INVESTIGATIONS INTO THE ENHANCEMENT OF COW'S MILK OXIDATIVE STABILITY

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Abstract
The quality and nutritional value of milk and dairy products are considerably influenced by the stability of its constituents. **The aim** of the present study was to evaluate the possibility of enhancing oxidative stability of cow's milk fat and vitamin B_2 using carrots and palm oil feed supplement CAF 100 as sources of natural antioxidants in cow feed. Milk samples were collected after 25-day period of feed supplementation. The intensity of riboflavin losses during its photo oxidative degradation in sunlight was measured by the fluorometric method. A slight and significant ($p<0.05$) difference in higher vitamin B_2 stability was seen in carrot-supplemented group (TG1) milk, where the losses of vitamin B_2 were by 3.01% less compared to CG (CG). The oxidative stability of butter oil samples stored in 60 °C temperature was analyzed by peroxide value (PV) method. The oxidative stability of samples initially affected by light from both trial groups was significantly ($p<0.05$) higher compared to CG showing the good potential of the cow diet enrichment with carotenoid additives. The longest induction period (> 14 days) was observed in CAF-100-supplemented (TG2) cow milk fats, which can be explained by carotenoids and tocopherol presence and its possible synergism in fat protection. The induction periods of the TG1 and CG were 12.03 and 10.97 days, respectively.
Key words: antioxidants, dairy products, feed additives, milk fat, peroxide value, vitamin B_2.

Introduction

The quality and nutritional value of milk and dairy products are considerably influenced by the stability of its constituents. Oxidative reactions in milk are detrimental because these reactions reduce the nutritional value of milk and contribute to a reduction in shelf life (van Aardt et al., 2005). Oxidative stability of milk is important not only for the lipid components, but also for other constituents, as, for example, for water soluble vitamin B_2, which can be easily affected by light influence (Eitenmiller et al., 2008). Riboflavin plays a key role in all problems related to the photosensitivity and photo degradation of milk and dairy products (Bosset et al., 1994). Besides the decrease of nutritional value, the formation of off-flavours is occurring. For example, light-activated riboflavin is an agent to the development of sunlight flavour in milk via methionine oxidation to methional. Other amino acids, besides methionine, may be affected by the presence of light and riboflavin (Dairy Science and Technology Handbook, 1993; MacGibbon and Taylor, 2006).

Carotenoids – the colored pigments ranging from light yellow through orange to deep red – are used as colorants for human food and nutrition supplements, or as feed additives to enhance the pigmentation of fish and eggs. The defensive role of carotenoids as natural antioxidants in food protection is known as well (Namitha and Negi, 2010). Carotenoids, especially those with nine and more conjugated double bonds in molecule, can offer good defense against oxidative deterioration, especially arisen from exposure to light (O'Connor and O'Brien, 2006). However, the protective role of carotenoids against singlet oxygen and light-caused deterioration is not employed enough in dairy product quality and nutritional value improvement. The cow feed supplements containing natural antioxidants – carotenoids – are not frequently used and well-known in Latvia.

The aim of the present study was to evaluate the possibility of enhancing oxidative stability of cow's milk fat and vitamin B_2 using carrots and palm oil feed supplement CAF 100 as sources of natural antioxidants in cow feed.

Materials and Methods

Experimental design. Feed of different carotenoid concentrations was fed to 3 groups of cows – control group (CG) and 2 trial groups (TG1, and TG2) of 5 cows in each that were selected in a conventional dairy farm in Latvia. The average stage of lactation (5.3 months), the average lactation number (i.e. 2.8) and cow breed (Latvian Brown, Danish Red, and crossed) were as similar as possible in all groups. Feed supplementation was implemented at the end of the indoor period (April). The basic feed was equal in all groups at least 2 weeks before and during supplementation period and it was haylage, mixed feed concentrate and hay. The amounts of the basic and supplemental cow feed and the content of total carotenes in each group's feed are given in Table 1.

CAF 100 that was supplemented to TG2 group's feed is a lightly orange colored powder, containing > 99% palm stearin (Carotino, 2006). It is rich in carotenes and vitamin E (approximately 120, and 300 ppm, respectively), providing 120 mg of vitamin E to the TG2 group per cow per day additionally.

Table 1

Cow feed composition

Cow groups	Basic feed, per cow per day	Supplemental feed, per cow per day	Total carotenes, mg per cow per day
Trial group 1 (TG1)	Haylage – *ad libitum*, mixed feed concentrate – 2 kg, hay – 2 kg	Carrots – 7 kg	387
Trial group 2 (TG2)		CAF 100[a] – 400 g	292
Control (CG)		-	242

[a] Animal Feed produced by Carotino SDN. BHD, J.C. Chang Group, Malaysia

Milk sample collection and storage. Individual cow milk samples were obtained from the morning milking on day 25 from the start of feed supplementation. Equal amounts of each group's individual cow milk (5 L) were pooled together resulting in one bulk milk sample per group. After collection, milk samples were transported to the laboratory. For analysis of riboflavin, milk was separated in subsamples of 100 mL that were put in 200-mL clear glass beakers and stored in direct sunlight for 1.5 and 3 h at room temperature stirring each 10 min. A blank analysis was made without milk exposure to light (0 h in light).

Chemicals. Water was purified with Simplicity (Millipore SAS, France). Sodium acetate and potassium iodide were from Stanchem, Poland; glacial acetic acid was from Lach-Ner, Czech Republic; chloroform was from Riedel-De-Haën, Germany; sodium thiosulphate was from AVSISTA, Lithuania. All reagents were of analytical or higher purity.

Vitamin B_2 content in milk was determined in accordance with the fluorometric method described by Havemose et al. (2004). Milk samples (5 mL) were mixed with 0.5 mL of 2 *M* sodium acetate and 1.5 mL of 2 *M* acetic acid. The samples were slowly agitated for 5 min before centrifugation at 1500 × g for 10 min. The supernatant was filtered through a 0.45 mm Nylon filter (Membrane Solutions), and the fluorescence was measured 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 fat extraction and storage. Right after transportation to the laboratory, milk for fat extraction was warmed up to 40-45 °C temperature subsequently separating cream with a conventional milk separator to approx. 30% fat content. Cream was ripened at temperature of 4-6 °C, 20 ± 1 h, then churned till formation of butter. The buttermilk was removed and butter was rinsed with cold distilled water for 5 times. Subsequently, butter was warmed up to 40-50 °C and centrifuged 15871 × g, 10 minutes at 40 °C to separate the clear butter oil layer that afterwards was carefully split into smaller (20 g) sub-samples for peroxide value (PV) determinations, e.g. fat was poured into appropriate number of transparent plastic one-way Petri plates. A half of the fat sub-samples were subjected to direct sunlight action at room temperature for 3 hours to hasten the fat ageing, while other samples were stored at the temperature of 4-6 °C in dark for 3 hours. After that all samples were placed into thermostatic oven at the temperature of 60 °C for fat ageing. The duration of ageing was 25 days for fat samples unaffected by light, or 14 days for fat samples affected by light for which the oxidation process was much faster.

Peroxide value (PV) of the milk fat. The PV test was carried out in accordance with iodometric titration method described by Охрименко и др. (2005). One g of the fat was put in a 100-mL Erlenmeyer glass flask, and mixed with 6 mL solution containing chloroform and glacial acetic acid (2:1, v/v). Parallel blank analysis was performed without fat sample. Then 1 mL of saturated potassium iodide solution and 30 mL of deionized water were added. After that, flasks were sealed and carefully shaken for exactly 3 min. Next, 3-5 drops of 1% starch solution to the reaction mixture were added, followed by the titration against 0.01 *M* sodium thiosulphate solution. The equation (1) was used for the calculation of PV:

$$PV = (V_0 - V) \times 0.00127 \times 100 / m, \quad (1)$$

where:

PV – the peroxide value, expressed as % of iodine utilized for the reduction of 100 g fat;

$(V_0\text{-}V)$ – the difference between a blank titration and the titration with the fat;

0.00127 – the iodine mass that corresponds to 1 mL of 0.01 *M* sodium thiosulphate solution, g;

100 – the conversion factor to 100 g amount of fat;

m – the mass of the fat sample, g.

Statistical analyses. The results were calculated, analyzed, and graphs were made using MS Office program Excel or Microsoft Windows for SPSS (SPSS 17.0, SPSS Inc. Chicago, Illinois, USA). Differences

between treatments were tested for significance ($p<0.05$) by ANOVA. Data are presented as means ± confidence interval ($p<0.05$).

Analyses were carried out in the Scientific Laboratory of Biochemistry and Microbiology of the Research Institute of Biotechnology and Veterinary Medicine 'Sigra', and in the Scientific Laboratory of Microbiology of the Faculty of Food Technology of the LLU.

Results and Discussion

The photo-oxidative stability of vitamin B_2 in milk

To compare the vitamin B_2 photo-oxidative stability in milk obtained from differently fed cow groups, in this test the light influence was used. It is known that β-carotene is 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 (Mortensen et al., 2004; Nozière et al., 2006). The highest total carotenes content was in TG1 feed (see Table 1). Carrots are one of the richest sources of carotenoids containing mainly α- and β-carotenes (Kotecha et al., 1998). The greater effects from the carotenoids supplementation or the lower losses of vitamin B2 in the TG1 milk were anticipated.

The initial content of vitamin B_2 in raw milk was 2.07-2.35 mg L^{-1} (see Fig. 1), and it was similar to the quantities showed in literature – 1.0-2.8 mg kg^{-1} (Горбатова, 2004), above literature data 1.8 mg kg^{-1} (Miller et al., 2007), or below the average vitamin B_2 content obtained in the study of Zagorska (2007) in Latvia conventional agriculture raw milk – 2.65 ± 0.10 mg L^{-1}.

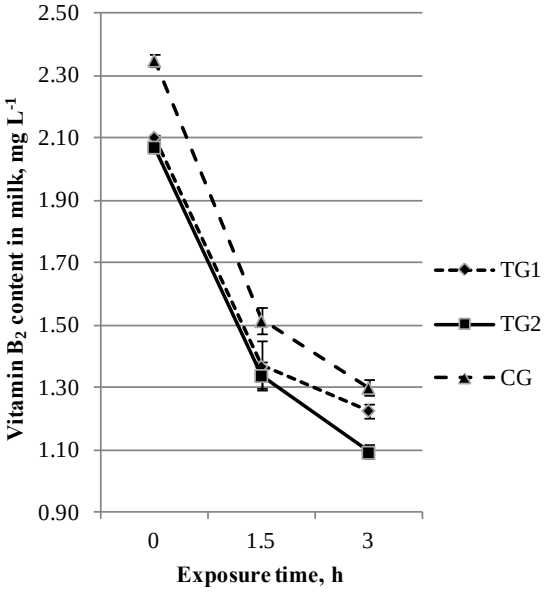

Figure 1. Decrease in vitamin B_2 content during exposure to direct sunlight in milk obtained after 25-day feed supplementation.

As the initial content of vitamin B_2 in milk obtained from 3 groups was significantly different ($p<0.05$), the oxidation intensity of vitamin B_2 was compared by its losses from the initial concentration (%). During the milk storage in direct sunlight for 1.5 and 3 h, the degradation of vitamin B_2 occurring in milk samples of all groups was high (see Table 2). After milk exposure to light during first 1.5 h, the losses of vitamin B_2 in all samples of milk were considerable – 34.9-35.5%, but did not differ significantly ($p>0.05$). During the remaining 1.5 h, the decrease in the content of vitamin B_2 was less intense, reaching 41.6-47.18%. A slightly and significantly higher ($p<0.05$) vitamin B_2 stability was seen in carrots-supplemented group (TG1) milk, where the losses of vitamin B_2 was by 3.01% less compared to CG. Regarding CAF-100-supplemented group (TG2) milk, there was not observed any superiority of vitamin B_2 stability over CG milk.

The results can be explained by the concentrations of such antioxidative compounds in milk as carotenoids and ascorbic acid that inhibit the degradation of vitamin B_2. The total carotenes content in TG1 (carrots-supplemented group) feed was considerably higher compared to CG, and also to TG2, and this can be the possible explanation of the slightly and significantly ($p<0.05$) higher stability of the vitamin B_2 in TG1 group milk samples as anticipated previously.

However, the carotenoids are not the only compounds giving protection to the riboflavin during photo-oxidation. It has been reported that ascorbic acid also has strong quenching ability against active-oxigen species and it effectively prevents the light-activated off-flavour formation and reduction of riboflavin in milk and aqueous solution (Trang et al., 2008; Lee et al., 1998). Lee et al. (1998) demonstrated that addition of 0.1% ascorbic acid resulted in 50% and 25.5% inhibition of reduction of riboflavin in whole milk and skim milk, respectively, after 10 h illumination at 3300 lux (Trang et al., 2008). Due to the significant role of ascorbic acid its average content in the raw milk that was obtained from differently fed cow groups was compared. It was 16.70±0.10, 16.99±0.10, and 17.20±0.09 mg L^{-1} in TG1, TG2, and CG milk, respectively, showing that both trial groups had a significantly ($p<0.05$) lower content of ascorbic acid that was by 0.5% less for TG1 and by 0.2% less for TG2, compared to CG. This can be one of the reasons why the riboflavin oxidative stability of carotenoids-supplemented group milks was only slightly higher (TG1-3h) or even lower (TG2-3h) compared to CG ($p<0.05$). The significantly lower content of ascorbic acid in TG2 possibly extinguished the positive effect of carotenoids giving a negative result as slight (2.57%) but significantly ($p<0.05$) higher losses of vitamin B_2 in this group's milk after 3-h-sunligt exposure compared to CG.

Table 2

Losses in vitamin B_2 content in milk during storage in direct sunlight (mean±SD)

Exposure time, h	1.5			3.0		
Groups	Experimental		Control	Experimental		Control
	TG1	TG2	CG	TG1	TG2	CG
Decrease in vitamin B_2, %	34.87 ± 2.54	35.31 ± 2.04	35.50 ± 0.87	41.60 ± 0.65	47.18 ± 0.81	44.61 ± 0.37

A more pronounced positive tendency that cow feed supplementation with carotenoids additives has potential to increase the stability of vitamin B_2 against photo-oxidative degradation in milk was seen in our previous investigation, showing that the vitamin B_2 photo-oxidative stability was higher in milk obtained from carotenoids-supplemented cows, saving 10-11% of vitamin B_2 during 1.5 h period, and 5-8% during 3 h period of milk exposure to sunlight (Antone et al., 2011).

However, the average losses of vitamin B_2 in our study were higher – 34.87-35.50% and 41.60-47.18% compared to the results from our previous investigations, namely, 10.03-21.22% during first 1.5 h and 15.66-23.48% during 3-h period of the milk exposure to sunlight (Antone et al., 2011). The differences in results can also be explained by the differences in light intensity (Bosset et al., 1994) and in the initial antioxidative capacity of milk as well (Walstra et al., 1999). The losses of vitamin B_2 in fluorescent light are also significant. Unpackaged pasteurized milk exposed to cool white fluorescent light for 7 h undergoes riboflavin losses of over 75% (Bosset et al., 1994). For the further research it would be valuable to take into consideration that the changes in vitamin B_2 content should be monitored earlier – during the first hour of sunlight exposure due to its extremely high sensitivity to photo-oxidation, thus possibly allowing detecting more pronounced effects from cow feed supplementation with different carotenoids supplements.

As mentioned previously, the photo-oxidative degradation is the main cause of riboflavin losses. This should be considered when choosing the packaging materials for food products containing vitamin B_2, among which milk and dairy products are rich and excellent natural dietary sources (Eitenmiller et al., 2008). Milk storage in packages made from glass or other transparent materials is quite often practiced in Latvia. Cardboard packaging is also frequently used, and while it is not transparent it is certainly not always impermeable to light (Walstra et al., 2006). The intensity of light and lengths of storage time of products containing riboflavin and other photo-sensitive vitamins should also be chosen cautiously to minimize the losses of nutritional value of food products.

The oxidative stability of milk fat measured as PV changes

The oxidative stability of milk fat was measured by changes in PV during storage at the temperature of 60 °C. PV is a sensitive indicator of oxidative and photooxidative changes in fats and oils. PV shows the concentration of primary oxidation products – hydroperoxides and peroxides – in the fat, however these are highly unstable substances producing secondary oxidation products – mainly carbonyl compounds (Bosset et al., 1994). In our test, the main cause of oxidation was the influence of temperature (60 °C) and the partial contact of fat with air, but for a half of samples – also the contact with sunlight at the beginning of the ageing process.

As seen from Figure 2, the oxidation process of milk fat that was not affected by light was very slow. During the 25-day period, only slow changes in the PV or induction period were observed showing the relatively high oxidative stability of butter oil. Significant ($p<0.05$) differences between samples of the three groups were not established, and PV did not exceed 0.012% of iodine at the end of the 25-day period. The induction period of the butter oil samples from all the three groups initially unaffected by light and stored at 60 °C temperature was > 25 days. Such a good stability of milk fat can be explained by the fact that it has a relatively low polyunsaturated fatty acid content and high proportion of saturated fatty acids compared to many other edible fats. Milk fat also contains natural fat-soluble antioxidants – carotenoids and vitamin E (tocopherols and tocotrienols) that increases the induction period (MacGibbon and Taylor, 2006). Vitamin E functions as the primary antioxidant and as the peroxy free radical scavenger. It is the primary, lipid-soluble, autooxidation chain-breaking action mechanism antioxidant that combines actions with other lipid- and water-soluble antioxidants to provide an efficient defense against free radical damage (Eitenmiller et al., 2008). The induction period usually is very slow until hydroperoxides are formed. Initially antioxidants are consumed; after

this has been achieved, peroxides are first liberated and subsequently broken down to form perceptible amounts of flavour products (Walstra et al., 1999).

Concerning the samples of milk fat exposed to light – the oxidative deterioration was much faster. At the beginning fat oxidation possessed with low intensity (induction period) followed by rapid increase in hydroperoxide concentration (Fig. 3) accordingly to the theory (Kamal-Eldin and Yanishlieva, 2005). For each type of fat, the end of the induction period was determined by the tangent method. The tangents as linear function lines of the phase of active hydroperoxide formation (B) of CG and TG1, respectively, were drawn, and the crossing points with corresponding linear function lines of induction period (A) were found.

The established induction periods are showed in Table 3. The induction periods were 10.97 and 12.03 days, for the CG and TG1 respectively. The induction period of TG2 was > 14 days, because the phase of active hydroperoxide formation (exponential phase) during the 14-day period was not observed yet. Induction periods of milk fat samples from both trial groups were longer than induction period of control group's milk fat, and a tendency of the impact of antioxidant protection on fat stability through the enrichment of cow's diet by its antioxidant content was evident.

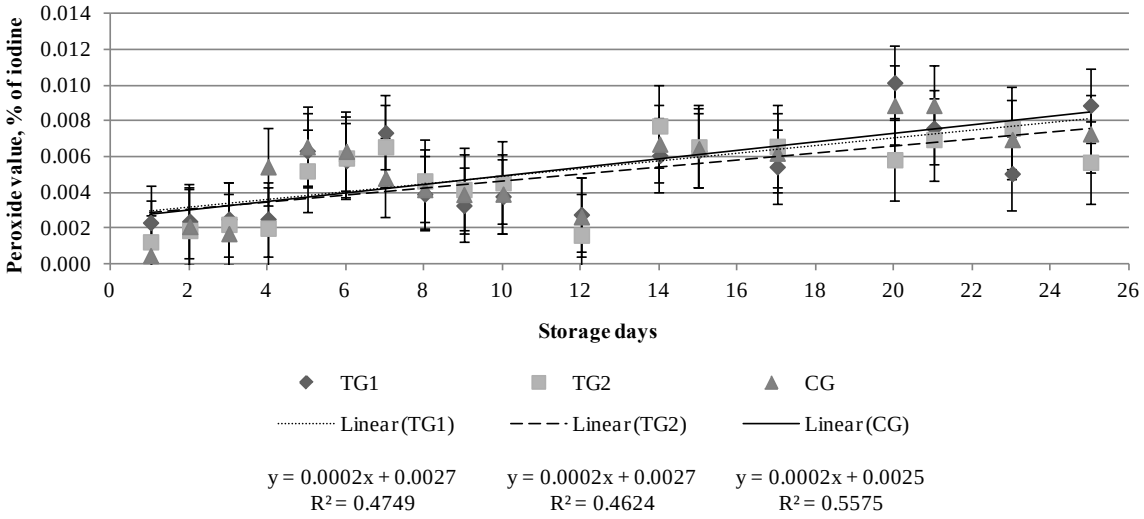

Figure 2. Peroxide value changes of milk fat stored in the dark.

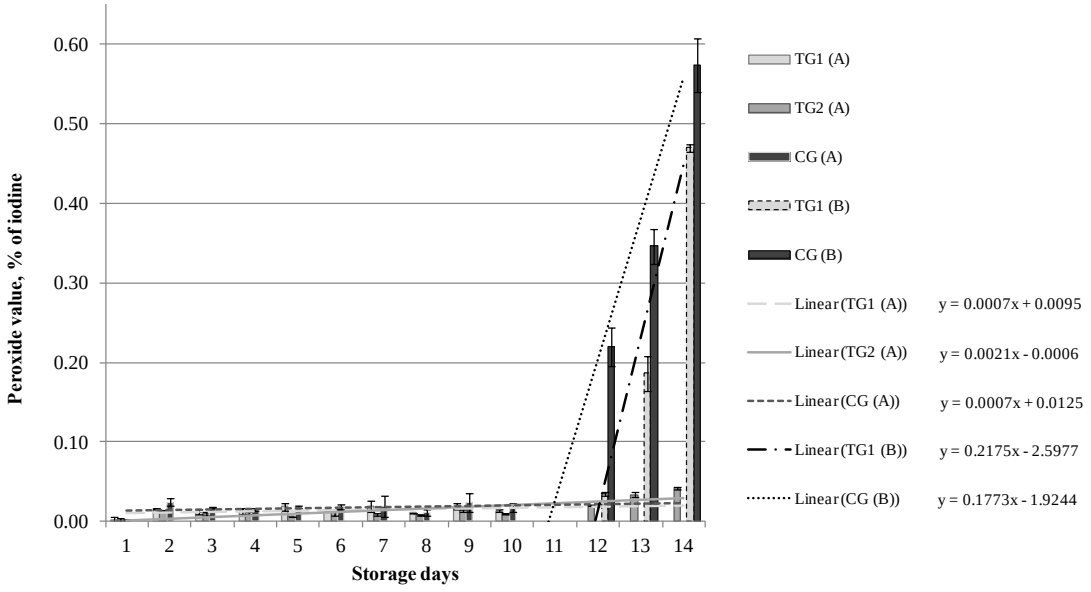

Figure 3. Peroxide value changes of milk fat exposed to light:
(A) – the induction period, (B) – the phase of active hydroperoxide formation.

Table 3

The established induction periods for the milk fat exposed to light

	Experimental groups		Control group
	TG1	TG2	CG
Induction period, days	12.03	> 14.00	10.97

The longest induction period and the highest ($p<0.05$) protection against lipid oxidation were observed in CAF-100-supplemented (TG2) cow milk fat, which can be related with increased tocopherol concentration in palm oil supplement and with the possible synergism between carotenoids and tocopherols in fat protection. The oxidative stability of the carrot-supplemented (TG1) group's milk fat was also higher compared to CG, showing the good potential of the enrichment of cow diet with carotenoids additives. The observed advantages of CAF 100 feed supplement were: longer shelf life, as well as easier storage and portioning compared to carrots. However, the benefits of carrots as feed supplements are that they are cheaper and locally grown vegetable products in Latvia.

Conclusions

1. A slightly and significantly higher ($p<0.05$) vitamin B_2 stability was seen in carrots-supplemented group (TG1) milk, where the losses of vitamin B_2 during 3-h sunlight exposure was by 3.01% less compared to CG.
2. The highest ($p<0.05$) protection against lipid oxidation was observed in CAF-100-supplemented cow milk butter oil.
3. The established induction periods for the fat samples initially affected by light and stored in 60 °C temperature for the CG and TG1 were 10.97 and 12.03 days, respectively. The induction period of TG2 was > 14 days.
4. The induction period of the butter oil samples from all the three differently fed cow groups initially unaffected by light and stored in 60 °C temperature was > 25 days and did not differ significantly.

Acknowledgments

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References

1. Antone U., Šterna V., Zagorska J. (2011) Potential to increase the stability of milk riboflavin against photo-oxidative degradation. *Proceedings of the 6th Baltic Conference on Food Science and Technology 'Innovations for Food Science and Production 'FOODBALT-2011",* LLU, Jelgava, pp. 50–54.
2. Bosset J.O., Gallmann P.U., Sieber R. (1994) Influence of light transmittance of packaging materials on the shelf-life of milk and dairy products – a review. In: Mathlouthi M. (ed.) *Food packaging and preservation,* 1st ed., Chapman & Hall, New York, pp. 230.
3. Carotino group (2006) Available at: http://www.soulproject.com.my/actualsite/carotinoMY/23.html, 16 February 2012.
4. *Dairy Science and Technology Handbook* (1993) Vol. 1: Principles and Properties. Hui Y.H. (ed.), VCH, New York etc., p. 25.
5. Eitenmiller R.R., Lin Ye W.O., Landen Jr. (2008) *Vitamin analysis for the health and food sciences.* 2d ed., CRC Press, Boca Raton, pp. 325–334.
6. Havemose M.S., Weisbjerg M.R., Bredie W.L.P., Nielsen J.H. (2004) Influence of feeding different types of roughage on the oxidative stability of milk. *International Dairy Journal,* 14, pp. 563–570.
7. Kamal-Eldin A., Yanishlieva N. (2005) Kinetic analysis of lipid oxidation data. In: Kamal-Eldin A. and Pokorny J. (eds) *Analysis of Lipid Oxidation,* AOCS Press, Champaign, Illinois, USA, pp. 234–237.
8. Kotecha P.M., Desai B.B., Madhavi D.L. (1998) Carrot. In: Salunke D.K. and Kadam S.S. (eds) *Handbook of Vegetable Science and Technology,* Marcel Dekker, NY, pp. 119–139.
9. Lee H., Jung Y., Kim Y. (1998) Effects of ascorbic acid on the light-induced riboflavin degradation and color changes in milks. *Journal of Agricultural and food chemistry,* 46, pp. 407–410.
10. MacGibbon A.K.H., Taylor M.V. (2006) Composition and structure of bovine milk lipids. In: Fox P.F., Mc. Sweeney P.L.H. (eds) *Advanced Dairy Chemistry,* 3rd ed., vol. 2, Springer, New York, NY, pp. 1–9.
11. Miller G.D., Jarvis J.K., McBean L.D. (2007) *Handbook of dairy foods and nutrition.* 3rd ed., National Dairy Council, CRC Press, Boca Raton, p. 11.
12. Mortensen G., Bertelsen G., Mortensen B.K., Stapelfeldt H. (2004) Light-induced changes in packaged cheeses - a review. *International Dairy Journal,* 14, pp. 85–102.

13. Namitha K.K., Negi P.S. (2010) Chemistry and biotechnology of carotenoids. *Critical reviews in Food Science and Nutrition,* 50, pp. 728–760.
14. Nozière P., Graulet B., Lucas A., Martin B., Grolier P., Doreau M. (2006) Carotenoids for ruminants: From forages to dairy products. *Animal Feed Science and Technology,* 131, pp. 418–450.
15. O'Connor T.P., O'Brien N.M. (2006) Lipid Oxidation. In: Fox P.F. and Mc. Sweeney P.L.H. (eds) *Advanced Dairy Chemistry,* 3rd ed., vol. 2. Lipids, Springer, New York, NY, pp. 557–585.
16. Trang V.T., Kurogi Y., Katsuno S., Shimamura T., Ukeda H. (2008) Protective effect of aminoreductone on photo-degradation of riboflavin. *International Dairy Journal,* 18, pp. 344–348.
17. van Aardt M., Duncan S.E., Marcy J.E., Long T.E., O'Keefe S.F., Nielsen-Sims S.R. (2005) Effect of antioxidant (a-tocopherol and ascorbic acid) fortification on light-induced flavor of milk. *Journal of Dairy Science,* 88, pp. 872–880.
18. Walstra P., Geurts T.J., Noomen A., Jellema A., van Boekel M.A.J.S. (1999) *Dairy Technology: Principles of Milk Properties and Processes.* Marcel Dekker, New York, pp. 58–62.
19. Walstra P., Wouters J.T.M., Geurts T.J. (2006) *Dairy Science and Technology,* 2d ed., CRC Press, Boca Raton, p. 415.
20. Zagorska J. (2007) The evaluation of organic milk quality. Summary of doctoral thesis for acquiring the Doctor's degree of Engineering Sciences in sector of Food Sciences, LLU, Jelgava, p. 48 (in Latvian and English).
21. Горбатова К.К. (2004) *Химия и физика молока* (Milk chemistry and physics), ГИОРД, Санкт-Петербург, 288 с. (in Russian).
22. Охрименко О.В., Горбатова К.К., Охрименко А.В. (2005) *Лабораторный практикум по химии и физике молока* (Laboratory practical classes on milk chemistry and physics), ГИОРД, Санкт-Петербург, 189 с. (in Russian).