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SHORT COMMUNICATION

Influence of a dry fractionation of butterfat on the content of fatty acids including conjugated linoleic acids

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Abstract There is a growing demand among consumers for food products with natural nutritional–physiological advantages over comparable conventional products. As part of an EU project, a process using dry fractionation is evaluated that enables the targeted low-input enrichment of conjugated linoleic acids (CLA) in milk fat. Furthermore, the distribution of CLA isomers in the fat fractions was analysed. In the olein fraction for highland butter a CLA enrichment of 15.3% was obtained. The yield of the CLA rich olein fraction was 44.5% of the total amount of olein and stearin. There were significant increases during the first fractionation step of highland butter for the concentration of the CLA isomer *cis*-9, *trans*-11 ($P \leq 0.05$) and during the second fractionation step for the concentration of CLA isomers *cis*-9, *trans*-11; *trans*-11, *cis*-13 ($P \leq 0.05$) and *trans*-7, *cis*-9 ($P \leq 0.01$). Experiments carried out demonstrate that the selected physical separation process enables CLA enrichment but the increase is too minor to achieve any decisive positive impact on human health and therefore too costly as an industrial CLA enrichment process.

Keywords Dry fractionation · Butterfat · Olein fraction · Conjugated linoleic acid · Enrichment · Isomers

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Introduction

The dairy industry has the opportunity to meet the demand for foods with properties that promote human health by developing new dairy products for the functional food market.

Based on animal experiments conjugated linoleic acids (CLA) exhibit several important health-promoting attributes [1] which have not been exhaustively elucidated yet on human health. CLA isomers are isomers of linoleic acid in which the double bonds are conjugated. The term CLA is understood to cover all the 28 known CLA isomers.

Only few studies have examined the CLA content of milk fat fractions, whereas the content of the increase of the main CLA-isomers in fat fractions have not been investigated yet. A study [2] reported the investigation of the effect of dry fractionation of bovine milk fat on CLA content in the resulting fractions.

Triglycerides in milk fat exhibit widely divergent melting points, therefore they can be separated by means of crystallisation processes. The most commonly used process is physical dry fractionation. This process involves the purely thermal–mechanical separation of triglycerides based on their melting points. In collaboration with university and industry, a fractionation process for low-input CLA enrichment of highland butter was evaluated and some samples were analysed. Highland butter had been chosen as a suitable raw material because it has a significantly higher CLA content than conventional butter [3]. Its utilisation as raw material for a milk fat fraction rich in CLA would result in a higher added value of this dairy product. The aim of the work carried out was

the evaluation of a process which allows the targeted, low-input enrichment of CLA in milk fat with a commercially interesting yield and to acquire knowledge about the distribution of CLA isomers in milk fat fractions.

Materials and methods

Samples

The raw materials were anhydrous butterfat and highland butter.

Highland (or alpine) butter, which contained 85.9% fat, originated from the Alp Mutten (Graubünden, Switzerland, 2,100 m altitude). The butter consisted of 2 kg portions that were produced in summer 2004 and stored until use at –20 °C. Anhydrous butterfat is produced from butter and consists of more or less pure milk fat (>99.8%). Anhydrous butterfat has been provided by Emmi Butterzentrale AG (Lucerne, Switzerland).

The fatty acid and CLA composition of the two milk fat samples is shown in Tables 1 and 3.

Fractionation

Technologically suitable fractionation conditions were evaluated using anhydrous butterfat.

The water-free butterfat was first melted at a temperature of 75 °C to liquefy all triglycerides, then cooled during 1.5 h to 24 °C at ambient temperature.

Two fractionation steps were performed. For the first fractionation step different temperatures ranging from 32 to 9.5 °C were applied. The best results were obtained by cooling the water bath to 20 °C at which temperature the crystal-liquid butterfat was tempered for 4 h, meanwhile the high-melting triglyerides crystallize (stearin fraction) and low-melting triglycerides remain liquid (olein fraction). In all experiments the water-free butterfat was warmed up or cooled down in a water bath at a rate of 0.2 °C per minute to the required crystallisation temperature without stirring. After crystallisation, the crystal-liquid butterfat suspension was separated into an olein and stearin fraction using vacuum filtration (vacuum pump AEG Type RE 2, suction capacity 1.8 m^3/h, folded filter S&S 597 HY 1/2). For the second fractionation step the resultant olein fraction was warmed again at 75 °C and

Table 1 Total CLA content, sum of several fatty acids and content of selected CLA isomers in anhydrous butterfat (g/100 g fat): first fractionation

Product	*T* (°C)	*n*	CLA	Short chain fatty acids [a]	Saturated fatty acids [b]	Unsaturated fatty acids [c]	Omega-3 fatty acids [d]	Omega-6 fatty acids [e]	C18:2 c9 t11	C18:2 t11 c13	C18:2 t7 c9
Anhydrous butterfat	-	1	0.768b	10.16b	63.61a	26.21b	1.13b	2.16b	0.622b	0.042c	0.031c
First fat fractions	20	8	0.866a	10.93a	59.77b	28.93a	1.28a	2.37a	0.706a	0.045b	0.039a
		SD	0.009	0.17	0.69	0.46	0.02	0.04	0.008	0.001	0.001
First fat fraction	22	1	0.866a	10.99a	59.58b	28.83a	1.29a	2.40a	0.709a	0.045a, b	0.037b
First fat fractions	24	3	0.868a	10.79a	61.20b	28.60a	1.25a	2.35a	0.705a	0.046a	0.039a, b
		SD	0.020	0.01	0.71	0.25	0.04	0.02	0.016	0.001	0.001
First fat fraction	28	1	0.872a	10.97a	59.74b	29.15	1.28a	2.44a	0.712a	0.044a, b, c	0.039a
Average difference compared to anhydrous butterfat		13	0.1	0.74	–3.53	2.66	0.14	0.21	0.085	0.003	0.008
		SD	0.01	0.15	0.87	0.40	0.03	0.04	0.009	0.001	0.001
ANOVA			***	*	**	**	**	**	***	*	***

n number of experiments, *SD* standard deviation, *ANOVA* analysis of variance, *t test* students *t* test

*$P \le 0.05$; **$P \le 0.01$; ***$P \le 0.001$; ns (not significant)

a, b, c different letters indicate significant differences (Fisher LSD test $P \le 0.05$)

[a] C4 to C10:1

[b] C4 to C10, C12, C12 iso, C12 aiso, C13 iso, C14, C14 iso, C14 aiso, C15, C15 iso, C16, C16 iso, C16 aiso, C17, C17 iso, C17 aiso, C18, C19, C20 and C22

[c] C10:1, C14:1 ct, C16:1 ct, C17:1 t, C18:1 t4 to C18:1 c14t16, C18:2 ttNMID to C18:2 c9c15, C20:1 t to C20:2 cc, C20:3 (*n*-6) to C22:6 (*n*-3)

[d] C18:2 t11c15 + C18:2c9c15, C18:3 c9c12c15, C20:3 *n*-3, C20:5, C22:5 and C22:6

[e] C18:1 t12, C18:1 c12, C18:2 t9t12, C18:2 c9t12+(c,c-MID + t8c13), C18:2c9c12, C18:3 c6c9c12, C20:2 cc, C20:3 *n*-6 and C20:4 *n*-6

Table 2 Total CLA content, sum of several fatty acids and content of selected CLA isomers in anhydrous butterfat (g/100 g fat): second fractionation

Product	T (°C)	n	CLA	Short chain fatty acids [a]	Saturated fatty acids [b]	Unsaturated fatty acids [c]	Omega-3 fatty acids [d]	Omega-6 fatty acids [e]	C18:2 c9 t11	C18:2 t11 c13	C18:2 t7 c9
First fat fraction	20	1	0.860	10.69	59.55	28.51	1.24	2.31	0.699	0.045	0.038
Second fat fraction	14	1	0.943	10.92	57.39	31.3	1.44	2.59	0.769	0.049	0.042
Second fat fraction	12.5	1	1.017	11.42	54.12	34.65	1.63	2.95	0.831	0.053	0.046
Difference compared to first fraction (at 12.5 °C)		1	0.16	0.73	–5.43	6.14	0.39	0.64	0.132	0.008	0.008

[a] C4 to C10:1

[b] C4 to C10, C12, C12 iso, C12 aiso, C13 iso, C14, C14 iso, C14 aiso, C15, C15 iso, C16, C16 iso, C16 aiso, C17, C17 iso, C17 aiso, C18, C19, C20 and C22

[c] C10:1, C14:1 ct, C16:1 ct, C17:1 t, C18:1 t4 to C18:1 c14t16, C18:2 ttNMID to C18:2 c9c15, C20:1 t to C20:2 cc, C20:3 (*n*-6) to C22:6(*n*-3)

[d] C18:2 t11c15 + C18:2c9c15, C18:3 c9c12c15, C20:3 n-3, C20:5, C22:5 and C22:6

[e] C18:1 t12, C18:1 c12, C18:2 t9t12, C18:2 c9t12+(c,c-MID + t8c13), C18:2c9c12, C18:3 c6c9c12, C20:2 cc, C20:3 *n*-6 and C20:4 *n*-6

Table 3 Total CLA content, sum of several fatty acids and content of selected CLA isomers in highland butter (g/100 g fat): first fractionation

Product	T (°C)	n	CLA	Short chain fatty acids [a]	Saturated fatty acids[b]	Unsaturated fatty acids[c]	Omega-3 fatty acids[d]	Omega-6 fatty acids[e]	C18:2 c9 t11	C18:2 t11 c13	C18:2 t7 c9
highland butter	–	1	2.159	8.77	55.73	34.82 [b]	2.17	2.38	1.843	0.141	0.048
First fat fractions	20	2	2.277	9.49	53.26	36.22 [a]	2.39	2.29	1.962	0.140	0.049
		SD	0.005	0.04	0.35	0.05	0.01	0.01	0.005	0.001	0.001
Average difference		2	0.12	0.72	–2.47	1.40	0.22	-0.1	0.119	–0.001	0.001
compared to highland butter		SD	0.005	0.04	0.35	0.05	0.01	0.01	0.005	0.001	0.001
ANOVA			*	*	NS	*	*	NS	*	NS	NS

SD standard deviation, *NS* not significant

[a] C4 to C10:1

[b] C4 to C10, C12, C12 iso, C12 aiso, C13 iso, C14, C14 iso, C14 aiso, C15, C15 iso, C16, C16 iso, C16 aiso, C17, C17 iso, C17 aiso, C18, C19, C20 and C22

[c] C10:1, C14:1 ct, C16:1 ct, C17:1 t, C18:1 t4 to C18:1 c14t16, C18:2 ttNMID to C18:2 c9c15, C20:1 t to C20:2 cc, C20:3 (*n*-6) to C22:6 (*n*-3)

[d] C18:2 t11c15 + C18:2c9c15, C18:3 c9c12c15, C20:3 n-3, C20:5, C22:5 and C22:6

[e] C18:1 t12, C18:1 c12, C18:2 t9t12, C18:2 c9t12+(c,c-MID + t8c13), C18:2c9c12, C18:3 c6c9c12, C20:2 cc, C20:3 *n*-6 and C20:4 *n*-6

cooled down to 24 °C during 1.5 h and then cooled down to different temperatures ranging from 22 to 9.5 °C. The best results were obtained by cooling down to 16 °C and tempering for 4 h and another 15 h at 12.5 °C, after which it was vacuum-filtered. Tests of the second fractionation process at temperatures above 12.5 °C did not produce any comparable CLA content, tests below 12.5 °C have shown difficulties in subsequently separating the two fractions.

Methods of analysis

Fatty acids and CLA content of the milk fat fractions were determined using GC (gas chromatography). CLA isomers were analysed by Ag^+-HPLC (high performance liquid chromatography).

Methylation

After dissolution of milk fat in hexane, the glycerides were transesterified (5 min at room temperature) to the corresponding fatty acid methyl esters (FAME) by a solution of potassium hydroxide (2.0 mol/l) in methanol according to an international standard [4].

GC Analysis

Reference FAME were obtained from Matreya Inc. (Pleasant Gap, PA). FAME of butterfat were analysed

Table 4 Total CLA content, sum of several fatty acids and content of selected CLA isomers in highland butter (g/100g fat): second fractionation

Product	*T* (°C)	n	CLA	Short chain fatty acids [a]	Saturated fatty acids [b]	Unsaturated fatty acids [c]	Omega-3 fatty acids [d]	Omega-6 fatty acids [e]	C18:2 c9 t11	C18:2 t11 c13	C18:2 t7 c9
First fat fraction A	20	1	2.281	9.52	53.01	36.18	2.4	2.29	1.966	0.141	0.049
Second fat fraction A1	12.5	1	2.512	9.59	47.25	40.21	2.7	2.51	2.170	0.153	0.055
Second fat fraction A2	12.5	1	2.447	9.86	50.8	38.74	2.57	2.43	2.111	0.150	0.054
First fat fraction B	20	1	2.274	9.46	53.51	36.25	2.38	2.28	1.959	0.140	0.049
Second fat fraction B1	12.5	1	2.51	9.74	48.96	40.09	2.68	2.58	2.169	0.152	0.054
Average difference		3	0.21	0.23	-4.17	3.48	0.26	0.22	0.186	0.011	0.005
compared to first fraction		SD	0.04	0.14	1.81	0.80	0.08	0.08	0.036	0.002	0.001
*t*test			*	ns	ns	*	*	*	*	*	**

[a] C4 to C10:1

[b] C4 to C10, C12, C12 iso, C12 aiso, C13 iso, C14, C14 iso, C14 aiso, C15, C15 iso, C16, C16 iso, C16 aiso, C17, C17 iso, C17 aiso, C18, C19, C20 and C22

[c] C10:1, C14:1 ct, C16:1 ct, C17:1 t, C18:1 t4 to C18:1 c14t16, C18:2 ttNMID to C18:2 c9c15, C20:1 t to C20:2 cc, C20:3 (*n*-6) to C22:6 (*n*-3)

[d] C18:2 t11c15 + C18:2c9c15, C18:3 c9c12c15, C20:3 *n*-3, C20:5, C22:5 and C22:6

[e] C18:1 t12, C18:1 c12, C18:2 t9t12, C18:2 c9t12+(c,c-MID + t8c13), C18:2c9c12, C18:3 c6c9c12, C20:2 cc, C20:3 *n*-6 and C20:4 *n*-6

using an Agilent 6890 GC equipped with an on column injector and a flame ionisation detector [5]. The fatty acids (FA) were separated on a capillary column CP-Sil 88 (100 m × 0.25 mm i.d. × 0.20 μm) and quantified using nonanoic acid as internal standard. The results were expressed in absolute values, as g FA per 100 g fat.

Ag⁺-HPLC analysis

The methylesters of *cis*-9, *trans*-11 (98%); *trans*-10, *cis*-12 (98%), and technical-grade *cis*-9, *trans*-11 (75–78%) were obtained from Matreya Inc. (Pleasant Gap, PA, USA). Other CLA isomers were synthetized by isomerisation of the commercially available reference (technical grade) with Iod [6]. The analysis were performed on a LC series 1100 (Agilent Technologies, Switzerland, Basel) equipped with a Photodiode array detector using three ChromSpher 5 Lipids columns in series (stainless steel, 205 × 4.6 mm, 5 μm particle size, Chrompack Middleburg, The Netherlands) according to Rickert et al. [7], as modified by Kraft et al. [8]. The solvent consisted of UV-grade hexane with 0.1% acetonitrile and 0.5% ethyl ether (flow rate 1 ml/min), freshly prepared daily. The column was pre-treated daily by elution with 1% acetonitrile/hexane for 30–60 min prior to sample analysis. Usual injection volumes were 10–20 μl, representing <250 μg lipid. *Trans, trans* CLA isomers were eluted first, followed by *cis, trans*/*trans*, *cis* and finally *cis*, *cis* isomers. The HPLC peak areas for CLA isomers *trans*-7, *cis*-9 and *trans*-8, *cis*-12 and *cis*-9, *trans*-11 were added and used for calculation compared with the peak of these three isomers from GC chromatogram [7, 8]. The results were expressed as absolute values in gram per 100 g fat.

Statistical analysis

Principal component analysis, *t* test and analysis of variance (ANOVA) were performed with Systat for Windows [9].

Results and discussion

From the various fractionation conditions tested, technologically optimal time and temperature of the selected CLA enrichment process were obtained. The conditions for maximum CLA enrichment were found to be a first fractionation temperature of 20 °C followed by a second fractionation at 12.5 °C, see Tables 1, 2, 3 and 4. The cooling process takes a correspondingly long time since the released crystal heat must be bled off at controlled intervals in order to prevent re-melting of already existing crystals. If cooling is too fast and uncontrolled, it is difficult to subsequently separate the two fractions due to inclusion of liquid fat in the crystals. On the other hand, it had to be suitable for industrial application. The cooling was without stirring as in previous experiments

stirring did not show a favourable effect on CLA content [2].

The first fractionation of anhydrous butterfat showed a significant enrichment of 13.0% total CLA ($P \leq 0.001$), 10.2% unsaturated fatty acids, 13.3% omega-3 fatty acids, 10.6% omega-6 fatty acids (all $P \leq 0.01$) and 7.5% short chain fatty acids ($P \leq 0.05$) and a significant decrease of 5.6% saturated fatty acids ($P \leq 0.01$) compared to the raw material (Table 1). With the second fractionation step of anhydrous butterfat an increase of 18.6% total CLA, 21.5% unsaturated fatty acids, 31.5% omega-3 fatty acids, 27.7% omega-6 fatty acids and 6.8% short chain fatty acids and a decrease of 9.1% saturated fatty acids was observed at 12.5 °C compared to first fraction (Table 2). With the first fractionation step of highland butter significant increases were observed of 5.6% total CLA, 4.0% unsaturated fatty acids, 10.1% omega-3 fatty acids and 8.2% short chain fatty acids (all $P \leq 0.05$), furthermore a decrease of 4.4% saturated fatty acids and 4.2% omega-6 fatty acids (all $P > 0.05$) was noticed at 20 °C (Table 3). With the second fractionation step highland butter showed significant average increases of 9.2% total CLA, 9.6% unsaturated fatty acids, 10.9% omega-3 fatty acids and 9.6% omega-6 fatty acids (all $P \leq 0.05$) and 2.4% short chain fatty acids ($P > 0.05$) and a decrease of 7.8% saturated fatty acids ($P > 0.05$) compared to first fraction (Table 4). Concerning the CLA isomer distribution the most important isomers of milk fat, *cis*-9, *trans*-11; *trans*-7, *cis*-9 and *trans*-11, *cis*-13 showed a significant increase in the first fractionation step of anhydrous butterfat with *cis*-9, *trans*-11 of 13.8%; *trans*-7, *cis*-9 of 25.8% (all $P \leq 0.001$) and *trans*-11, *cis*-13 of 7.1% ($P \leq 0.05$) (Table 1). With the second fractionation step of anhydrous butterfat at 12.5 °C the CLA isomers increased by 18.9% *cis*-9, *trans*-11, 17.8% *trans*-11, *cis*-13 and 21.2% *trans*-7, *cis*-9 (Table 2). During the first fractionation step of highland butter, the concentration of the CLA isomer *cis*-9, *trans*-11 increased by 6.5% ($P \leq 0.05$), the two other CLA isomers *trans*-7, *cis*-9 and *trans*-11, *cis*-13 were not significantly influenced (Table 3). In the second fractionation step, the three CLA isomers increased significantly, *trans*-7, *cis*-9 by 10.2% ($P \leq 0.01$), *cis*-9, *trans*-11 by 9.5% and *trans*-11, *cis*-13 by 7.8% (all $P \leq 0.05$) (Table 4). Both fractionation steps showed higher percentile increases of the total CLA content and the concentrations of the three CLA isomers *cis*-9, *trans*-11 and *trans*-7, *cis*-9 in anhydrous butterfat compared to highland butter (Tables 1, 2, 3, 4).

In our investigations the resultant olein fractions with two fractionation steps, first at 20 °C and the second at 12.5 °C, for highland butter achieved an average increase of CLA of 0.33 g, corresponding to an increase of 15.3% in the olein fraction compared to the raw material highland butter. The yield of the CLA rich olein fraction was 44.5% of the total amount of olein and stearin. For standard anhydrous butterfat the resultant CLA concentration after the second fractionation step at 12.5 °C achieved an average increase of CLA of 0.26 g which is an increase of 33.9%. The maximum CLA enrichment obtained in the study of O'Shea et al. [2] was 63% over the parent fat. The yield of the soft fraction was 30%. Refractionation of the soft fraction using the same fractionation conditions reduced the CLA content by 10%. That study highlighted that a milk fat fraction enriched in CLA may be achieved by dry fractionation whereas our study showed that only little CLA enrichment is possible with the selected physical separation process. Triglycerides containing CLA are found in the olein and the stearin fraction. The test results show that a higher content is found in the olein fraction in both types of butter. Conjugated linoleic acids are bound in triglycerides of milk fat and thereby combined with a range of widely differing fatty acids. These may be long-chain, short-chain, unsaturated or saturated. Hence triglycerides with CLA exhibit different melting and crystallization points depending on the other two fatty acids. Depending on the composition and the related crystallization point of the triglyceride, during fractionation the bound CLA isomer makes its way either to the stearin or to the olein fraction, which makes enrichment difficult by purely physical–mechanical methods. One possible reason for the varying enrichment of CLA content within our experiment in anhydrous butterfat compared to highland butter could be the different configuration of triglycerides in both butter types. The differences between our investigations and the study of O'Shea et al. [2] may be due to differences in cooling rates with an increased cooling rate within our investigations. The faster cooling rate may have entrapped the triglycerides containing the conjugated double bond of CLA in the stearin fraction and so included liquid fat in crystals. Based on the physical separation process, industrial-volume CLA enrichment is too minor and furthermore too costly to achieve any decisive positive impact on human health.

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