POTENTIAL TO INCREASE THE STABILITY OF MILK RIBOFLAVIN AGAINST PHOTO-OXIDATIVE DEGRADATION

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Abstract
Milk and other dairy foods are excellent natural sources of riboflavin (vitamin B_2). Exposure of milk to light as one of the most serious dangers of riboflavin degradation, can take place at several stages from milking to the consumers, initiating the sensibilization of riboflavin, and resulting in the oxidation of milk, losses of nutritional value, and strong off-flavours that can make the oxidized milk unacceptable to consumers. The stability of milk components is dependent of a delicate balance between the anti- and pro-oxidative factors. Carotenoids are known as one of the strongest natural antioxidants. The aim of this study was to compare the influence of cow feed carotenoids on the milk riboflavin stability against photo-oxidative degradation. Milk was obtained from three experimental and one control cow groups. Experimental group's feed was supplied with carotenoid sources: carrots, red palm oil and red palm oil concentrate. Milk samples were collected after 6 week long feed supplementation and exposed to direct sunlight for 1.5 and 3 hours. The losses of riboflavin measured by the fluorometric method was significantly lower ($p<0.05$) in all experimental groups milk when cow diet was enriched with carotenoids from carrots, red palm oil, and red palm oil carotenoid concentrate, compared to the control group (respectively, 10.03, 10.82, 11.13, and 21.22% after 1.5 h storage, and 15.66%, 18.95%, 18.64%, and 23.48% after 3 h storage). A strong positive correlation between milk yellow colour intensity and its riboflavin stability was also observed.
Key words: dairy products, vitamin B_2, cow feeding, antioxidants, carotenoids.

Introduction
Milk and other dairy foods are excellent natural sources of vitamin B_2 (riboflavin), and other water-soluble vitamins: B_1 (thiamine), B_6 (pyridoxine), B_{12} (cianocobalamin), niacin (nicotinic acid), and pantothenic acid. Light is one of the most serious dangers of riboflavin degradation that is extremely sensitive to wavelengths of 420–560 nm (Eitenmiller et al., 2008, Dairy Science and Technology Handbook, 1993). Exposure of milk to light can take place at several stages from milking to the consumers and initiate the sensibilization of riboflavin, and result in the oxidation of milk. The oxidation processes cause the deterioration of nutritional quality of dairy products, and give rise to strong off-flavours, making them unacceptable to consumers (Havemose et al., 2004). The water-soluble vitamin riboflavin present in milk acts as a potent photosensitizer (the ability of riboflavin to generate singlet oxygen in milk in its capacity as a photosensitizer has been confirmed earlier) and has been implicated in the photooxidation of milk fat (MacGibbon and Taylor, 2006). Light-activated riboflavin is an agent in the development of sunlight flavor in milk via methionine oxidation to methional. Other amino acids, besides methionine, may be affected by the presence of light and riboflavin. It also catalyzes the photodegradation of ascorbic acid (Dairy Science and Technology Handbook, 1993, MacGibbon and Taylor, 2006). The extent of off-flavour development is a function of the wavelength involved, and the intensity and duration of exposure. Light has been shown to penetrate milk to an appreciable depth (MacGibbon and Taylor, 2006). The high sensitivity of flavins to light degradation is a significant factor influencing food packaging. The light induced loss of riboflavin in fluid milk packaged in glass containers and subjected to sunlight was one of the first nutrient losses in food products documented by scientific study (Eitenmiller et. al. 2008). However, glass and other transparent material bottles or other packages are often used as packing of milk and dairy products still nowadays, perhaps due to preference of consumers. The stability of milk components are dependent of a delicate balance between the anti- and pro-oxidative processes in milk influenced by different factors. One of these factors is the presence of antioxidants. Carotenoids and vitamin E act as fat-soluble antioxidants in, for example, the milk fat globule membrane which is regarded as a major site of

auto-oxidation. β-carotene (BC) is also particularly involved in prevention of photo-oxidation, as it absorbs light in a concentration-dependent manner that would otherwise be absorbed by riboflavin, thereby inducing quality changes (Noziere et al., 2006). Factors influencing the potential for oxidized flavor development can be manipulated by changing the cow's diet. Numerous studies have shown that antioxidants, as tocopherols and carotenoids, can be transferred from the feed to milk, and thereby improve the oxidative stability of milk (Havemose et al., 2004). However the potential of feed carotenoids, as defence mechanism against photo-oxidative degradation of milk components such as riboflavin, as well as against following oxidation of milk lipids, that was mentioned earlier, is not as clearly shown by previous investigations. Furthermore – carotenoid content in the cow feed often is not sufficiently estimated, especially in winter and spring months when cow feed is poorer in it. Amongst the richest sources of carotenoids crude palm oil (0.05 to 0.2%) (Stołyhvo, 2007) and carrots (0.006 to 0.055%) (Kotecha et al., 1998) are considered, containing mainly α- and β-carotenes. The aim of this study was to compare the influence of supplementation of cow feed by different carotenoid sources on the milk riboflavin stability against photo-oxidative degradation and colour.

Materials and Methods

Experimental design. For this experiment 20 cows were selected in a conventional type dairy farm "Strautiņi", and divided into 4 groups – control group (G1) and 3 experimental groups (G2, G3, and G4) by 5 cows in each. The stage of lactation (1^{st} – 3^{rd} month), cow breed (Holstein, Latvian Brown and crossed) and lactation number (i.e. 1–5) were as similar as possible in all groups. The basic feed was equal in all groups, i.e. silage was fed to ad libitum and rapeseed animal feed – 2 kg per cow per day. In the G2 diet additionally 7 kg carrots per cow per day were included. In the G3 diet additionally red palm oil NVRSO – 100 g per cow per day was included. In the G4 diet additionally red palm oil carotenoid concentrate 5 g per cow per day was included. Groups 1, 2, and 4 also received rapeseed oil 100 g per cow per day, mixed in dry forage.

Milk sample collection and storage. Individual cow milk samples were obtained in day 42 of feed supplementation from afternoon milking. Equal amounts of each group's cow's milk were pooled together getting 1 pooled sample of each cow group. Pooled samples were immediately cooled to the temperature of 4–8 °C, and transported to the laboratory next morning. A total 4 pooled milk samples were divided into smaller subsamples and stored at 4–6 °C temperature until analyses of raw milk colour. Other subsamples for analysis of milk riboflavin were stored in direct sunlight for 1.5, and 3 h at room temperature or in dark at 4–6 °C temperature for 3h, and sub sequentially frozen at temperature of –18 °C until analysis not longer than 1 month.

Total carotenes in feed were determined in accordance with ГОСТ 13496.17–95 method, by measuring the concentration on the photometer FEK-56 M by the wave length 450 nm. The extraction of total lipids from feed for *analyses of α- and β- carotene* concentration was performed by the method of Hara and Radin, 1978. Concentration of α- and β- carotene in feed was determined by HPLC using the technique consisting of a Waters Alliance 2695 HPLC with photodiode array detector, monitoring between 280 and 600 nm, using a 150 x 4.6 mm, RP C18 column and Empower Pro software. The flow rate was 2 ml min^{-1} and the mobile phase consisted of acetonitrile, methanol-acetate ammonium 50 m*M*, dichloromethane and water (70:15:10:5). Concentration of carotenoids was calculated by using external standards. The above mentioned feed and milk analyses were carried out in the Scientific Laboratory of Biochemistry and Microbiology of the Research Institute of Biotechnology and Veterinary Medicine 'Sigra' of the LLU.

Milk riboflavin content was determined based on a method described by Havemose et al., 2004. Milk samples (5 ml) were mixed with 0.5 ml of 2 *M* sodium acetate (Poliskie Odczynniki

Chemiczne) and 1.5 ml of 2 *M* acetic acid (Chemipur, Poland). The samples were slowly agitated for 5 min. before centrifugation at 1500 x g for 10 min. The supernatant was filtered through a filter (Filtra acido hidrohlorico extracta 90) and the fluorescence was read using a TD-700 Fluorometer (Turner Designs, Sunnyvale, CA), emission 520 nm. All analytical procedures were conducted using glassware wrapped in aluminum foil to avoid light exposure resulting in additional riboflavin degradation during sample preparation.

Milk colour analysis. The colour of milk samples was measured using a ColorTec – PCM colour meter, USA (CIE 1976 L*a*b* colour model), which has been calibrated with a standard. The milk riboflavin and colour analyses were carried out in the Research Laboratory of Food Packing Materials and in the Scientific Laboratory of Microbiology of the Faculty of Food Technology of the LLU.

Milk fat content was determined by automated infrared analysis using Milcoscan equipment (method ISO 9622-1999) in the laboratory of Milk Quality Control of the Sigulda CMAS.

All analytical reagents used in analysis were of analytical or higher purity. Samples were analyzed at least in duplicate. The results were calculated, analyzed, and graphs were made using MS Office program Excel.

Results and Discussion

Milk riboflavin content and stability in relation to cow feeding.

The content of total carotenes in cow group's G1, G2, G3 and G4 feed was measured, and it was 225, 1325, 275, and 320 mg per cow per day, respectively.

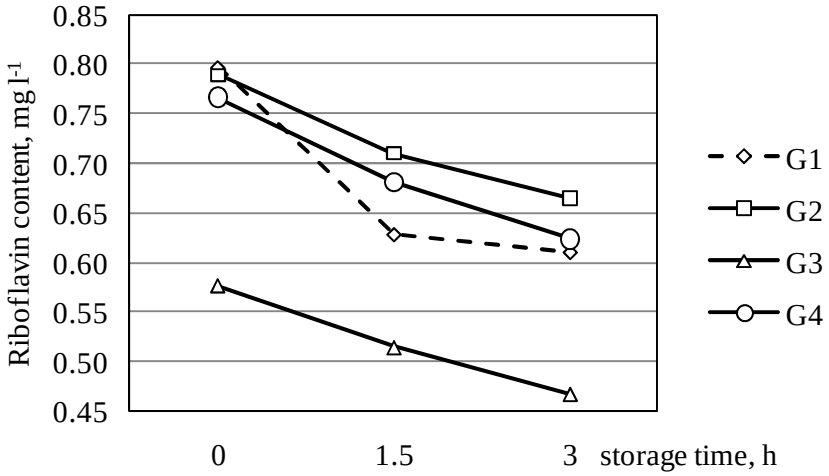

Figure 1. Decrease of riboflavin content in milk during storage in direct sunlight

The riboflavin content in raw milk was from 0.58 till 0.80 mg l^{-1} what is below the quantities showed in literature: 0.8–1.2 mg kg^{-1} (Рогожин, 2006), 1.0–2.8 mg kg^{-1} (Горбатова, 2004); the average riboflavin content of fluid whole milk according to Miller et al., 2007, is about 1.8 mg kg^{-1}, but in the study of Zagorska, 2007, the average riboflavin content in Latvia raw milk, obtained in conventional agriculture was 2.65±0.10 mg l^{-1}. Such of our experiment can be explained by the very high sensitivity of riboflavin to different implications of mechanical, light and other influence from milking and sample collection to laboratory analysis. Comparing the riboflavin content in all group milk, the significantly lowest ($p<0.05$) riboflavin content was in G3 milk (0.567 mg l^{-1}). The riboflavin content in other group's raw milk before storage in direct sunlight was rather similar (0.77–0.80 mg l^{-1}). During the storage in direct sunlight the degradation occurred in all groups milk (Fig. 1).

Already after 1.5 h storage in direct sunlight, the losses of riboflavin were considerable (10.03–21.22%), and, moreover, by approximately 10% higher in G1 milk than in the three

experimental group's milk (Table 1). The losses of riboflavin measured 3h after milk storage in direct sunlight continued to increase, and in G1 milk still were the most expressed – by approximately 5–8% higher, as compared to G2, G3, and G4. So, it can be assumed that feed supplementation with carotenoids helps to prevent the photo-oxidative degradation of riboflavin. The most pronounced positive effect was observed when feed carotenoid content was the highest, and cow feed was supplemented by carrots 7 kg per cow per day.

Table 1

The influence of milk storage in direct sunlight to the riboflavin degradation.

Groups	Losses of riboflavin, %, from the initial amount	
	Storage time 1.5 h	Storage time 3.0 h
G1 (control)	21.22	23.48
G2	10.03	15.66
G3	10.82	18.95
G4	11.13	18.64

Milk riboflavin stability in relation to milk colour.

According to Noziere, Graulet et al., 2006, carotenoids are responsible for the yellow coloration of cattle milks derived from breeds or diet regimens that have a high carotenoid concentration. The yellow colour intensity (the b* component value) that represents the entire carotenoid pigment amount in milk was significantly lowest in G1 milk among all groups (6.42±0.188). The b*components of all the three experimental groups milk were significantly higher ($p<0.05$) and rather close, respectively, 7.82±0.095 for G2, 8.10±0.057 for G3, and 8.47±0.095 for G4. This can be explained by the significantly higher carotenoid concentration in all the three experimental cow groups feed. The correlation between yellow colour intensity and losses of milk riboflavin was calculated, results showed in Figure 2. Correlation was stronger (r=- 0.93) after the first 1.5 h of storage in the sunlight, and decreased later – after 3 h of storage in sunlight (r=- 0.74).

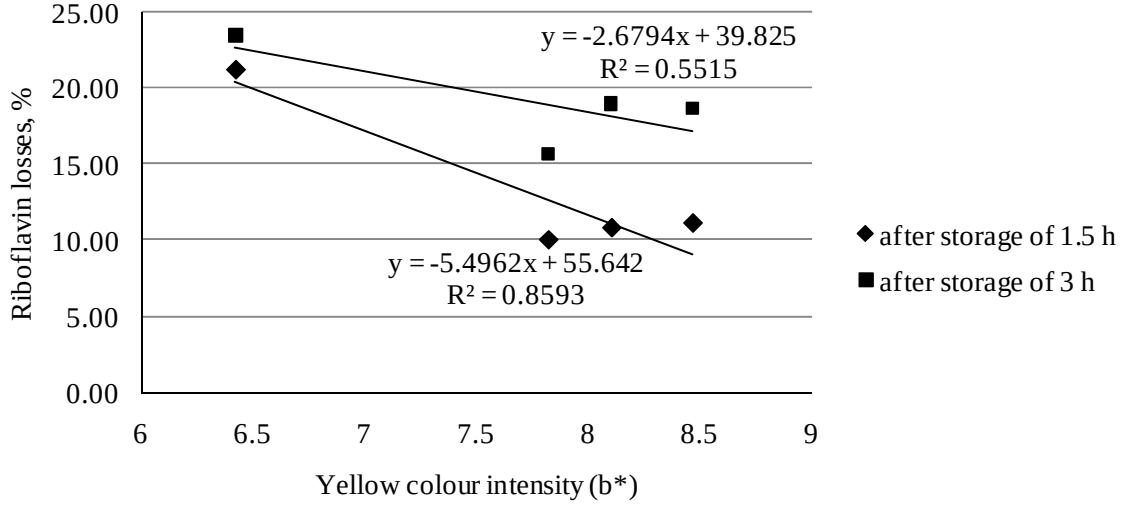

Figure 2. Correlation between losses of milk riboflavin and the intensity of its yellow colour

The fat content was measured, and it was 3.81, 4.02, 3.91, and 4.58 % in G1, G2, G3, and G4 milk, respectively. Recalculating the colour intensity in all groups to average 4.08% fat content, the correlation between yellow colour intensity and losses of milk riboflavin was smaller, but also rather tight: R=-0.83 and -0.69 after 1.5 h and 3 h storage in sunlight, respectively.

However, it should be necessary to collect more data to appreciate the feed and milk carotenoid influence on milk riboflavin stability.

Conclusions

1. Milk riboflavin photo-oxidative stability strongly depends on cow's diet, i.e. – its carotenoid content. The degradation of vitamin B_2 in direct sunlight was significantly lower ($P<0.05$) in all the three experimental cow groups milk when cow diet was enriched with carotenoids from carrots, red palm oil, and red palm oil carotenoid concentrate, compared to the control group (respectively, 10.03, 10.82, 11.13, and 21.22% after 1.5 h storage, and 15.66%, 18.95%, 18.64%, and 23.48% after 3 h storage).
2. A strong positive correlation between milk yellow colour intensity and its riboflavin stability was observed. The correlation between milk colour intensity and losses of milk riboflavin after 1.5 h and 3 h storage in direct sunlight was r=- 0.93, and - 0.74, respectively.

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