yoghurt at 5°C for 16 days.

The same analysis also observed a decrease in the biotin, and PP contents. They attributed these losses to the combined effect of microbial catabolism during the incubation period and chemical decomposition of these vitamins during cold storage.

Vitamins which increase during the actual manufacture of yoghurt with sea buckthorn are PP and folic acid, because they are actively synthesized by the starter cultures. According to Reddy, Shahani, and Banerjee (1973) , the increases in folic acid and PP in yoghurt (made from whole milk fortified with 10 g of sea buckthorn and incubated for 3 hours at 42°C) amounted to 3.95 and 22mg100g-1, respectively (see also Table no 2); losses in storage may exceed these gains in due course.

Although there was a general agreement in the literature that vitamin B12 decreases during yoghurt production, Tamime and Robinson (1999) found that some species of *Lactobacillus* and strains of yoghurt starter culture synthesize vitamin B12.

However, enhanced synthesis of vitamins in yoghurt can be achieved by using different combinations of starter cultures.

The biological activity of *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus* and biostarter cultures during the manufacture of yoghurt with sea buckthorn and related products is highly complex. This scientific work has elucidated some general information about the content of vitamins.

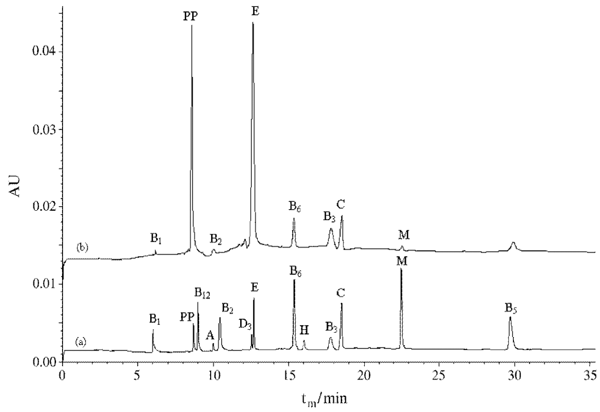

Fig.1.*Cromatogram of standard solutions* (a) and extracts of yogurt (b). The concentration of standard solution: vitamin A, 400 mg / ml, vitamin B12, PP, D3, B6 and E, 50 mg / ml, and the other vitamins 100 mg / ml.

The relative availability of vitamins in yoghurt is much more difficult to assess because, unlike minerals, many vitamins are sensitive to the conditions of processing. Thus, the method of fortification, for example, the addition of yogurt powder or membrane processing, the heat treatment of the milk base, the strains of starter bacteria used and the conditions of fermentation can all alter the concentrations of the more important vitamins (Tamime & Robinson, 1999).

Vitamin C is very sensitive to light and oxygen because the under the light action reduced form of ascorbic acid present in yogurt was reversibly oxidized to dehydro-ascorbic acid. Vitamin C varies between 400 and 1500 mg per 100 g fruit, content superior in strawberries, kiwi, oranges, tomatoes and carrots.

Table no.1 *Effect of incubation temperature upon vitamin synthesis in yoghurt*

Vitamin (mg 100 g-1)	Milk + 2% SMP	Incubation temperature (°C) for 3 hours
		37 40 42 45
Folic acid	0.37	3.74 4.04 4.32 3.94
Niacin	120	126 130 142 136

Table no.2. *The main features that were found to separate* vitamins (n = 3, P = 0.95)

Vita-mins	Migration time,min	A*	R5**	$Nx10^{-3}$***	Cmin,mg/ L****
A	28,5	1,60	68	320	0.4
B12	8,8	1,92	26	180	0.5
D3	31,8	1,02	2.7	850	0.3
PP	9,2	-	-	230	0.8
E	32,3	1,05	3.3	630	0.3
C	30.6	1,02	6.8	290	2.4

* pick area ration
** time retention, min
*** determination coefficient,
**** relative migration time (migration time of vitamin/internal standard

Sea buckthorn has been estimated that in the whole world there was enough vitamin C,A,D,B as required to meet the needs of a balanced diet for the entire population of the planet. Vitamin C increases the body defense against infections by stimulating the immune system, accumulation of calcium in bones, promotes iron absorption from the digestive tract and stimulates the formation of hemoglobin.

4. CONCLUSIONS

The quantities of vitamins are dependent on the rate of inoculation, the strain of yoghurt starter and the conditions of fermentation (Friend, Fiedler, & Shahani, 1983; Kilara & Shahani, 1976)

We find that vitamin C will decrease slightly after 1 day or 3 days and after 7 days, 14 days the amount is less obvious.

We observed that the needs of sea buckthorn fruit will grow according the determination of vitamin C in yogurt, and also determined from milk as raw material and for this is necessary a higher addition of fruit in the winter because vitamin C and other vitamins content in raw material is influenced also by animal diet, season and lactation period. According to Reddy, et al. (1973), the increases in PP in yoghurt (made from whole milk fortified with 10 g of sea buckthorn and incubated for 3 hours at 42°C) amounted to 3.95 and 22mg100g-1);

The vitamins which are lost in storage may exceed these gains in due course. Although there is a general agreement in the literature that vitamin B12 decreases during yoghurt production, (Tamime & Robinson, 1999) found that some species of Lactobacillus and strains of yoghurt starter culture synthesize vitamin B12.

This latter aspect was confirmed in yoghurt with sea Buckthorn made by the direct acidification method rather than by microbial fermentation. A long incubation of yoghurt (i.e. incubation at 30°C for 14–16 hours) decreased the synthesis of vitamins, but increased the content of C,B1,B12 and nicotinic acid in the final product.

Final product is superior compared with plain yogurt that most vitamins decrease with few exceptions.

Soluble vitamins are those that grow in general according to optimum and maximum content of vitamins to ensure stability and choice of final product optimal storage temperature.

Microorganisms and specific cultures and different combinations of these influence increases or decreases of these vitamins

Therefore, it is important to use selected strains of the yoghurt starter cultures and processing conditions in order to maintain the nutritional properties of yoghurt.

If the consumer appeal of both natural and fruit sea buckthorn /bio-yoghurts is also placed on record, together with their excellent performance in respect with public health, then it is obvious why the market for these products is an expanding one.

ACKNOWLEDGEMENTS

The authors are grateful to the project "Romanian Research Integration in European Research Context, POSDRU/88/1.5/S/60370 " financed from European Social Fund by Operational Sectoral Programme for Human Resources Development 2007-2013".

6. REFERENCES

[1] **Andrews, T**. (2000). Nectar and Ambrosia: An Encyclopedia of Food in World Mythology. 250 Accessible online: http: foodtimeline. org/foodfaq2html. *Accessed, 8*(21), 2006.

[2] **Anonymous.** (1997). *Nutritional Benefits of Yogurt and Other Fermented Milk Products*: National Dairy Council, London (UK).

[3] **Beaton, G. H., Martorell, R., L'abbe, K., Edmonston, B., & McCabe, G**. (1992). Effectiveness of vitamin A supplementation in the control of young child morbidity and mortality in developing countries: Summary report.

[4] **Eitenmiller, R. R., Ye, L., & Landen, W**. (2008). *Vitamin analysis for the health and food sciences*: CRC.

[5] **Friend, B., Fiedler, J., & Shahani, K**. (1983). Influence of culture selection on the flavor, antimicrobial activity, beta-galactosidase and B-vitamins of yogurt. *Milchwissenschaft, 38*, 133-136.

[6] **Kilara, A., & Shahani, K**. (1976). Lactase Activity of Cultured and Acidified Dairy Products1. *Journal of dairy science, 59*(12), 2031-2035.

[7] **Kosikowski, F. V., & Mistry, V. V**. (1997). Creamed cottage cheese. In *Cheese and fermented milk foods* (Vol. 1, pp. 131–145): F.V. Kosikowski.

[8] **Reddy, G., Shahani, K., & Banerjee, M.** (1973). Inhibitory effect of yogurt on Ehrlich Ascites tumor-cell proliferation. *Journal of the National Cancer Institute, 50*(3), 815.

[9] **Tamime, A. Y., & Robinson, R. K.** (1999). *Yoghurt: science and technology*: CRC Press.