

## EVALUATION OF BUTTER OIL OXIDATIVE STABILITY AND NUTRITIONAL VALUE AFFECTED BY COW FEEDING

Unigunde Antone, Vita Sterna, Jelena Zagorska

Latvia University of Agriculture  
u.antone@gmail.com

### Abstract

Nutritional value and shelf life of milk and dairy products depend on the composition and stability of their constituents. The **aim** of the present study was to evaluate the effects of carrots as cow feed carotenoid source on butter oil fatty acid (FA) composition and oxidative stability. Milk was obtained from one trial group (TG; n=5) and one control cow group (CG; n=5) in a conventional dairy farm in Latvia. TG cows received carrots 7 kg per cow per day; the length of the supplementation period was 39 days. The stability of butter oil exposed to sunlight (3h) and held for 14 days in the temperature of 60 °C was analyzed by peroxide value method.

The changes of the FA content and ratios in TG milk fat were more positive with respect to the fat nutritional value as observed in CG – stronger increase in the content of polyunsaturated FA ( $p<0.05$ ), and in the ratio between stearic and palmitic acids ( $p<0.05$ ); also a tendency was seen of increasing content of short and monounsaturated FA, as well as decreasing the ratio between  $\omega 6$  and  $\omega 3$  FA groups.

Oxidative stability of the carrot supplemented cow group's milk butter oil samples that were collected after 25-day trial period, exposed to sunlight (3h) and stored at a temperature of 60 °C was significantly ( $p<0.05$ ) higher compared to CG samples. After the 39-day long trial period stability difference was not significant, despite the tendency that average polyunsaturated FA content in TG samples was higher compared to the control.

**Key words:** butter oil, fatty acid, peroxide value, carotenoids, cow feed, carrots.

### Introduction

Nutritional value and shelf life of milk and dairy products depend on the composition and stability of their constituents. Milk lipids confer distinctive nutritional, textural and organoleptic properties to dairy products, such as cream, butter, whole milk powder and cheese (MacGibbon and Taylor, 2006). However, the health benefits of milk fat is the cause for the debate among scientists that continues for years. Unbalanced fat composition with predominance of saturated fat is related to increased rates of heart disease (German and Dillard, 2006). Therefore trials are made to achieve a more healthful milk fat composition by altering the cow's diet (Bobe et al., 2007). Natural antioxidants – carotenoids are known well in health and food protection amongst which  $\beta$ -carotene is particularly involved in prevention of photo-oxidation, hindering unfavourable quality changes (Namitha and Negi, 2010). However, the potential of carotenoids is employed insufficiently in dairy. Carrots are one of the richest sources of carotenoids containing mainly  $\alpha$ - and  $\beta$ -carotenes (Kotecha et al., 1998). The **aim** of the present study was to evaluate the effects of carrots as cow feed carotenoid source on butter oil fatty acid (FA) composition and oxidative stability.

### Materials and Methods

*Experimental design.* Milk was obtained from one trial group (TG; n=5) and one control cow group (CG; n=5) 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 and May). The basic feed (equal in both groups) was haylage, mixed feed concentrate and hay. TG received 7 kg of carrots, additionally providing 145 mg of total carotenes per cow per day.

*Milk sample collection and storage.* Individual cow milk samples were obtained from the morning milking before the feed supplementation (D0) and on days 25 (D25) and 39 (D39) from the start of the feed supplementation. One bulk milk sample per each group was obtained pooling 5 L of individual cow's milk and transported to the laboratory.

*Butter oil extraction and storage.* Milk was warmed up to 40-45 °C subsequently separating cream with a milk separator to approximately 30% of fat content. Cream was ripened at 4-6 °C, for  $20 \pm 1$  h, than churned till the formation of butter. The buttermilk was removed and butter was rinsed with cold distilled water. Then butter was warmed up to 40-50 °C and centrifuged  $14360 \times g$ , for 10 minutes at 40 °C to separate the pure butter oil, that was used for fatty acid analysis or split into smaller (20 g) sub-samples for peroxide value analyses, e.g. fat was poured into the appropriate number of transparent plastic Petri dishes and subjected to direct sunlight at  $20 \pm 1$  °C for 3 h to hasten the fat ageing. Then, the samples were placed into thermostatic oven at  $60 \pm 1$  °C for 14 days.

*Peroxide value (PV) of the milk fat* was determined in accordance with the iodometric titration method

(Охрименко и др., 2005). The length of the induction period was established by setting the point of intersection of lines of linear functions corresponding to the induction period and active phase of peroxide and hydroperoxide development.

*Analyses of fatty acid (FA) composition were performed according to the method of Semporé and Bézard (1996) with some modifications.* Extracted butter oil was transesterified to methyl esters in a sodium methylate solution, e.g., 7-15 mg of the oil was mixed with 1 ml of hexane (with 50 ppm of butylated hydroxytoluene) and 10 µL of Na methylate solution (12.5 g 100 mL<sup>-1</sup>) (self-made, containing 5.32 g of sodium in methanol 100 mL<sup>-1</sup> solution (wv<sup>-1</sup>)), shaken 1 min, left for 10 min in 20 ± 3 °C and centrifuged for 5 min at 4 °C and 14360×g. The upper layer containing FA methyl esters was used for further analysis by gas-liquid chromatography using the ACME model 6100, GLC (Young Lin Instrument Co.) gas chromatograph fitted with the flame ionization detector, and a 30 m long, 0.25 mm i.d. Alltech AT-FAME analytical column. The carrier gas (He) flow rate was 2 ml min<sup>-1</sup>. The injector and detector temperatures were 225 °C and 250 °C, respectively. The oven temperature was programmed from 50 °C (4 min) till 170 °C at a rate of 8 °C min<sup>-1</sup> (held 15 min), till 240 °C at a rate of 6 °C min<sup>-1</sup>. Peaks were identified using standard mixture Supelco FAME Mix C4-C24, Sigma Aldrich. Results were evaluated with an integrator program (Autochro-2000, Young Lin Instrument Co.) The sum of FA groups' content was calculated according to the following formulas (1-6):

- **Saturated FA (SFA)** = sum of C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0, C24:0 (1)
- **Short and medium FA** = sum of C4:0, C6:0, C8:0, C10:0 (2)

- **Monounsaturated FA (MUFA)** = sum of C14:1, C15:1, C16:1, C18:1w9c, C18:1w9t, C20:1w9 (3)

- **Polyunsaturated FA (PUFA)** = sum of C18:2cw6, C18:2t, C18:3w6, C18:3w3, C20:4w6, C20:5w3, C22:5w3, C22:6w3; (4)

- **w3 FA** = sum of C18:3w3, C20:5w3, C22:5w3, C22:6w3 (5)

- **w6 FA** = sum of C18:2cw6, C18:3w6, C20:4w6 (6)

Changes (%) in fatty acid content or ratios in milk fat before and during the supplementation were calculated using the following formula (7):

$$\text{Changes} = \frac{(\text{FA content}_{\text{AVER}_{(D25, D39)}} \times 100)}{\text{FA content}_{D0}} - 100 (\%) \quad (7)$$

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.

*Chemicals* were of analytical or higher purity. Water was purified with Simplicity (Millipore SAS, France). Potassium iodide was from Stanchem, Poland, glacial acetic acid from Lach-Ner, Czech Republic, chloroform from Riedel-De-Haën, Germany, sodium thiosulphate from AVSISTA, Lithuania, sodium from Charlau Chemie, methanol and hexane (HPLC grade) from Chromasolv.

*Statistical analyses* were made using Microsoft Office program Excel and Microsoft Windows for SPSS (SPSS 17.0, SPSS Inc. Chicago, Illinois, USA). Differences between the groups were tested for significance (p < 0.05) by ANOVA.

## Results and Discussion

The FA content and ratios before and after the cow feed supplementation are given in Table 1.

Table 1

Content of FA groups and ratios before and after the cow feed supplementation

Sampling	Cow groups	Content of fatty acid groups (% of total FA)				Fatty acid ratios	
		short and medium FA	SFA	MUFA	PUFA	ω6 / ω3	C18:0 / C16:0
Before supplementation (D0)	TG (carrots)	9.22	74.80	18.81	1.54	2.21	0.11
	±SD	0.52	1.86	0.89	0.15	0.77	0.02
	CG (control)	10.38	73.62	20.30	1.70	1.82	0.13
	±SD	1.50	2.14	2.17	0.21	0.29	0.01
After supplementation (average result of the D25 and D39 samples)	TG (carrots)	9.37	71.05	21.11	2.08 <sup>a</sup>	1.77	0.16 <sup>a</sup>
	±SD	0.15	1.99	1.69	0.14	0.09	0.01
	CG (control)	9.79	71.56	21.25	1.92	1.71	0.17 <sup>a</sup>
	±SD	0.15	0.56	0.65	0.07	0.14	0.00

<sup>a</sup> – the difference between parameters before and after the supplementation is significant (p<0.05).

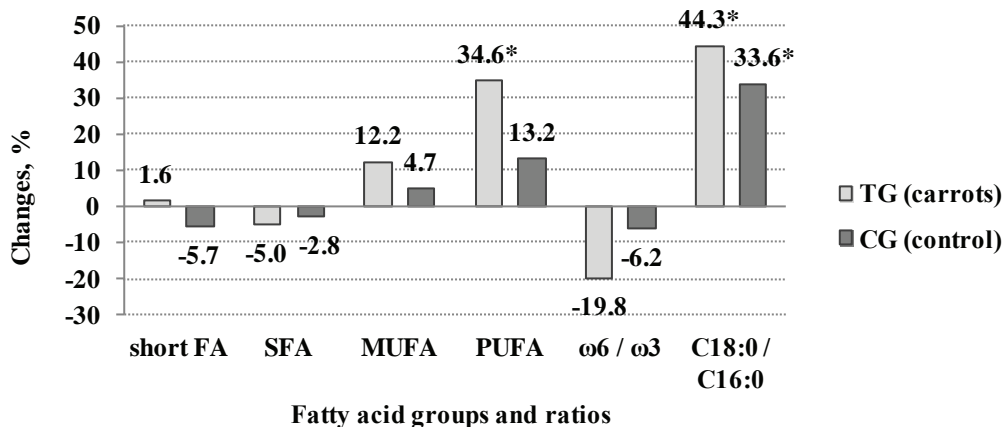


Figure 1. Changes in the fatty acid content and ratios in milk fat of cows fed differently  
\* – changes of parameters are significant ( $p < 0.05$ ).

The content of the short and medium FA, SFA, MUFA, and PUFA, as well as ratios between  $\omega 6 / \omega 3$  and C18:0 / C16:0 did not differ significantly between the groups before the feed supplementation (D0).

After the feed supplementation, there was a significant increase observed in the milk fat PUFA content of the trial group by 34.6%, as well as in the ratio of stearic and palmitic acids (C18:0 / C16:0) of both groups (see Fig. 1). The changes of stearic and palmitic acid ratio were more pronounced in the trial group. Other parameters did not change significantly in either group, however, the tendency of the mean value changes was more positive in the trial group with regard to the increase of short and medium FA, MUFA content, and the decrease of SFA content and ratio between  $\omega 6 / \omega 3$  FA.

In summary, the FA content and ratios changes in the trial group were more positive as observed in CG. Higher content of short and medium FA, MUFA, PUFA content in milk fat, as well as higher ratio between stearic and palmitic acids are considered to be more favorable for human health (Chillard et al., 2000; German and Dillard, 2006). The lower ratio of the  $\omega 6 / \omega 3$  FA also is considered to be favourable for the prevention of number of diseases (Gebauer et al., 2005)

To our knowledge, little studies have been carried out on FA composition of milk as affected by the supplementation of cow forage with carrots. In the study of Nałęcz-Tarwacka et al. (2003), cow feed was supplemented with 5 kg of carrots per cow per day. The supplementation was started at the beginning of the cow dry period till the 20<sup>th</sup> day after calving. Their observations about the positive changes of FA composition were similar to our results. There was a significant increase ( $p \leq 0.01$ ) in the content of linoleic acid, conjugated linoleic acid (CLA), as well as in MUFA and PUFA contents in milk. Also the content

of oleic acid increased significantly ( $p \leq 0.05$ ), but the content of lauric-, palmitic acids and the total SFA content decreased significantly ( $p \leq 0.01$ ).

The possible explanation of results can be related to the effects of carrot forage components as, for example, fiber or sugars, altering rumen biohydrogenation and milk FA synthesis processes in cows. The effect of carrot supplementation has to be investigated further. Many studies assure that milk FA composition is affected by feed components. For example, higher contents of CLA and C18:3n3 have been related to the use of grass-based forage rather than maize silage and to higher proportions of clover and other legume species than grasses (Larsen et al., 2010).

#### *Oxidative stability of milk fat*

The oxidative stability of milk fat was compared by PV changes affected by the initial storage of butter oil in the light (3h) and further storage at a temperature of  $60 \pm 1$  °C, and by measuring the concentration of primary oxidation products (hydroperoxides and peroxides) in the fat. The oxidative stability of food system can be characterized by the length of the induction period when low oxidation intensity is followed by rapid increase in hydroperoxide concentration (O'Connor and O'Brien, 2006).

The changes of the peroxide value of samples collected after 25-day feed supplementation period and stored in the light and at a temperature of  $60 \pm 1$  °C are represented in Fig. 2.

The oxidative stability of the fat depends on the FA composition, fat-soluble antioxidant content and other anti- and prooxidative factors (O'Connor and O'Brien, 2006). Its relation to the fat PUFA content was analyzed, however, the length of induction period also can be related to the antioxidant content of butter oil and other factors. The established induction

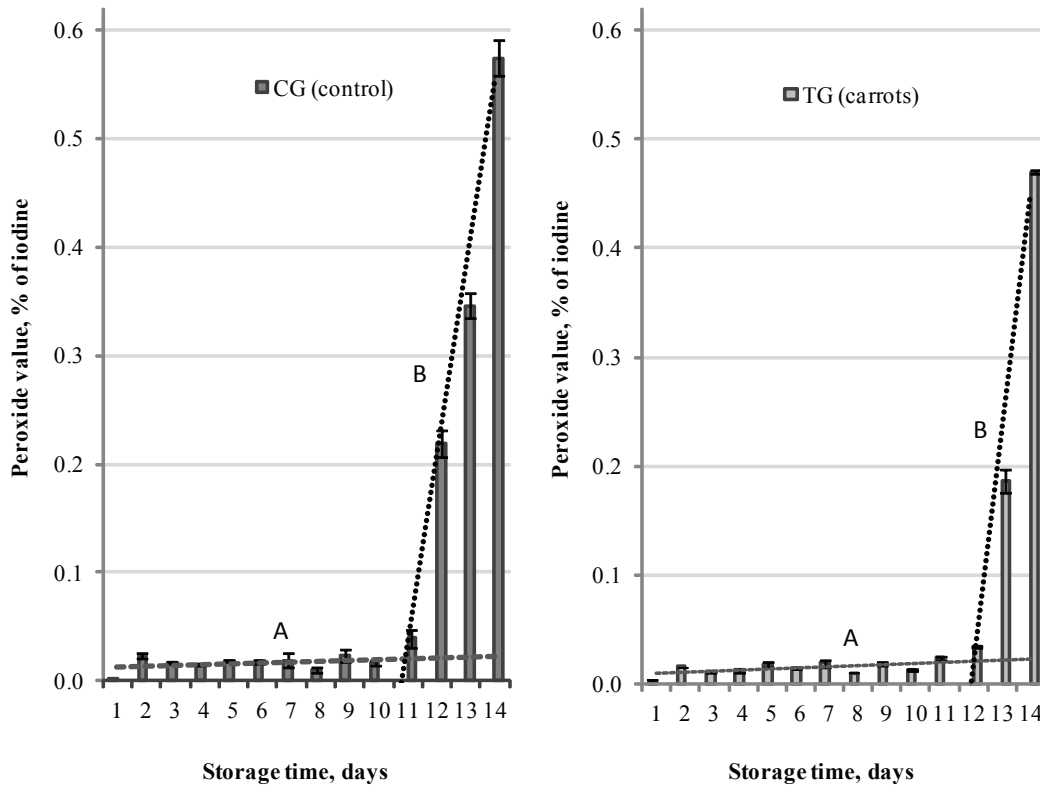


Figure 2. Changes of peroxide value of butter oil samples stored in light (3 h) and at 60 ± 1 °C A– induction period, B – active phase of peroxide and hydroperoxide development.

periods of light-affected butter oil samples and their PUFA content are showed in Tab. 2.

benefits of milk fat through its FA composition and stability improvement.

Table 2  
Induction periods established by PV method and PUFA contents of butter oil samples affected by light and stored at a temperature of 60 °C

Sampling days	PUFA content (mean ± SD), % of total FA		Induction period, days	
	TG	CG	TG	CG
D25	1.98±0.38	1.96±0.27	12.03	10.97
D39	2.17±0.24	1.87±0.23	12.00	11.85

After the 25-day trial period the induction period of TG samples was significantly (p<0.05) longer compared to CG. At the same time the PUFA content was similar in the butter oil of both groups.

Concerning D39 samples, the average PUFA content was higher in TG fat, consequently leading to a higher susceptibility to oxidation, however, the length of the induction period was insignificantly (p>=0.05) longer compared to CG. Therefore it can be assumed that cow feed enrichment with carotenoid supplements can leave a certain impact on health

**Conclusions**

1. A significant (p<0.05) increase of the polyunsaturated fatty acid content in the trial group’s milk fat by average 34.6% was observed after 25 and 39-day cow feed supplementation period with carrots compared to D0.
2. The changes in the fatty acid content and ratios in the trial group’s milk fat were more positive with respect to the fat nutritional value as observed in the control group – stronger increase in the content of polyunsaturated fatty acids (p<0.05), and in the ratio between stearic and palmitic acids (p<0.05); also the tendency was seen of increasing content of short and monounsaturated fatty acids, as well as decreasing the ratio between ω6 and ω3 fatty acid groups.
3. Oxidative stability of the carrot supplemented cow group’s milk butter oil samples that were collected after 25-day trial period, exposed to sunlight (3h) and stored at a temperature of 60 °C was significantly (p<0.05) higher compared to the control group samples. After 39-day long trial period the butter oil stability difference was not significant, despite the tendency of average

polyunsaturated fatty acid content in the trial group samples to be higher compared to the control.

#### Acknowledgments

This investigation was financially supported by the Latvia Council of Science project No.

09.1561 *Investigations of lipid composition and enzymes of cow's milk, their role in quality and functionality of milk products*, and co-funded by the European Social Fund through the project 1.1.2.1.2. *Support for doctoral studies in LLU* (Agreement 2009/0180/1DP/1.1.2.1.2/09/IPIA/ VAAA/017).

#### References

1. Bobe G., Zimmerman S., Hammond E.G., Freeman A.E., Porter P.A., Luhman C.M., Beitz D.C. (2007) Butter composition and Texture from Cows with Different Milk Fatty Acid Compositions Fed Fish Oil or Roasted Soybeans. *Journal of Dairy Science*, 90, pp. 2596-2603.
2. Chilliard Y., Ferlay A., Nansbridge R.M., Doreau M. (2000) Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated, trans and conjugated fatty acids. *Annales De Zootechnie*, 49, pp. 181-205.
3. Gebauer S., Harris W.S., Kris-Etherton P.M., Etherton T.D. (2005) Dietary n-6: n-3 Fatty Acid Ratio and Health. In: *Healthful lipids*. AOCS Press, CRC.
4. German J.B., Dillard C.J. (2006) Composition, Structure and Absorption of Milk Lipids: A Source of Energy, Fat-Soluble Nutrients and Bioactive Molecules. *Critical reviews in Food Science and Nutrition*, 46: pp. 57-92.
5. Kotecha P.M., Desai B.B., Madhavi D.L. (1998) Carrot. In: Salunke D.K., Kadam S.S. (eds) *Handbook of Vegetable Science and Technology*, Marcel Dekker, NY, pp. 119-139.
6. Larsen M.K., Nielsen J.H., Butler G., Leifert C., Slots T., Kristiansen G.H., Gustafsson A.H. (2010) Milk quality as affected by feeding regimens in a country with climatic variation. *Journal of Dairy Science*, 93, pp. 2863-2873.
7. MacGibbon A.K.H., Taylor M.V. (2006) Composition and structure of bovine milk lipids. In: Fox P.F. and Mc. Sweeney P.L.H. (eds) *Advanced Dairy Chemistry*, 3<sup>rd</sup> ed., vol. 2, Springer, New York, NY, pp. 1-9.
8. Nałecz-Tarwacka T., Karaszewska A., Zdziarski K. (2003) The influence of carrot addition to cow's ration on the level of vitamins and fatty acids in cow milk. *Polish Journal of Food and Nutrition Sciences*, 12/53 (2), pp. 53-56.
9. Namitha K.K., Negi P.S. (2010) Chemistry and biotechnology of carotenoids. *Critical reviews in Food Science and Nutrition*, 50, pp. 728-760.
10. 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*, 3<sup>rd</sup> ed., vol. 2. Lipids, Springer, New York, NY, pp. 557-585.
11. Semporé B.G., Bézard J.A. (1996) Analysis of neutral lipids: fatty acids. In: Nollet L.M.L. (ed) *Handbook of Food Analysis*, vol. 1. New York: Marcel Dekker, pp. 331-394.
12. 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).