Danish Research Centre for
Organic Food and Farming

Final Report

For DARCOF II research projects financed by grants from
The Directorate for Food, Fisheries and Agro Business
under the Danish Ministry of Food, Agriculture and Fisheries

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1. Research program

Research in organic farming 2000-2005 (DARCOF II)

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2. Project title and number

Production of organic milk of high quality considering the future demands for use of organically produced feed and natural vitamins (II-2)

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3. Head of project

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4. Participating institutes

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Slutrapporten sendes elektronisk til Forskningscenter for Økologisk Jordbrug *foejo@agrsci.dk* senest 3 måneder efter projektets afslutning.

Slutrapporten vedlægges et dansk resumé.

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6. Project period (month, year)

Start of project: 01 2002
End of project: 12 2006

7. Final report

A. Project summary

Future regulations for organic production of milk stipulate that all feed must be organically produced, and that the cow can only be fed natural vitamins and antioxidants. This leads to feeding changes of such importance that it must be presumed to influence the antioxidative capacity and thus the shelf-life of the milk. The project aims at elucidating the nature of the consequences that these new regulations for organic feeding and use of natural vitamins and antioxidants for cows in organic milk production will have on the antioxidative capacity of the milk. New studies show that uric acid in milk is an important antioxidant of significance for the oxidative stability of milk. During the project, it is the aim to increase the supply of selenium and the development of endogeneous antioxidants (uric acid and glutathione peroxidase) in order to produce oxidatively stable milk and dairy products.

Table A.1: Work package list (from application)

No.	Work package title	Participants*	Budget (1.000 DKK)	Start	End	Deliverable no(s)
1	Antioxidative capacity of raw milk	<u>Jacob Holm Nielsen</u>	1668	0102	1205	D1-D11
2	Cheese production and oxidative stability of cheeses with high levels of uric acid and selenium	<u>Leif H. Skibsted</u>	330	0104	1205	D5, D8 &D12

* Responsible participants are underlined

B. Objectives and expected achievements

To elucidate the effect of introducing new rules for organic feeding and use of natural vitamins and antioxidants on the antioxidative capacity of the milk from cows producing milk under organic conditions. Furthermore to increase the selenium supply and the formation of endogenous antioxidants (uric acid and glutathione peroxidase) of the cow in order to produce oxidatively stable milk and dairy products.

The aim consists of the below intermediate aims:

- To study the variation in content of pro- and antioxidants in organic milk from a number of herds in relation to regional variation, feeding and season
- To study whether the natural isomer of vitamin E is a more effective antioxidant than the synthetic isomer of vitamin E
- To study whether the natural carotenoid content in milk has an antioxidative effect
- To study whether cheese exposed to light shows improved antioxidative characteristics if it is based on milk with a high uric acid content
- To make guidelines on how to improve the oxidative stability of the organic milk through feeding

Achievements

The project aims at investigating the possibility to create processed dairy products (cheese) with an oxidative stability up to the same standards as conventionally produced dairy products. The project will introduce the possibility of using endogenously formed antioxidants (uric acid and glutathione peroxidase) and natural vitamin E to improve shelf-life of organic dairy products. The project is holistic and comprises the chain from stable to table. It involves inter-institutional cooperation and is based on expertise at both KU and at AU.

C. Progress and results

C.1 Description (summary) of main results and conclusions

Glutathione peroxidase activity in milk
A method for analysis of the selenium-dependent glutathione peroxidase (GPX) in milk has been developed. It has not been possible to detect any specific activity of these enzymes in milk. We have tried to induce expression of GPX in milk through a feeding experiment where dairy cows were fed seleno-methionine. The amount of selenium increased with a factor 5, however, it was still not possible to detect any GPX activity in milk. Further, there is very little endogenous glutathione in milk to be used as reducing substrate for glutathione peroxidase. In fact, glutathione added to milk is rapidly metabolized, probably by the concerted action of γ-glutamyl transpeptidase and sulfhydryl oxidase. Thus, if activity of GPX is of importance for the oxidative stability of milk, alternative substrates must be utilized. The results have been published in International Dairy Journal.

For the first time glutathione S-transferase (GST) has been isolated and identified (by MALDI-TOF MS) in milk. This enzyme is normally synthesized in the liver, and recent results where cows were fed a high content of tannins (often used by organic farmers to increase bypass of proteins in the rumen of the cow) indicate that this induces a higher level of GST in the milk. This enzyme is a potential candidate as antioxidant in milk; however, as GPX will it need glutathione as substrate. A paper on this subject will be published in the near future.

Development of a method to detect stereoisomers of α-tocopherol
A method for detection of the stereoisomers of α-tocopherol in milk has been developed. It was possible to extract α-tocopherol from the milk and afterwards derivate the stereoisomers of α-tocopherol with ethylenglycol dimethylether and dimethylsulphate as described by Riss *et al.* (1994). The derivates of the stereoisomers were separated by chiral-chromatography and separated into five peaks, where the first one was all of the *2S*-stereo-isomers and the next four peaks were the *2R*-stereo-isomers in the following order: *RSS*-, *RRS*-, *RRR*- and *RSR*-α-tocopherol. By this method we have been able to differentiate between the four *2R*-stereo-isomers of all-rac α-tocopherol and the *2S*-stereo-isomers. It was found that milk from cows contains about 85-95% *RRR*-α-tocopherol and about 5-15% of the synthetic *2R*-stereo-isomers (*RSS*-, *RRS*- and *RSR*-α-tocopherol). It was not possible to find any *2S*-stereo-isomers in milk from cows.

Content of synthetic stereoisomers of α-tocopherol in milk
Organic and conventional milk from retail were examined for the content and distribution of the stereoisomers of α-tocopherol. The results indicate that the ratio of *RRR*-α-tocopherol was higher in organic milk (92-97%) compared with conventional milk (85-87%). This result indicates that the use of synthetic α-tocopherol was higher in the conventional milk production. Furthermore the results have provided a basis for a survey of the composition of organic and of conventional milk.

Survey of composition of conventional and organic milk from a Danish dairy plant

In the conventional milk production in Denmark, the use of corn silage has increased during recent years at the expense of the use of grass silage. This change in feeding strategy has affected the composition of the milk in a direction, where milk fat is more saturated while the content of important antioxidants as tocopherols and carotenoids has declined. However, in the organic milk production the use of grass silage is still very important, and on this background we have found it interesting to make a survey of the general composition of untreated conventional and organic raw milk samples collected from silo tanks on organic and conventional dairy plant. The milk has been examined for the content of the following important pro- and antioxidants: Fatty acid composition, Tocopherols and Carotenoids. The results clearly show that organic milk has a lower content of the synthetic isomers of α-tocopherol, and organic milk contains significantly higher concentrations of *RRR*-α-tocopherol, β-carotene, lutein and zeaxanthine, and there is no significant difference in the content of CLA in the two milk types. A paper on this subject is about to be submitted.

Detection of α-tocopherol in isolated MFGM

Data from oxidation studies indicate that α-tocopherol in the milk is located in different compartments. To investigate and explain these observations we have isolated the milk fat globule membrane (MFGM) from the core of the milk fat globule, and the fatty acids and α-tocopherol are being quantified in the membrane and in the core of the globule prior and after light oxidation. It seems like there is more unsaturated lipid in the membrane than in the triglycerides of the core of the fat globule. There is also a tendency towards protection of α-tocopherol in the membrane of the fat globule by light, when the milk has been exposed to light for 24 hours. It is suggested that storage of the milk at 4°C makes the milk fat more crystalline, so movement between the membrane and the core of the fat globule and within the membrane is restricted. No more research will be done in this area in this project.

Metal catalysed- and photooxidation in milk

A preliminary study of metal catalysed oxidation and photooxidation in milk has been conducted. When the milk was added copper alone there was a little accumulation of hexanal, but no degradation of α-tocopherol. When the milk was both added copper and exposed to light, the hexanal accumulation was somewhat higher, but there was still no degradation of α-tocopherol. Was the milk exposed to light alone, the greatest accumulation of hexanal and the greatest degradation of α-tocopherol was observed. It is necessary to perform further studies of the oxidative mechanism of α-tocopherol to understand the observations – why was there no degradation of α-tocopherol observed, when copper was in place?

Oxidation in milk with high and low content of α-tocopherol

An experiment was conducted to investigate the effect of synthetic α-tocopherol on the oxidative stability of milk. The content of the α-tocopherol was determined in the milk from cows, which had been fed with normal feed (barley whole crop 10%, rapeseed cakes 8%, maize silage 36%, grass silage 34%, rolled barley 6%). The standard supplementation of the feed with *all-rac*-α-tocopheryl acetate was withdrawn in a six day wash-out period followed by a supplementation period of three days with high concentrations of α-tocopherol of approximately 10 times the normal dose (2600 IU per day of *all-rac*-α-tocopheryl acetate). Milk samples from the morning milking were collected on the last day of the wash-out period (day 0) and on the last day of the supplemental period (day 3). The milk samples from the four cows in each sample day were pooled and analysed for the content of α-tocopherol. These two pooled milk samples, before and after supplementation, were exposed to fluorescent light at 2200 lux, and the development of the oxidation product hexanal and the degradation of α-tocopherol were followed for 24 h at 4°C. The secretion of α-tocopherol to the milk was found to vary between the individual cows. The content of α-tocopherol in the milk was declining during the period where the feed was depleted for synthetic α-tocopherol, and the content of α-tocopherol in the milk increased when synthetic α-tocopherol (2600 IU) was added to the feed again.

The ratio of the synthetic *2R*-stereo-isomers (*RSS*-, *RRS*- and *RSR*-) in the milk was found to decrease during the depletion period of vitamins from the feed. However, after supplementation of 2600 IU per day of synthetic α-tocopherol to the feed it increased again. When the feed did not contain supplemental *all-rac*-α-tocopheryl acetate, the proportion of the synthetic *2R*-stereo-isomers of α-tocopherol (*RSS*-, *RRS*- and *RSR*-α-tocopherol) was 2.4%, 2.9% and 2.1%, respectively, which was rather low. The native *RRR*-α-tocopherol dominated in the milk with 92.6% of the total α-tocopherol content. When the feed was supplemented, the contributions of the three synthetic *2R*-isomers were increased to 6.4%, 4.9% and 4.4%, respectively. None of the *2S*-stereo-isomers were detected in the milk before or after supplementation by *all-rac*-α-tocopheryl acetate.

It was found relevant to investigate the oxidative stability of milk with high (approx 600 µg/L) and low (approx 400 µg/L) content of α-tocopherol. Milk with high and low content of α-tocopherol was exposed to fluorescence light with an intensity of 2200 lux for 24 hours, and the degradation of α-tocopherol and accumulation of hexanal in the milk was followed for 24 hours.

The formation of hexanal was not significantly different (P=0.79) in the two milk types during the storage period under fluorescent light, indicating that the high concentration of α-tocopherol could not inhibit the lipid oxidation. The α-tocopherol concentration in milk sampled before and after dietary α-tocopherol supplementation declined over the 24 h storage period. This decline was apparently largest in milk from cows given supplemental *all-rac*-α-tocopherol. Nevertheless, the final α-tocopherol concentration was still significantly higher in milk taken from cows after supplementation (P<0.01).

These new results can explain results of previous studies, in which cows were given a dietary supplementation of *all-rac*-α-tocopheryl acetate without any consistent increase in the α-tocopherol concentration in milk. Accordingly, it may be concluded that supplementation of the feed with *all-rac*-α-tocopheryl acetate is an ineffective way to increase the content of α-tocopherol in milk, and it can be concluded that the supplementation of the feed with 2600 IU day^{-1} of *all-rac*-α-tocopheryl acetate is not capable of improving the oxidative stability of milk.

Oxidation in milk with high content of unsaturated fatty acids and high content of α-tocopherol

Based on the conclusion from the experiment above, i.e. that there was no effect of supplementation by *all-rac*-α-tocopheryl acetate on the oxidative stability of milk despite different levels of α-tocopherol present, a new experiment was designed in which dairy cows received feed with a high content of unsaturated fatty acids. The feed was depleted of *all-rac*-α-tocopheryl acetate supplementation for a longer period prior to supplementation with higher amounts by *all-rac*-α-tocopheryl acetate than in the first experiment. Three Holstein cows in mid lactation were fed a diet with toasted soy beans as a feed with high content of unsaturated fatty acids, and no supplementation was given for 26 days, followed by a period of 16 days in which the feed was supplemented with 3400 IU of *all-rac-α*-tocopheryl acetate per day. The feed consisted of grass silage [77.2%], barley straw [2.8%], dried beet pulp [7.4%], oats [4.2%], toasted soy beans [8.1%] and minerals [0.24%]. The milk was collected just before supplementation (day 0) and 16 days after supplementation (day 16) had begun. Again the milk from each sample day was pooled, analysed for the content of *α*-tocopherol and sub-sampled. The sub-samples were subjected to two pro-oxidative treatments. One milk sub-sample was exposed to fluorescent light at 2200 lux for 24 h at 4°C, and one milk sub-sample was mixed with copper(II) sulphate to an end concentration of 25 µM for 24 h at 4°C in order to initiate oxidation. The development of hexanal and the degradation of *α*-tocopherol were followed in these two sub-samples during the 24 h storage period. The depletion of ascorbyl radicals in the milk with added copper(II) sulphate was also followed but with an concentration of 100 µM of copper(II) sulphate.

Fatty acid concentrations in the pooled milk samples before and after supplementation by *all-rac-α*-tocopheryl acetate for both experiments were not different. However, there was a clear effect of the feeding strategy in this experiment, where the content of the unsaturated fatty acids, especially oleic acid (C18:1; $P<0.001$) and linoleic acid (C18:2; $P<0.001$), was significantly higher than in the first experiment.

Before supplementation the content of *α*-tocopherol in the pooled milk samples was approximately 700 µg L^{-1}. After 7 days of supplementation, the content of *α*-tocopherol in the milk had increased to approximately 800 µg L^{-1}, and the content of *α*-tocopherol remained constant throughout the rest of the feeding experiment. This gave a total increase of 29% of *α*-tocopherol after supplementation.

The longer depletion period prior to the period of supplementation in the second experiment compared with the first experiment and the different types of feed affected the distribution of *α*-tocopherol stereo-isomers. The *RRR-α*-tocopherol isomer in the second experiment constituted as much as 97.1% of the total *α*-tocopherol in the milk. The remaining 2.9% of synthetic stereo-isomers could have originated from the small amount of *α*-tocopherol present in the mineral supplementation, determined by analysis in our laboratory and which was not declared on its recipe. After 16 days of supplementation by *all-rac-α*-tocopheryl acetate the ratio of the synthetic *α*-tocopherol stereo-isomers increased to 11.2% of the total *α*-tocopherol content in the milk. This was less than in the first experiment; it should be noted that the concentration of natural *α*-tocopherol in the feed was higher in the soy bean-based feed compared with the feed used in the first experiment. Furthermore, it should be emphasized that none of the *2S*-stereo-isomers was detected in the milk in any of the experiments.

The three cows received on average 907 mg natural *α*-tocopherol from the feed per cow per day and 2151 mg *all-rac-α*-tocopherol from the vitamin supplement, which gave a total of 3058 mg *all-rac-α*-tocopherol per cow per day. The distribution of the *α*-tocopherol stereo-isomers in the *all-rac-α*-tocopheryl acetate supplement was as follows: 269 mg day^{-1} for the *RRR*-stereo-isomer, 807 mg day^{-1} for the synthetic *2R*-stereo-isomers and 1075 mg day^{-1} for the *2S*-stereo-isomers. Based on this estimated intake, the expected distribution of the *α*-tocopheryl stereo-isomers in milk would be 38.5%, 26.4% and 35.1%, respectively, provided that there was no discrimination between the stereo-isomers. However, the actual distribution found for the isomers in the milk was 88.8%, 11.2% and 0.0%, respectively, again indicating a high degree of discriminative absorption.

The accumulation of hexanal during 24 h of exposure to fluorescent light at 2.200 lux was significantly lower in the milk without any supplementation by *all-rac-*α-tocopheryl acetate, compared with the accumulation of hexanal in milk produced after supplementation (P<0.001). This was in marked contrast to the results obtained in the first experiment. The content of α-tocopherol decreased more rapidly in the milk from cows receiving the supplemented feed (P<0.001). During the 24 h storage period the levels approached each other and were not significantly different after 24 h, irrespective of whether the cows had been receiving *all-rac-*α-tocopheryl acetate supplementation or not.

In milk stored for 24 h with added copper(II) sulphate there was a significantly higher accumulation of hexanal in milk collected after 16 days of supplementation compared with the milk collected before supplementation (P<0.001). Only a small decrease in the content of α-tocopherol was detected in both milk types. The content of α-tocopherol in the milk collected after 16 days of *all-rac-*α-tocopheryl acetate supplementation was initially higher, but in milk collected after 16 days of supplementation following initiation of oxidation with Cu^{2+} the decrease in the content of α-tocopherol during storage was significantly lower (P<0.01).

Cu^{2+} induces oxidation and radical formation in the aqueous phase of the milk and in order to monitor those processes, the formation and subsequent depletion of the ascorbyl radical were followed by ESR-spectroscopy. The initial rate of depletion of the ascorbyl radical was higher in the milk from cows receiving the supplement than in milk from cows not receiving the supplement. Furthermore, the time required for complete depletion of the ascorbyl radical was shorter in milk collected from the cows receiving supplement by *all-rac-*α-tocopheryl acetate (13 min) than in the milk collected prior to supplementation (17 min; P<0.05). There were no ascorbyl radicals present in either of the two milk types after 5 h at 4°C, when the milk had copper(II) sulphate added to an end concentration of only 25 µM.

The results of this experiment and the one described before are accepted for publication: “The difference in transfer of *all-rac-*α-tocopherol stereo-isomers to milk from cows and the effect on its oxidative stability” by Tina Slots, Leif H. Skibsted and Jacob Holm Nielsen.

Oxidation in milk with supplement of natural α-tocopherol to the feed

In a study where the cows have received supplement of the natural isomer of α-tocopherol and selenium as seleno-methionine, the uptake or excretion of α-tocopherol and β-carotene seems to compete, when the content of β-carotene in milk, where the cows have received α-tocopherol supplement to the feed, is lower than in the milk, where the cows didn’t receive any supplement. Selenium seems to have a positive effect on the uptake or excretion of β-carotene. Furthermore have selenium no effect on the uptake or excretion of α-tocopherol, when the content of α-tocopherol is the same in these two milk types. There are no significant differences between the oxidation products of the fatty acid oxidation and lipid hydroperoxides, in the milk, when the milk is exposed to fluorescent light at 2,000 lux.

Survey of milk composition in relation to shelf life in Danish milk

The objective of the study was (i) to compare the nutritional composition of milk produced in organic and conventional production systems taking into consideration: different proportions of grass or grass silage, maize silage and different amount of concentrate (cereals), and (ii) to define the variation in content of pro- and antioxidants in milk in relation to regional variation, feeding and season. There is overall a positive correlation between the content of pasture in the cows' feed and the content of β-carotene in the milk. There is also a positive correlation between the amount of grass silage in the feed and the content of α-tocopherol in the milk. High amounts of cereal in the feed promote de novo syntheses of the short chain fatty acids in the udder, and the amount of the short chain fatty acids in the milk correlate positive with the amount of corn in the feed. Unsaturated fat from for example pasture is contributing to high amount of CLA and trans fatty acids in the rumen, and these contributes to a low fat percent in the milk, when CLA and the trans fatty acids contribute to low de novo syntheses. Pasture is positively correlated to unsaturated fatty acids as linolenic acid and CLA but opposite correlated to the fat percent of the milk.

Pasture, grass silage and cereals are counting for a high part of the feed for the organic cows, where the feed for the conventional cows mainly consist of maize silage, concentrate, by-products, minerals and hay/straw, and this correlation is also seen in these data. Organic milk contains more α-tocopherol, RRR-α-tocopherol and β-carotene than conventional milk, and these parameters have an opposite correlation to the incident of mastitis and to the overall health status. Many mastitis incidents and high negative health status are mostly widespread between the conventional cows. There is variation over the season for the organic farms especially in respect to the composition of the feed and composition of the milk.

Survey of milk composition in relation to shelf life in milk from DK, SE and UK

This survey had the same objective as the Danish survey. The feeding regime from 25 farms from Denmark, 20 farms from Sweden and 25 farms from United Kingdom was registered and milk samples were collected and analysed for the content of antioxidants and fatty acid composition. There were significant differences in the feeding regimes of the milk production systems between Denmark, Sweden and United Kingdom. The organic and conventional low input milk production in United Kingdom used significantly more pasture than organic and conventional milk production systems in Denmark and Sweden and in the conventional milk production in United Kingdom. Particularly, the feed in the conventional low input milk production system nearly consisted of pasture alone. The organic and conventional milk production in Denmark used very high amounts of maize silage, compared to the two systems in Sweden (P<0.0001). Cereals made up a large part of the feed in organic and conventional milk production systems in Sweden compared to Denmark and United Kingdom (P<0.0001) and the amount of concentrates was high in all systems except in organic and conventional low input production systems in United Kingdom. Overall, milk production systems of the United Kingdom used high amounts of pasture, and the milk production systems in the Scandinavian countries used high amounts of grass silage, maize silage, and cereals. The daily milk production in the conventional low input and organic milk production systems in United Kingdom was significantly lower than in the other five milk production systems evaluated in the survey (P=0.0025). This agreed with the literature, which emphasize that a high amount of pasture in the diet derives a reduced daily milk yield. When looking at the vitamins and antioxidants, as α-tocopherol and carotenoids, the content in milk from organic and conventional low input production systems in United Kingdom were significantly higher than in other production systems (P=0.0218). As emphasized in the literature, milk from production systems using high amount of pasture, such as milk from the organic and conventional low input production systems, also had high content of C18:1 (t11), C18:2 (c9,t11) CLA and C18:3 (c9,c12,c15). This content was significantly higher in milk from the organic and conventional low input systems in United Kingdom (P<0.0001 for both variables) than in the other systems. Also a significant linear relationship between pasture and C18:1 (t11) and between pasture and C18:2 (c9,t11) CLA in Denmark (P<0.0001 for both fatty acids) and in Sweden

(P=0.0004 for both fatty acids) was observed. In United Kingdom there seemed to be a correlation between the farms using pasture all day and the content of C18:1 (t11) and C18:2 (c9,t11) CLA in the milk. The content of C18:1 (c9) did not differ during the survey, but the content of C18:2 (c9,c12) was significantly higher in milk from the organic and conventional milk production systems from Denmark and Sweden and in milk from conventional high input systems from United Kingdom compared with the other systems (P=0-0023). A linear correlation between maize silage and the content of C18:2 (c9,c12) in milk was observed in Denmark (P<0.0001) which corresponds with the literature. The data was analysed multivariate by principal component analysis, and the conventional low input and organic production systems in United Kingdom deviated by correlating to pasture and the components in the milk relevant to pasture. The other five systems were more isolated in a cluster, which correlated to maize silage, grass silage and cereals. There seemed to be a tendency towards a correlation between the conventional systems and maize silage, the synthetic isomers of α-tocopherol (2R-a-toc) and C18:2 (c9,c12). On the contrary, the organic milk production systems correlated with cereals, grass silage and short chain fatty acids.

In conclusion, different feeding regimes in the three countries, with pasture as the main part of the diet in some countries and maize silage as a main part along with pasture in the diet in other countries, had a huge impact on the composition of milk from these production systems

Light-induced processes in cheese

Cheeses with 4 different contents of selenium (control, low concentration, medium concentration and high concentration) were made. The cheeses were stored at 4-6^{o}C for 8 weeks exposed to darkness and to light, respectively. Samples were taken continuously, and measurements of colour, secondary oxidation products, determination of peroxide value, measurements of fluorescence for detection of protein and determination of riboflavin contents were made. No significant difference was found in colour on the surface of the cheeses with different selenium content. An incipient increase in the peroxide value was observed in cheeses after 24 days of storage in light, and after 56 days of storage in light the cheeses with high selenium content had a significantly lower peroxide value. No change in peroxide value was observed between 0 and 56 days of storage in darkness. The oxidation product hexanal was not formed in cheeses with high selenium content, while hexanal was formed between day 37 and 56 in the other samples. No such tendency was observed with the other oxidation products such as octanal and acetaldehyde. The results are going to be published in the near future.

Conclusion

- Glutathione peroxidase acitivity are not found in milk, but glutathione S-transferase are found in milk, both antioxidative enzymes need gluthatione as substrate, however there is very little endogenous glutathione in milk to be used as reducing substrate for glutathione peroxidase. In fact, glutathione added to milk is rapidly metabolized, probably by the concerted action of γ-glutamyl transpeptidase and sulfhydryl oxidase. Thus, if activity of GPX is of importance for the oxidative stability of milk, alternative substrates must be utilized.

- Supplementation of the feed with synthetic vitamin E showed that only the natural isomer RRR-stereo-isomer and to a minor extent the 2R-stereo-isomers are excreted into milk where it can act as antioxidant. Supplementation of feed with high levels of synthetic α-tocopherol and subsequent high level of α-tocopherols in milk with high content of unsaturated fatty acids α-tocopherol seems to become prooxidative.

- The survey of orgasnic and conventional milk production including 25 Danish farms showed feeding cows with pasture, there is a tendency towards higher amount of β-carotene, C18:3 and CLA in the milk. The fat content and the daily yield on the contrary seem to fall. When using grass silage in the feed, the content of α-tocopherol seems to rise, and when using cereals in the feed the content of short chain fatty acids also rise. Organic production systems use high amounts of pasture, grass silage and cereals, which gives high content of the natural stereo-isomer of α-tocopherol, β-carotene, lutein, zeaxanthine, C18:1 (t11), CLA, C18:3 and short chain fatty acids in the milk. Conventional milk production systems use high amount of maize silage, concentrate, by-products, minerals and hay, and this give high amounts of the synthetic 2R-stereo-isomers of α-tocopherol and C18:2 in the milk. United Kingdom use higher amount of pasture, and Sweden and Denmark use higher amount of cereals, maize silage and concentrates.

- Cheese made with high content of selenium is found to be oxidative more stable after 56 days of storage in light.

C.2 Fulfilment of deliverables and milestones

(To be completed for each work package)

WP1 Antioxidative capacity of raw milk, DIAS	Time schedule	Deviations, if any*
Deliverables		
1 Report for the Danish Dairy Board	10/2002	Finished
2 Report for the Danish Dairy Board	04/2003	Finished
3 Report for the Danish Dairy Board	10/2003	Finished
4 Report for the Danish Dairy Board	04/2004	Finished
5 Report for the Danish Dairy Board	10/2004	Finished
6 Final report for FØJO and the Danish Dairy Board	12/2005	03/2007
7 Paper for Mælkeritidende	2003	Published
8 Paper for Mælkeritidende	10/2005	05/2008
9 The shelf-life of organic milk in relation to feeding (paper)	8/2003	Accepted

10 The antioxidative capacity of natural vitamin E in milk (paper)	6/2005	This part has been transferred to the FØJO III programme
11 Mechanistic study of vitamin E i MFGM (paper)	8/2005	This part has been transferred to the FØJO III programme
13 Report for the Danish Dairy Board	4/2005	Finished
14 Report for the Danish Dairy Board	10/2005	Finished
15 Report for the Danish Dairy Board	4/2006	Finished
16 Report for the Danish Dairy Board	10/2006	Finished
17 Defence of Ph.D. thesis	12/2006	05/2008
Milestones		
1 Characterization of milk from organic herds finished	5/2005	Finished
2 Variation in the oxidative stability of milk	5/2005	Finished
3 Feeding experiment with natural vitamin E finished	3/2005	Finished
4 Model experiments with vitamin E incorporated in the fat globule membrane of milk	3/2005	Finished

WP2 Cheese production and oxidative stability of cheeses with high level of uric acid and selenium	Time schedule	Deviations, if any*
Deliverables		
5 Report for the Danish Dairy Board	10/2004	Finished
8 Paper for Mælkeritidende	10/2005	08/2008
12 Influence of urate and selenium on light-induced oxidative changes in cheese	12/2004	Are going to be published medio 2007
Milestones		
5 Light-induced processes in cheese are described in relation to the level of natural antioxidants	12/2004	Are going to be published medio 2007

** Deviations are to be further discussed in D*

D. Description of deviations and subsequent adjustments of plans

- Due to maternity leave of the Ph.D.-student Tina Slots (formerly known as Tina Lund-Nielsen) will the project be prolonged with 12 months (from February 2004 to January 2005) and 11 months (from March 2006 to February 2007), totally to June 2008. There will in stead be created two papers with the results from (i) the survey of milk composition in DK, SE and UK, and (ii) the oxidation of α-tocopherol in presence of riboflavin. The studies regarding mechanistic studies of vitamin e has been transferred to the FØJO III programme where there is produced milk with different levels of vitamin E and we will be able to follow oxidation and further investigate the pro-oxidative effects of vitamin E observed in the present studies, before we come up with guidelines on how to improve the oxidative stability of the organic milk through feeding.

E. Project publications and other products

1. Products from Organic Eprints archive

Østdal, Henrik; Weisbjerg, Martin; Skibsted, Leif and Nielsen, Jacob H. (2007) Antioxidative capacity of milk with a high urate content. Accepted for publication Milchwissenschaft

Nielsen, Jacob Holm; Lund-Nielsen, Tina and Skibsted, Leif Higher antioxidant content in organic milk than in conventional milk due to feeding strategy . Online at <http://www.darcof.dk/enews/sep04/milk.html>. Newsletter *

Slots, T.; Skibsted, L.H. and Nielsen, J.H. (2007) The difference in transfer of all-rac-a-tocopherol stereo-isomers to milk from cows and the effect on its oxidative stability. *International Dairy Journal*.*

Slots, Tina; Leifert, Carlo; Butler, Gillian; Kristensen, Troels and Nielsen, Jacob Holm (2006) Effect of dairy management on quality characteristics of milk . Paper presented at Joint Organic Congress, Odense, Denmark, May 30-31, 2006..

Stagsted, J. (2006) Absence of both glutathione perioxidase activity and glutathione in bovine milk. *International Dairy Journal* 16(6):pp. 662-668.*

Stagsted, Jan and Nielsen, Jacob H. (2004) Purification of glutathione-binding proteins from bovine milk and identification of glutathione S-transferase. Working Paper.*

This list was generated on **Fri Mar 30 06:47:55 CEST 2007**.

2. Other products (oral presentations, public meetings, field days, etc.)

Oral presentation: *Effect of Dairy Management on Quality Characteristics of Milk* at Joint Organic Congress in Odense, Denmark at the 30th and 31st of May 2006.

* 25-75% financed by DARCOF
** 5-25% financed by DARCOF

F. Scientific education

Ph.d-student Tina Slots is employed at the project.

G. National and international cooperation

Since March 2004 we have been participating in the EU programme “Improving quality and safety and reduction of cost in the European organic and “low input” food supply chains” co-ordinated by Professor Carlo Leifert, University of Newcastle upon Tyne. Jacob Holm Nielsen and Tina Slots will be responsible for a survey of milk composition in relation to shelf life in Danish, Swedish, English and Italian milk.

H. Critical reflection on the project

The dairy industry is very interested in the results from the survey of organic and conventional milk and there will in the future be applied for money to continue developing feeding strategies in order to produce organic milk with another composition than the conventional milk.

8. Budget

A. Account for any change in budgets

B. Budget for the whole project (1.000 DKK)

Year:	**Original budget**	**Consumption before 2005**	**Consumption 2005**	**Consumption 2006**	**Total**
Man-months					
Scientific personnel		14	9,1	5,3	28,4
Technical personnel		0,9	5,9	4,2	17

Year:	**Original budget**	**Consumption before 2005**	**Consumption 2005**	**Consumption 2006**	**Total**
Salaries					
Scientific personnel	1010	608	189	256	1053
Technical personnel	390	244	90	0	334
Other operational costs	265	198	60	20	278
Equipment	0	0	0	0	0
Others (please specify)	0	0	0	0	0
Direct costs	1665	1050	339	276	1665
Indirect costs (20% of direct costs)	333	225	62	46	333
Total	1998	1275	401	322	1998

Comments:

9. Signatures and stamps

Name	Institute	Date	Signature
Head of project			

Appendix I. Detailed budget

A. Budget for each participating institute (1.000 DKr)

Name of Institute: Department of Food Science, KVL

Year:	Original budget	Consumption before 2005	Consumption 2005	Consumption 2006	Total
Man-months	-	5,1	1,9	0	7
Scientific personnel	-	5,1	1,9	0	7
Technical personnel	-	0	0	0	0

Year:	Original budget	Consumption before 2005	Consumption 2005	Consumption 2006	Total
Salaries	225.000	169.728	77.829	0	247.557
Scientific personnel	225.000	169.728	77.829	0	247.557
Technical personnel	0	0	0	0	0
Other operational costs	50.000	27.443	0	0	27.443
Equipment	0	0	0	0	0
Others (please specify)	0	0	0	0	0
Direct costs	275.000	197.171	77.829	0	275.000
Indirect costs (20% of direct costs)	55.000	39.434	15.566	0	55.000
Total	330.000	236.605	93.395	0	330.000

Comments:

B. Budget for each participating department (1.000 DKK)

Name of Institute: DIAS

Year:	**Original budget**	**Consumption before 2005**	**Consumption 2005**	**Consumption 2006**	**Total**
Man-months					
Scientific personnel		8,9	7,2	5,3	21,4
Technical personnel		0,9	5,9	4,2	17

Year:	**Original budget**	**Consumption before 2005**	**Consumption 2005**	**Consumption 2006**	**Total**
Salaries					
Scientific personnel	785	438	111	256	805
Technical personnel	390	244	90	0	334
Other operational costs	215	171	60	20	251
Equipment	0	0	0	0	0
Others (please specify)	0	0	0	0	0
Direct costs	1390	853	261	276	1390
Indirect costs (20% of direct costs)	278	186	46	46	278
Total	1668	1039	307	322	1668

C. Budget for co-financing from each participating institute (1.000 DKK)

Name of Institute:

Year:	**Original budget**	**Consumption before 2005**	**Consumption 2005**	**Consumption 2006**	**Total**
Man-months					
Scientific personnel					
Technical personnel					

Year:	Original budget	Consumption before 2005	Consumption 2005	Consumption 2006	Total
Salaries Scientific personnel Technical personnel					
Other operational costs					
Equipment					
Others (please specify)					
Direct costs					
Indirect costs (20% of direct costs)					
Total					

Comments: