

## Assessment of processing technologies which may improve the nutritional composition of dairy products – Overview of progress

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### Abstract

*Among consumers there is a growing demand for food products with a natural nutritional-physiological advantage over comparable conventional products. As part of an EU funded project, ALP is examining the possible impact of processing on nutritionally valuable milk components, using the example of conjugated linoleic acids (CLA). The extent to which processing influences the CLA content of the end product was determined by literature research and own investigations of organic and conventional butter. Furthermore, new chemical, sensory-based and bio crystallization methods were evaluated by ALP and the University of Kassel to determine the oxidation stability of butter. In a further step the storage stability of CLA enriched and conventional butter was examined and the different methods will be compared. As a third objective a process for low-input CLA enrichment of milk fat (with a focus on alpine butter) has been developed. Since the process selected for the work is a physical enrichment process, it is accepted by international organic farming and food groups. Among the many benefits ascribed to CLA, it is believed to be an effective agent against cancer. The demand for foods with properties that promote human health is growing. The dairy industry has the opportunity to meet this demand by developing new dairy products with a nutritional-physiological function for the functional food market.*

### Introduction

Recent studies indicate that conjugated linoleic acids (CLA) naturally present in milk and dairy products may have anti-mutagenic, anti-carcinogenic, anti-diabetic and anti-atherosclerotic effects on human health. Processing standards for organic food aim at preserving or enhancing specific bio-active or functional components of the raw material and discourage or prohibit processing methods which may have a detrimental impact on the nutritional quality. Our work package addresses the topic of processing strategies and examines the potential effects of processing on nutritionally high-value milk components, using CLA as an example, with a view to making the findings available to the food industry.

### Objective 1: Composition - Overview of progress

The aim of the study was giving an overview of the present knowledge on the influences of dairy processing and storage on the content of CLA and to pay attention to possible differences between milk products of organic origin compared to

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conventional milk products. A comparison of organic with conventional milk products shows a higher CLA content in organic dairy products, furthermore higher contents of linoleic acid, trans-vaccenic acid,  $\beta$ -carotene and  $\alpha$ -tocopherol. In newer studies on the effect of storage and heating steps during dairy processing, no changes of the CLA content or the CLA isomer profile was observed with the exception of microwaving. In commercial dairy products undergoing fermentation like yoghurt, butter made from fermented cream and cheese no effects of the fermentation on CLA content or the CLA isomer profile could be observed. CLA content was stable during butter making from CLA enriched milk. This was confirmed by own investigations on butter made from fermented cream both of conventional and organic origin. Within these investigations, significant differences in total CLA content between cream of organically produced milk and conventional milk have been shown (Table 1). Recent studies on cheese showed no changes of the CLA content during manufacturing or ripening. In several more recent investigations with probiotic bacteria (lactic acid bacteria like *Lactobacillus rhamnosus* or *Lactobacillus acidophilus*, propionibacteria and bifidobacteria like *B. breve* and *B. dentium*) or other strains of these bacteria groups in laboratory scale, an increase of CLA could be observed under the condition that free linoleic acid (LA) was available in the media. Specific procedures allow the increase of the CLA content in a fraction. These procedures are dry fractionation, fractionation using supercritical carbon dioxide and urea complexation, whereas micro filtration did not increase the CLA content.

### **Objective 2: Methods and Shelf-Life - Overview of progress**

PUFA/CLA-enriched butter was produced at the pilot plant of ALP to study its oxidative stability and its sensory quality versus conventional butter, also produced at the pilot plant of ALP. Different methods to assess oxidative stability and sensory quality of raw materials and end products with a focus on milk fat were evaluated and established by ALP and the University of Kassel, partner within WP 5.3. A gas chromatography olfactometry (GC-O) method, which enables the detection of the aroma-active compounds of butter originating from lipid oxidation (see paper "Aroma-active secondary oxidation products of butter" by Silvia Mallia) and a sensory-based method were evaluated by ALP. Within the sensory-method, terminology and the respective references were specified by the certified sensory panel of ALP to assess sensory changes during storage regarding olfactometry, flavour and texture (spreadability). In addition, a systemic (holistic) laboratory method was developed by the University of Kassel. The bio crystallization method is based on a crystallographic phenomenon generating biocrystallograms with reproducible crystal structures. This method enabled a significant differentiation of the two types of butter at all points of degradation (shelf-life). The developed methods were applied to monitor the oxidative stability of PUFA/CLA-enriched butter during storage. The results obtained by chemical, sensory and holistic methods will be compared and the shelf-life of the different butter samples will be evaluated and discussed.

### **Objective 3: Novel Processing - Overview of progress**

As a third objective, in cooperation with the University of Applied Sciences, College of Agriculture, Zollikofen and the dairy industry, a process for low-input CLA enrichment of milk fat (with a special focus on alpine butter) has been developed as part of a diploma thesis. Alpine butter provides a suitable raw material since it has a significantly higher CLA content than conventional butter. Suitable fractionation conditions were evaluated with anhydrous butterfat. The optimal time and temperature

of the selected CLA enrichment process were obtained from the various fractionation conditions tested. The milk fat crystallization was influenced by changing crystallization time (between one and 20 hours) and temperature (between 32°C and 9.5°C) as well as by means of multiple fractionation. The aim of the physical separation process was to obtain a higher CLA content in a fraction while optimally separating the two fractions, and to achieve a commercially interesting yield of the fraction with the higher CLA content. Fatty acids and the CLA content of the olein and stearin fraction were determined in the laboratory using gas chromatography. CLA isomers were analyzed by Ag+-HPLC (high performance liquid chromatography) and compared against the CLA content of the respective raw material. The resultant olein fraction achieved a CLA content for conventional anhydrous butterfat of 10.2 mg per gram of fat with two fractionation steps at the optimal times and temperatures. This is 2.5 mg CLA per gram of fat more than the CLA content of the raw material anhydrous butterfat. The resultant olein fractions of alpine butter achieved an average increase of CLA of 3.3 mg/g fat corresponding to an increase of 15.3% in the olein fraction compared to the raw material alpine butter (Table 2). Triglycerides containing CLA are found both in the olein and the stearin fraction. However, the test results show that a higher content is found in the olein fraction in both types of butter.

### **Conclusions**

Objective 1: Processing and storage of dairy products generally do not change the concentration of CLA in milk fat. A certain potential to increase the CLA level is given by using selected strains for fermented dairy products supplemented with free LA and different chemical and physical processes.

Objective 2: The GC-O analysis and sensory-based method show its ability to characterise the aroma and detect the odour differences from oxidative processes in the produced PUFA/CLA-enriched butter and conventional butter. The bio crystallisation method enabled a significant differentiation of the two types of butter at all points of degradation (shelf-life). These findings will be made available to food industry to assess oxidative stability and sensory quality of raw materials and end products with a focus on milk fat. The shelf-life of the two types of butter can be evaluated by these methods.

Objective 3: Tests conducted demonstrate that the selected physical separation process accepted by international organic farming and food groups enables CLA enrichment. The test results show that a higher content is found in the olein fraction in both types of butter. While a CLA enrichment of 32.5% was obtained in the olein fraction for conventional anhydrous butterfat, alpine butter shows only 15.3% enrichment. Given the costly and complex process involved, this is low, and furthermore too minor to achieve any decisive positive impact on human health.

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## Tables

**Tab 1: Own investigations of fermented butter (Objective 1)**

Origin	n	CLA cream [g/100 g fat]	CLA butter [g/100 g fat]	Difference butter - cream [g/100 g fat]
integrated farming	7	1.35 <sup>ax</sup>	1.31 <sup>cx</sup>	-0.04
organic	5	1.54 <sup>by</sup>	1.48 <sup>dy</sup>	-0.06

a, b and c, d: different letters in columns mean significant differences ( $p < 0.005$ ); x,y: different letters in rows mean significant differences ( $p < 0.01$ ); n: number of samples

**Tab 2: Extract of results of series of experiments (Objective 3)**

Product	T [°C]	n	CLA content [g/100 g fat]
<b>anhydrous butterfat</b>	-	<b>1</b>	<b>0.768 b</b>
First fat fractions	20	8 sx	0.866 a 0.009
First fat fraction	22	1	0.866 a
First fat fractions	24	3 sx	0.868 a 0.020
First fat fraction	28	1	0.872 a
average difference compared to anhydrous butterfat		13 sx	0.1 0.01
ANOVA			***
First fat fraction	20	1	0.860
Second fat fraction	12.5	1	1.017
difference compared to first fraction		1	0.16
<b>highland butter</b>	-	<b>1</b>	<b>2.159</b>
First fat fractions	20	2 sx	2.277 0.005
average difference compared to highland butter		2 sx	0.12 0.005
ANOVA			*
First fat fraction A	20	1	2.281
Second fat fraction A1	12.5	1	2.512
Second fat fraction A2	12.5	1	2.447
First fat fraction B	20	1	2.274
Second fat fraction B1	12.5	1	2.51
average difference compared to first fraction		3 sx	0.21 0.04
t-Test			*

n: number of experiments; sx: standard deviation; significance: \* $p \leq 0.05$ ; \*\* $p \leq 0.01$ ; \*\*\* $p \leq 0.001$ ;  
ns (not significant); a, b: different letters indicate significant differences (Fisher LSD test  $p \leq 0.05$ );  
ANOVA: analysis of variance; t-test: students t-test