

Stability of vitamin A and E in powdered cow's milk in relation to different storage methods

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Abstract. In this article, the influence of different ways of storage on the content of vitamin A and E in powdered cow's milk was studied. The cow's whole milk powder was taken directly from the manufacturer and stored for one year in 4 different ways – in the light at room temperature, in the dark at room temperature, in a refrigerator at 8°C and in a freezer at -20°C. The content of vitamins was measured 4 times during the first month and then once a month. The samples were stored for one year. Vitamins A and E were determined by HPLC using DAD and FLD detectors. Vitamin A was identified in all samples but only α -tocopherol (out of various forms of vitamin E) was detected in all samples. In all cases steeper decline of both vitamins in first 14 days of storage was identified. The highest losses of vitamin A and E in powdered milk occurred during storage in the light at room temperature. The value decreased by 91 resp. 95% of the original value.

Key words: stability, milk powder, storage, vitamin A, vitamin E.

INTRODUCTION

Milk in human nutrition has a nutritional, protective and detoxification function. It represents inter alia an important dietary resource of minerals and vitamins (Pánek, 2002). Convenient way to long-term storage of surplus milk is drying. Minimum durability extends this way up to two years.

Drying is a proven successful method of preserving with success also applicable to dairy products. Using this technological procedure obtain a product enabling economic transport of milk for a larger distance to areas, which are milk deficient. This product is easy to store and to recover. Powdered milk is stable due to the low water content, it is very slow in microbiological spoilage, oxidation and enzymatic processes. Dried milk products must be protected during storage, shipment and distribution from wetting or secondary contamination by microorganisms. Raw milk is considered a good source of vitamins A and E, but little information is known about the content of these vitamins in the long term storage of dried milk. In addition, the information is different (Ramalho et al., 2012, Yasmin et al., 2012, Michlová et al., 2015). The sustainability of these products depends on the type of product, quality, packaging, storage temperature and oxygen

content. It is known that, while the mineral content is stable over time, the content of vitamins is lost. Powdered milk is also an important commodity in international trade.

Vitamin A plays an important role in the biochemical pathways related to visual perception. It affects also the growth, differentiation and maturation of gametes, and is important for growth, fetal and bone development (Debier et al., 2005). It plays a crucial role in the synthesis of proteins, nucleic acids, and lipoproteins. Vitamin A is also an effective antioxidant. Vitamin deficiency is associated with vision disturbances (i.e. night blindness), inhibition of growth and deformities of bone and reproductive organs. High doses of vitamin A cause increased hepatic reserve. In pregnant women it may have teratogenic effects (Miller et al., 1998). According to Capita & Calleja (2006) the recommended daily dose of vitamin A ranges for an adult from 0.8 to 1 mg (2,600–3,300 IU) and for a child from 0.4 to 0.6 mg (1,300–2,000 IU).

Vitamin E is a very important antioxidant. It has a significant function in protecting the body against free oxygen radicals, which can lead to DNA damage. It is also a factor that slows down the ageing of the body and plays a role in the prevention of cardiovascular diseases and cancer (Eitenmiller & Junsoo, 2004). Vitamin E is present in food, being dissolved in fats, and is released and subsequently absorbed during its cleavage in the intestine. The recommended daily dose of vitamin E is reported as 10–15 mg (15–22 IU) for adult. This value is around 5–8 mg (7–12 IU) for child (Monsen, 2000). Vitamin E deficiency is often associated with disorders of fat absorption or distribution or with cystic fibrosis (Pekmezci, 2011).

Factors that affect the stability of vitamins in powdered milk vary depending on the monitored vitamin. As for the vitamins A and E the most important factors are heat, moisture, oxygen, light and pH (Ottaway, 2010).

The aim of our study was to determine the stability of these vitamins in powdered milk depending on different methods of storage during one year period and its subsequent use in nutrition.

MATERIALS AND METHODS

Experimental material

The vitamin A and E content was monitored in cow's whole milk powder, which had been obtained directly from the manufacturer (26% fat). The sample was divided into 4 x 16 parts. The first four of them were immediately analysed for the vitamin A and E content, and others were stored for one year in 4 different ways - in the light at room temperature, in the dark at room temperature, in a refrigerator at 8°C and in a freezer at -20°C. The content of both vitamins were analysed on the 7th, 14th, 21st, 28th, 59th, 90th, 120th, 151st, 181st, 212nd, 243rd, 274th, 304th, 335th, 365th days after storage. To ensure the homogeneity of the sample the reconstituted milk was thoroughly shaken for 2 minutes prior to the measurement.

Measurement of vitamin E and A content in milk samples

Vitamin A and vitamin E (or the individual tocopherols (T) and tocotrienols (TKT)) were determined by high performance liquid chromatography with spectrophotometric and fluorescence detection, respectively.

For the preparation of the analytical samples, the following standards and chemicals were used: DL- α -tocopherol, 98.2% (CALBIOCHEM, Canada), tocopherol set (CALBIOCHEM, Canada), retinol, > 99% (Sigma-Aldrich, Germany), pyrocatechol, > 99.5% (Sigma-Aldrich, Germany), potassium hydroxide, min. 85% (Lachema, Czech Republic), methanol, p.a., content 99.5% (Lachner, Czech Republic), hexane, clean min. 95.0%, Penta, Czech Republic, methanol, super gradient, content min. 99.9% (Lachner, Czech Republic) and treated distilled water (Milipore, France).

The content of both vitamins was extracted by the method of Sánchez-Machado et al. (2006) with minor modification. Approximately 1 g of reconstituted homogenized sample was weighted in a plastic tube with a lid. 200 ml of methanolic pyrocatechol (0.2 g ml^{-1}) and 5 ml 1M KOH was added. The mixture was vortexed for 20 seconds. The sample was saponified for 10 minutes on ultrasound. Then the mixture was vortexed again 20 seconds. 5 ml of hexane and 1 ml of distilled water were added to the mixture. The mixture was vortexed for 1 minute. Subsequently, 3 ml were taken from the upper hexane layer and evaporated until dry using a Büchi rotovapor R-215 (Büchi Labortechnik GmbH, Essen, Germany). The residue was dissolved in 0.5 ml of methanol and an aliquot was transferred through a nylon filter into 1 ml Eppendorf, which was kept for 30 minutes in the freezer (-20°C). The sample was centrifuged for 2 minutes (Eppendorf miniSpin plus, by 14.4 rpm) and drained off into a dark vial. Analysis was carried out using a High Performance Liquid Chromatograph Ultimate 3000 (Thermo Fisher Scientific, Dionex, Sunnyvale, CA, USA) with a quarternary pump, refrigerated autosampler, column heater, and FLD and DAD detector. Tocols and tocopherols in the sample were determined by HPLC-FLD under the following conditions: analytical column Develosil $5\mu\text{m}$ RP AQUEOUS ($250 \times 4.6 \text{ mm}$) (Phenomenex, Torrance, CA, USA), which allows the separation of all forms of tocopherols and tocotrienols (Fig. 1); precolumn Develosil $5\mu\text{m}$ C30 UG-100A ($10 \times 4 \text{ mm}$) (Phenomenex, Torrance, CA, USA); mobile phase methanol : dionized water ($97:3, \text{ v v}^{-1}$), HPLC super gradient methanol Lach-ner, Ltd. (Neratovice, Czech Republic) and water Milli-Q water, isocratic elution; flow rate 1 ml min^{-1} ; injection $10 \mu\text{l}$, column temperature 30°C ; detection FLD (excitation 292 nm, emission 330 nm).

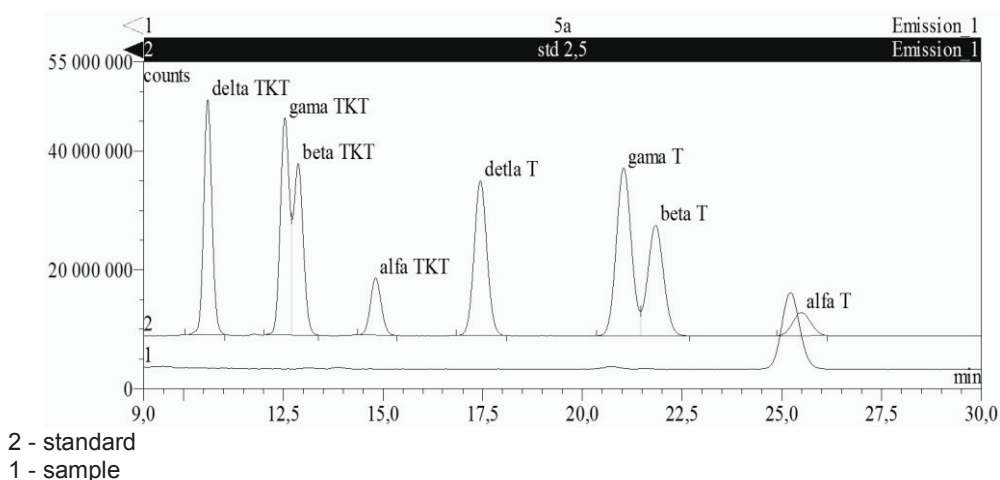


Figure 1. Chromatogram of vitamin E (compared to the standard).

Retinol was determined by the same chromatographic conditions using DAD detector ($\lambda = 325 \text{ nm}$) (Fig. 2).

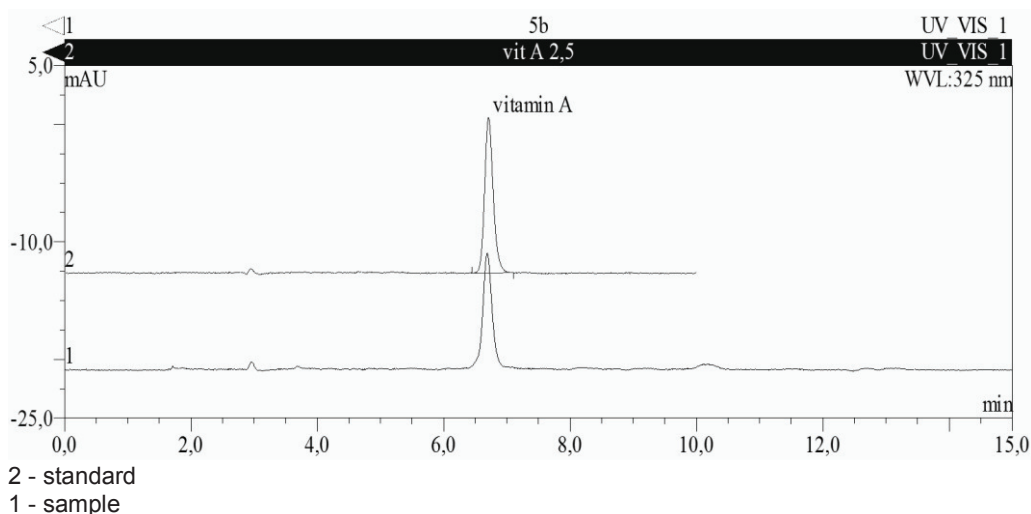


Figure 2. Chromatogram of vitamin A (compared to the standard).

The detection limit for vitamin A and each tocopherol and tocotrienol, expressed as a ratio of three times the value of the signal-to-noise ratio, were as follows: vitamin A $0.025 \mu\text{g ml}^{-1}$, δ -tocotrienol and δ -tocopherol $0.01 \mu\text{g ml}^{-1}$, β -tocotrienol, γ -tocotrienol, β -tocopherol and γ -tocopherol $0.025 \mu\text{g ml}^{-1}$, α -tocotrienol and α -tocopherol $0.05 \mu\text{g ml}^{-1}$ respectively. The results were processed with Chromeleon and MS Excel. All results were expressed as mean value (mg kg^{-1}) of three replicates.

RESULTS AND DISCUSSION

The content of vitamin E in freshly produced milk powder is about 1.5 times higher than the level of vitamin A. During storage, there was a decrease in the amount of both monitored vitamins. In addition, differences were observed in the decrease of particular vitamins.

Vitamin A

The decrease of vitamin A was almost identical with various ways of storage. The highest decrease occurred at the beginning of storage (about 14 days). In about 6–7 months of storage there was decline about 39% when stored in dark, 43% when stored in refrigerator and in freezer, and about 63% when stored in light. At the end of the storage period (1 year) there was a steep decrease in vitamin A. However, the swiftest decrease was detected by the storage in the light (about 51% during 28 days against 29–34% in the other cases). After one year of storage the value of vitamin A content in milk in all four cases of the storage was very similar and averaged 0.40 mg kg^{-1} (decrease by about 91%) (Fig. 3).

The effect of different storage conditions on the content of vitamin A is given by various authors. Frias et al. (2009) confirms that the storage period has considerable influence on vitamin A content. In their study value of vitamin A in milk powder decreased during 6 months of storage at 30°C by 68%. Their values are in good agreement with the data found in this study. Losses were determined by Chárvez Servín et al. (2008). They reported that vitamin A in milk powder at room temperature falls within the range from 5.4 to 28.9% after 70 days storage at room temperature (25°C). On the other hand, Duarte Fávoro et al. (2011) argue that vitamin A can be stored without significant losses when certain conditions are observed. The stability of vitamin A is dependent on production and storage conditions, e.g. absence of oxygen in contact with the packaged product or safe storage in the absence of light and at a temperature not exceeding 30°C. Boer et al. (2010) investigated the stability of vitamin A in powdered milk for 16 weeks at different temperatures in the dark and with exposure to fluorescent light. Samples stored in the dark lost 20–38% of the original value of vitamin A. The loss in the samples exposed to light was 70%. The highest vitamin loss occurred in this study during the first 10 weeks of storage. These data are consistent with the values reported in the present study.

As no significant differences were observed during storage of milk powder at room temperature neither in the dark nor in the refrigerator and the freezer, it is possible to store the milk powder in business and at home in conventional shelves. However, it should be stored in the dark and not in transparent plastic containers, since the influence of light occurs with the exception of end-stage monitoring significantly higher losses of vitamin A in powdered milk.

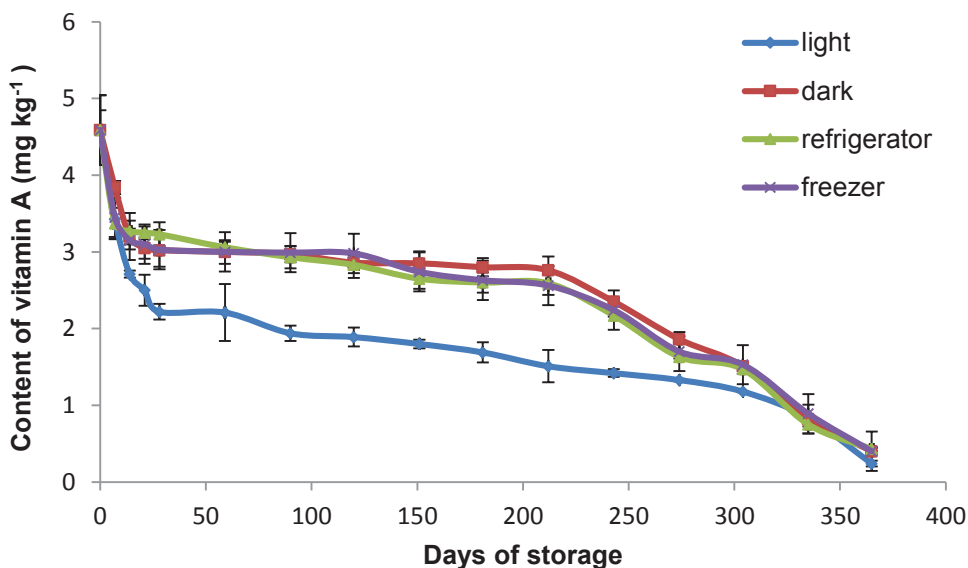


Figure 3. Average content of vitamin A in whole milk powder during different ways of storage.

Vitamin E

The amount of vitamin E also varies according to ways of storage. The highest decrease in the content of vitamin E occurs during storage of milk powder in the light at room temperature. In this way of storage of milk powder the vitamin E content dropped from the initial 6.61 mg kg⁻¹ to 0.19 mg kg⁻¹, which means an overall decrease of 97.1%. After six months of storage powdered milk contained about 57% of the original value of vitamin E when stored in dark, 48% when stored in refrigerator, 42% when stored in freezer and only 34% when stored in the light. In case of studied storage methods the level of vitamin E was at the end of the storage period approximately 0.50 mg kg⁻¹ (loss 92.4%) (Fig. 4).

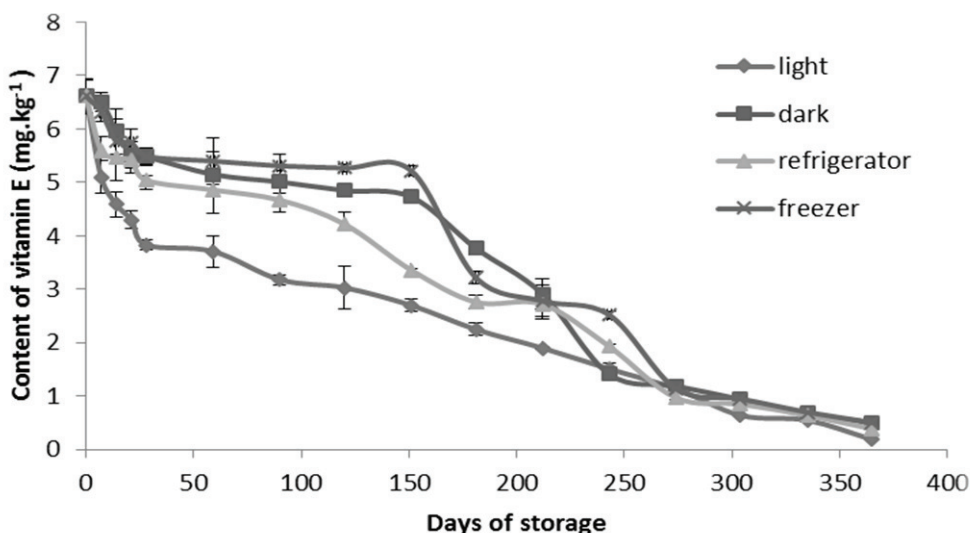


Figure 4. Average content of vitamin E in whole milk powder during different ways of storage.

The decrease in vitamin E during storage of powdered milk was also recorded by other authors. Valverde et al. (1993) claimed that loss of vitamin E occurs during the first month when stored at 30°C. After increasing the temperature from 30°C to 40°C the content of vitamin decreases even more. According to author, short time (60 days) of storage in the freezer does not cause any loss of vitamin E (resp. α -tocopherol). Losses occur in the course of 4 to 8 months of storage in the freezer. Their findings correspond with the results in this study, where the storage began at 25°C and the decrease of vitamin E up to 42% depending on the method of storage was found out. Lower losses were recorded by Chárvez Servín et al. (2008) who reported that vitamin E content in powdered milk decreases at room temperature in the range from 2.2 to 17.7% after 70 days storage at room temperature (25°C). Discussed results almost agree with the main findings of the study of Frias et al. (2009), who reported a reduction of vitamin E during the six-month storage from 3.98 ± 0.077 mg kg⁻¹ to 0.149 ± 0.004 mg kg⁻¹, which represents a decrease of 96% of the original value.

Decrease of the amount of vitamin E is slightly higher than the decrease of vitamin A under described conditions. A large drop in storage of vitamin E in the light may be

caused by considerable sensitivity to light of vitamin E. From the viewpoint of maintaining the quantity of vitamin E it is appropriate that the milk powder is stored in dark containers to prevent presence of light.

CONCLUSION

Significant differences in the content of vitamin A and E were found when four different ways of storage of whole cow's milk powder were used. The most rapid decrease in almost all cases was measured within the first 14 days of storage. The highest losses of vitamin A and E in powdered milk occurred during the storage in the light at room temperature. In other examined methods of storage (in the dark at room temperature, in the refrigerator and the freezer at -20°C) the content of vitamin A has declined almost identically, in contrast to the decline of vitamin E. The average reduction in vitamin A and E was 91%, resp. 95% after one year storage period. Powdered milk is a relatively good source of vitamin A and E about 5–6 months after production, but after 1 year of storage period, regardless of the storage method, is the content of both vitamins very low and this milk product cannot be recommended as a source of these vitamins in the diet.

To maintain the largest possible amounts of vitamins A and E, the powdered milk should be stored in dark containers and consumed before the expiry date of minimum durability, preferably within six months.

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