Danish Research Centre for Organic Farming

Midterm Status Report 2003 and Application for Continuation in 2004

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

1. Research program

Research in organic farming 2000-2005 (DARCOF II)

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)

3. Head of project

Head of Research Unit
Jacob Holm Nielsen
Department of Food Science
Danish Institute of Agricultural Sciences (DIAS)
P.O. Box 50
DK-8830 Tjele
Denmark
Tel.: +45 8999 1163/Mobile tel.: +45 2274 1163
Fax: +45 8999 1564
E-mail: jacobh.nielsen@agrsci.dk

4. Participating institutes

Danish Institute of Agricultural Sciences
Department of Food Science
Research Centre Foulum
P.O. Box 50
DK-8830 Tjele
Denmark

Tel.: +45 8999 1163/Mobile tel.: +45 2274 1163
Fax: +45 8999 1564
E-mail: jacobh.nielsen@agrsci.dk

Department of Dairy and Food Science
The Royal Veterinary and Agricultural University (KVL)
Rolighedsvej 30
DK-1958 Frederiksberg C
Denmark
Tel.: +45 3528 3221
Fax: +45 3528 3344
E-mail: ls@kvl.dk

5. Other project staff

Project leader
Head of Research Unit
Jacob Holm Nielsen
Department of Food Science
Danish Institute of Agricultural Sciences
P.O. Box 50
DK-8830 Tjele
Denmark
Tel.: +45 8999 1163/Mobile tel.: +45 2274 1163
Fax: +45 8999 1564
E-mail: jacobh.nielsen@agrsci.dk

Professor Leif Skibsted
Department of Dairy and Food Science
The Royal Veterinary and Agricultural University
Rolighedsvej 30
DK-1958 Frederiksberg C
Denmark
Tel.: +45 3528 3221
Fax: +45 3528 3344
E-mail: ls@kvl.dk

6. Project period (month, year)

Start of project:	01/2002
End of project:	12/2005

7. Midterm description of the project, its results and progress, and application for continuation in 2004

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	Jacob Holm Nielsen	1668	0102	1205	D1-D11
	Cheese production and oxidative stability of cheeses with high levels of uric acid and selenium	Leif H. Skibsted	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 KVL and at DIAS.

C. Midterm results and progress

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. Recently we have tried to induce expression of GPX in milk through a feeding experiment where dairy cows were fed selenomethionine. 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 glutathione peroxidases is of importance for the oxidative stability of milk, alternative substrates must be utilized.
The results have recently been submitted to International Dairy Journal (see publications)

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 dimethylsulfat 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-isomers and the next four peaks were the 2R-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-isomers of all-rac α-tocopherol and the 2S-isomers. It was found that milk from cows contains about 85-95% RRR-α-tocopherol and about 5-15% of the synthetic isomers (RSS-, RRS- and RSR-α-tocopherol). It was not possible to find any 2S-isomers in milk from cows.

Content of synthetic stereoisomers of α-tocopherol in milk
Organic milk 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.

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 are at the moment quantifying α-tocopherol in the membrane and in the core of the globule prior and after light oxidation.

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 contribution of synthetic α-tocopherol to the feed was removed for three days, and the content of α-tocopherol in the milk was analysed. After depletion, 3.300 IU of α-tocopherol were supplied to the feed per day. The content of α-tocopherol in the morning milk was subsequently followed during the period, of both depletion and after and at individual level every day. 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 (3.300 IU) was added to the feed again.

The ratio (in percentage) of the synthetic 2R-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 3.300 IU per day of synthetic α-tocopherol to the feed it increased again. It was relevant to investigate the oxidative stability of milk with high (700 μg/L) and low (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 3.200 lux for 24 hours, and the use of α-tocopherol and accumulation of hexanal in the milk was followed for 24 hours. The accumulation of hexanal in the two types of milk was identical, indicating that the high concentration of α-tocopherol could not inhibit the lipid oxidation.

Survey of composition of conventional and organic milk

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 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 plants.

The milk will be examined for the content of the following important anti- and prooxidants:
Fatty acid composition
Tocopherols
Carotenoids

Uric acid
Copper

Preliminary results indicate that organic milk has a higher content of carotenoids and to some extent also of α-tocopherol, but collection of milk samples from the dairy plant and the analysis of the milk samples is still on-going.

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	
2 Report for the Danish Dairy Board	04/2003	
3 Report for the Danish Dairy Board	10/2003	
4 Report for the Danish Dairy Board	04/2004	
5 Report for the Danish Dairy Board	10/2004	
6 Final report for FØJO and the Danish Dairy Board	12/2005	
7 Paper for Mælkeritidende	11/2002	The Danish Dairy Board has has asked us to prepare the paper in corporation with a paper from the ØFORSK Programme
8 Paper for Mælkeritidende	10/2005	
9 The shelf-life of organic milk in relation to feeding (paper)	8/2003	Se publication list
10 The antioxidative capacity of natural vitamin E in milk (paper)	6/2005	
11 Mechanistic study of vitamin E i MFGM (paper)	8/2005	
13 Report for the Danish Dairy Board	4/2005	
14 Report for the Danish Dairy Board	10/2005	
15 Defence of Ph.D. thesis	12/2005	
Milestones		
1 Characterization of milk from organic herds finished	5/2005	
2 Variation in the oxidative stability of milk	5/2005	
3 Feeding experiment with natural vitamin E finished	3/2005	
4 Model experiments with vitamin E incorporated in the fat globule membrane of milk	3/2005	
5 Light-induced processes in cheese are described in relation to the level of natural antioxidants	12/2004	

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	
8 Paper for Mælkeritidende	10/2005	
12 Influence of urate and selenium on light-induced oxidative changes in cheese	12/2004	
Milestones		
5 Light-induced processes in cheese are described in relation to the level of natural intioxidants	12/2004	

C. Description of deviations and subsequent adjustments of plans

Compared to the last report for 2002 there is no deviations. We have received a grant from SOAR as mentioned in the last status report

E. Project publications and other products

1. Articles in international, scientific journals with review procedures

Stagsted J & Nielsen J. H. (2003).Absence of both glutathione and activity of glutathione peroxidase from bovine milk *submitted to International dairy Journal*

2. Papers presented at congresses, symposiums, etc.

3. Reports, articles in agricultural journals, etc.

4. Oral presentations, public meetings, field days, etc.

F. Scientific education

In relation to this project a Ph.D. study is carried out.

G. National and international cooperation

We will in the future participate in the EU-programme "Improving quality and safety and reduction of cost in the European organic and "low input" food supply chains" coordinated by professor Carlo Leifert, University of Newcastle upon Tyne . Jacob Holm Nielsen will be responsible for a survey of milk composition in relation shelf life in Danish, Swedish, English and Italian milk.

H. Critical reflection on the project

As we not have been able to detect any activity of glutathione peroxidase in milk will the focus the investigation be focussed on more unspecific antioxidative properties of selenium.

8. Budget

A. Account for any change in budgets

B. Budget for the whole project (1.000 DKK)

Total consumption of funds from DARCOF and expected consumption this year and coming years

Year:	Consump-tion before 2003	Expected consumption 2003	2004	2005	Total
Man-months					
Scientific personnel	2.9	4	12.8	8.5	28.2
Technical personnel	0.9	5.9	6	4.2	17.0

Year:	Consump-tion before 2003	Expected consumption 2003	2004	2005	Total
Salaries					
Scientific personnel	100	180	450	280	1010
Technical personnel	20	130	140	100	390
Other operational costs	20	65	160	20	265
Equipment					
Others (please specify)					
Direct costs	140	375	750	400	1665
Indirect costs (20% of direct costs)	28	75	150	80	333
Total	168	450	900	480	1998

The project is furthermore supported with 1.6524 mill D.kr. by the Danish Dairy Board.

Comments:

9. Signatures and stamps

Name	Institute	Date	Signature
Head of project Jacob Holm Nielsen	Danish Institute of Agricultural sciences Department of Food Science	1/10-03	

Appendix I. Detailed budget

A. Budget for each participating institute (1.000 DKr)

Name of Institute: DIAS, Dept. of Food Science

Year:	Consump-tion before 2003	Expected consump-tion 2003	2004	2005	Total
Man-months					
Scientific personnel	2.9	4	6.0	8.5	21.4
Technical personnel	0.9	5.9	6.0	4.2	17

Year:	Consumption before 2003	Expected consump-tion 2003	2004	2005	Total
Salaries					
Scientific personnel	100	180	225	280	785
Technical personnel	20	130	140	100	390
Other operational costs	20	65	110	20	215
Equipment					
Others (please specify)					
Direct costs	140	375	475	400	1390
Indirect costs (20% of direct costs)	28	91	95	64	278
Total	168	546	570	384	1668

Comments:

B. Budget for each participating department (1.000 DKK)

Name of Institute and department: KVL

Year:	Consump-tion before 2003	Expected consumption 2003	2004	2005	Total
Man-months Scientific personnel Technical personnel			6.8		6.8

Year:	Consump-tion before 2003	Expected consumption 2003	2004	2005	Total
Salaries Scientific personnel Technical personnel			225		225
Other operational costs			50		50
Equipment					
Others (please specify)					
Direct costs			275		275
Indirect costs (20% of direct costs)			55		55
Total			330		330

Comments:

D. Budget for co-financing from external partners

(1.000 DKK)

The project is furthermore supported with 1.6524 mill D.kr. by the Danish Dairy Board.