

OLAJ SZAPPAN KOZMETIKA

A CEREOL NÖVÉNYOLAJIPARI RT. MAGYARORSZÁG

AZ UNILEVER ÉLELMISZER- ÉS MOSÓSZERGYÁRTÓ RT.

ÉS A MÉTE NÖVÉNYOLAJIPARI SZAKOSZTÁLYÁNAK LAPJA

Comparative study on conjugated linoleic acid content of dairy products

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ÖSSZEFoglalás

Az elmúlt években megnőtt az érdeklődés a konjugált linolsav (CLA) iránt, amit kedvező élettani hatásainak tulajdoníthatunk. A konjugált linolsav megtalálható különféle húsokban, főként a kérődzők húsában, sajiban, vajban és tejben, valamint ma már számos CLA termék kapható kereskedelmi forgalomban is. Négyfélle sajt, háromfélle vaj és egy tej konjugált linolsav tartalmát és összetételét vizsgáltuk, illetve hasonlítottuk össze néhány kereskedelmi forgalomban beszerezhető konjugált linolsav termékkel.

A vizsgált tejtermékek 5,2–11,0 mg/g zsír konjugált linolsavat tartalmaztak, melyek fő CLA komponense a 9Z,11E-CLA izomer. Ezzel szemben a kereskedelmi konjugált linolsav termékek összes CLA tartalma 43–80% volt, és a biológiai aktivitás szempontjából legfontosabb izomereket (9Z,11E-CLA és 10E,12Z-CLA) 14–75%-ban tartalmazták.

Abstract

Several conjugated linoleic acid (CLA) products are commercially available and there is an increasing interest in CLA due to the wide range of reported beneficial effects. Conjugated linoleic acid occurs in different meat and dairy products mainly in ruminant meat, cheese, butter and milk.

The conjugated linoleic acid content and composition of four samples of cheese, three samples of butter and one milk was determined and compared with some commercial conjugated linoleic acid products.

In dairy products the conjugated linoleic acid content was between 5.2 and 11.0 mg/g fat, and the main isomer was the 9Z,11E-CLA. On the other hand, in commercial conjugated linoleic acid products the total CLA content ranged from 43% to 80%, and the total amount of the two biological active isomers (9Z,11E-CLA and 10E,12Z-CLA) was between 14% and 75%.

Zusammenfassung

In den vergangenen Jahren verstärkte sich das Interesse für die konjugierte Linolsäure (CLA). Dies kann man ihrer günstigen biologischen Wirkung zuschreiben. Konjugierte Linolsäure findet man in verschiedenen Fleischchen, vorzüglich in Fleischern der Wiederkäuer, in Käse und Milch. Heutzutage findet man viele CLA-Produkte auch in dem Handelsverkehr. Der konjugierte Linolsäuregehalt und Zusammensetzung von vier verschiedenen Käsen, drei verschiedenen Buttern und einer Milch wurden untersucht und verglichen mit

einigen, in dem Handelsverkehr verfügbaren konjugierten Linolsäreprodukten. Die untersuchten Milchprodukte haben 5,2–11,0 mg konjugierte Linolsäure in 1 g Fett enthalten, mit 9Z,11E-CLA Isomer als Hauptkomponent. Dagegen war der gesamte CLA-Gehalt der Handels-CLA-Produkte 43–80%, und enthielten die hinsichtlich der biologischen Aktivität wichtigsten Isomere (9Z,11E-CLA und 10E,12Z-CLA) in 14–75%.

1. Introduction

Conjugated linoleic acid is a collective term for positional and geometrical isomers of linoleic acid. Conjugated linoleic acid was discovered in 1931, having a definitive UV absorbance at around 233 nm [1]. CLA is a physiologically active lipid with wide range of potential health benefit [2]. Naturally it occurs in ruminant's meat, dairy products [3, 4] but also found in breast-milk, human blood and tissues [4, 5].

The 9Z,11E-CLA and the 10E,12Z-CLA are the most important biological active isomers [2] (Fig.1.) CLA can significantly reduce body fat, while increasing lean muscle mass. CLA regulates the lipid/protein metabolism and increases the growth rate in animals [2, 6, 7, 8]. It has also shown promising results in reducing the risk of developing skin [9] and breast cancer [10] and coronary heart disease [11].

A number of gas chromatographic and HPLC methods are known for separation of conjugated linoleic acid isomers. GC methods have been generally performed by means of highly polar stationary phases (cyanopropylpolysiloxane [5, 6, 12, 13, 14], polyethylene glycol [15]) and long capillary columns (50 m or longer).

Ag⁺-HPLC column(s) proved to be useful in separation of the positional isomers of CLA using up to six columns con-

nected into series to improve selectivity and the resolution [6, 12, 14, 16, 17, 18].

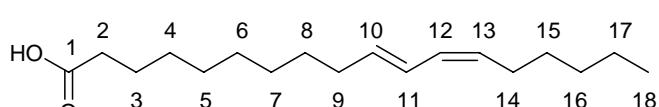
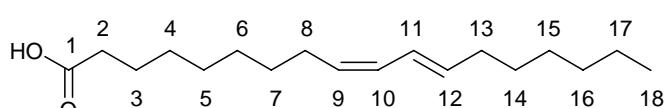
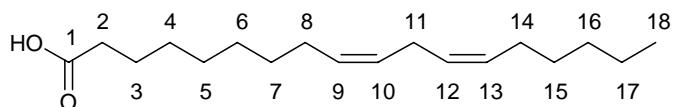


Fig. 1. Structures of the linoleic acid and its major conjugated isomers

Relatively low values of CLA have been reported in certain dairy products, for example in non-processed cheese [13]. Broad variation of CLA content have been found in cheese due to the ripening time, and the seasonal variation of conjugated linoleic acid content of milk, which also could lead to diverse results in different studies [13,15]. Increased CLA level have been reported in processed cheese compared to the natural cheese. This increase was attributed to the conditions of processing and/or the presence of food additives. Several factors including temperature and presence of air could influence the amount of CLA [13] in cheese.

2. Materials and methods

2.1. Chemicals

All solvents (hexane, methanol, diethyl ether, petroleum ether, ethanol) were of HPLC grade and obtained from Sigma Aldrich Co. (Steinheim, Germany). Dimethyl carbonate solution and sodium methylate was of synthetic grade and obtained from Merck-Schuchardt (Hohenbrunn, Germany).

A mixture of conjugated linoleic acid methyl esters as GC standard was also purchased from Sigma Aldrich Co. (Steinheim, Germany).

Commercial CLA samples:

- Sample 1: fatty acid mixture containing conjugated linoleic acid was obtained from Grünau Illertissen GmbH, Cognis (Illertissen, Germany)
- Sample 2: isomerised linoleic acid mixture was obtained from Loders & Croklaan lipid Nutrition Co., (Wormerveer, Netherlands)
- Sample 3: fatty acid mixture containing conjugated linoleic acid was obtained from Eastman Chemical Company (The Hague, Netherlands)

Sample 4: Fatloss capsule, conjugated linoleic acid product for human consumption (Matley Ltd. UK)

Dairy products was purchased from local grocery.

Sample 5–8: Cheese (Hungarian trappista, Hungarian goat's, French and Danish cheese)

Sample 9–11: Butter (Hungarian, French and Danish butter)

Sample 12: cow's milk, fat content 3,6 %

2.2. Lipid extraction

Fat from cheese and milk was extracted by acid digestion followed by solvent extraction. Cheese (3 g) or milk (10 g) was heated with 10 ml of concentrated HCl. After dissolving the sample slight boiling was kept for 10 minutes. After cooling a mixture of ethanol (10 ml), diethylether (25 ml) and petroleum ether (25 ml) was added and the whole mixture was allowed to stand for at least 4 hours. After separating the two phases, the aqueous layer was extracted with a mixture of petroleum ether – diethylether (1:1) (3 · 25 ml). The organic phases were combined and the solvent was removed under reduced pressure.

Butter (3 g) was melted and dried over anhydrous Na_2SO_4 and dissolved in n-hexane before preparation of methyl esters.

2.3. Preparation of fatty acid methyl ester

Free fatty acid sample (100 mg) was added to 8 ml of methanol-hexane (5:3 v/v) solution containing H_2SO_4 (0.25 %) and the mixture was stirred at room temperature for 15 minutes. The forming methyl esters were extracted with hexane (2 · 25 ml), and the combined hexane layers were washed with distilled water (2 · 25 ml) and brine (2 · 25 ml). The hexane phase was dried over anhydrous Na_2SO_4 and the solvent was removed by rotary evaporator.

To a solution of triglycerides (100 mg) in hexane (2 ml), dimethyl carbonate (1 ml) and sodium methylate – methanol solution (40 g/l NaOCH_3 in MeOH) (1 ml) were added. It was shaked for 5 min, then 10 ml of distilled water was added. The hexane phase was separated and dried over anhydrous Na_2SO_4 .

2.4. GC separation of fatty acid methyl esters

Fatty acid methyl esters were analyzed by GC (model: Hewlett Packard 6890, Hewlett Packard, Wilmington, USA) on a SGE BPX-70; 60 m · 0.22 mm, 0.25 mm 70% cyanopropylpolysiloxane (SGE Pty, Australia) column under the following conditions: sample amount 1 ml; oven was programmed from 120 °C to 210 °C at 1.3 °C/min. The injector and the FID detector were operated at 250 °C. The carrier gas was H_2 (0.6 ml/min), the split ratio was 100:1.

3. Results

CLA content and composition of four different commercial CLA products were compared. Identification of conjugated linoleic acid isomers was carried out by comparison with known standard mixtures and literature data (Fig. 2.) [14].

Our results showed a wide variability among the conjugated linoleic acid content and the isomer distribution of commercially available CLA products (see Table 1.). The total CLA

content ranged from 43% to 80%, and the total amount of the 9Z,11E-CLA and 10Z,12E-CLA was between 14% and 78%.

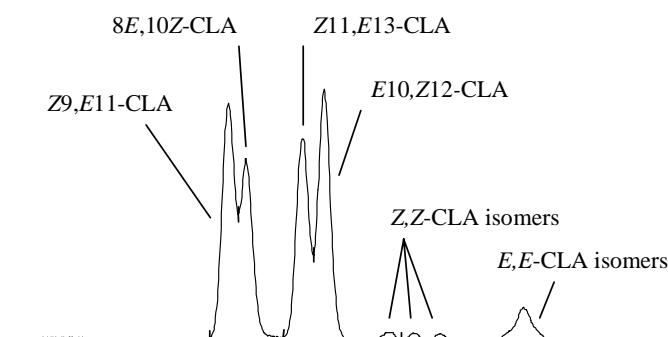


Fig. 2. Partial gas chromatograph of Sigma CLA GC standard

Table 1.
Conjugated linoleic acid content and composition of commercial products

Compound	Sample 1	Sample 2	Sample 3	Sample 4	Sigma std.
9Z,11E-CLA	14.1	48.7	17.1	46.8	26.0
8E,10Z-CLA	12.6	—	11.1	—	17.3
11Z,13E-CLA	16.2	—	16.0	—	21.5
10E,12Z-CLA	17.5	48.6	20.2	48.3	26.0
8Z,10Z-CLA	5.3	2.0 ^a	6.0	2.7 ^a	1.5
9Z,11Z-CLA	5.2		3.7		1.1
10Z,12Z-CLA	2.6		2.4		1.1
11Z,13Z-CLA	2.1		2.1		—
All E,E-CLA	18.0	0.8	18.1	2.2	5.5
Non identified CLA isomers	6.4	—	3.2	—	—
Total CLA ^b	43.6	79.7	66.5	65.5	—
Two biologically active isomers ^b	13.8	77.5	24.8	62.3	52.0

^a: total amount of the Z,Z isomers are given; ^b: based on the total fatty acid

Table 2 gives the amount of conjugated linoleic acid in different samples of cheese, butter and milk. Their CLA content ranged from 5.2 to 11.0 mg/g fat in cheese, from 6.3 to 7.8 mg/g fat in butter, and 5.6 mg/g fat in milk. In butter we detected only the 9Z,11E-CLA isomer, while in cheese and milk traces of E,E-CLA isomers have also been detected (Fig.3.). The amount of 9Z,11E-CLA was between 68–91% of total CLA in the analyzed dairy products.

Table 2.
Conjugated linoleic acid content in different dairy products

Sample	CLA content mg/g fat	
	9Z,11E-CLA	E,E-CLA isomers
Trappista cheese	3.9	1.3
Goat's cheese	10.0	1.0
French cheese	5.0	1.2
Danish cheese	6.0	2.8
Hungarian butter	6.9	—
French butter	6.3	—
Danish butter	7.8	—
Milk	4.4	1.2

4. Discussion

A broad variability of isomer distribution and conjugated linoleic acid content was found in different commercial CLA products. The differences in the isomer distribution can be

explained by different processes. Although the details of production methods of the analyzed samples were not known, several methods are discussed in the literature [20, 21, 22]. Different reaction conditions (e.g. solvent, temperature and pressure) may result in diverse isomer composition. Elevated temperature (200–250 °C) [23] and long reaction time (5–6 hours) [22, 23] can result in a complex mixture of conjugated linoleic acid isomers. To produce pure 9Z,11E-CLA an enzymatic method applying linoleate isomerase enzyme, which can be found in the intestinal flora of ruminants was developed and patented [24].

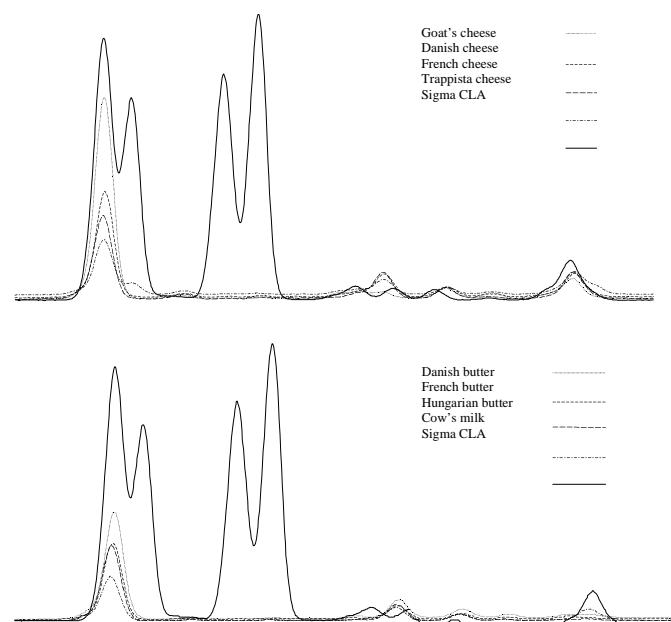


Fig. 3. Partial gas chromatograms of dairy products and CLA standard

The CLA products can be classified in two groups: bulk products and capsules. The bulk products – having very often a series of isomers – are mainly used for animal feeding stuffs or for further processing. Capsulated linoleic acid is used for human consumption as a dietary supplement for losing weight and improving body mass index. These products – originating mostly from safflower or sunflower oil – contain mainly the two biological active compounds (9Z,11E-CLA and 10E,12Z-CLA) in an approximately 1 to 1 ratio. These dietary supplements consist of conjugated linoleic acid in the form of free fatty acid with a few exceptions containing conjugated linoleic acid in triacylglycerol form.

Conjugated linoleic acid is a naturally occurring constituent in milk, cheese and butter. Besides the 9Z,11E-CLA isomer traces of several other CLA isomers (E7,Z9-, E8,Z10-, E10,Z12-, E11,Z13-, E12,Z14-18:2) can also be found in milk. All of these isomers are originated from the biohydrogenation of linoleic acid in rumen [5,13,25]. The 9Z,11E-18:2 conjugated linoleic acid isomer – the major CLA isomer in milk fat – is formed from linoleic acid (9Z,12Z-18:2) in an isomerization reaction catalysed by linoleate isomerase (E.C.5.2.1.5). Only a small amount of the 9Z,11E conjugated linoleic acid is absorbed from the rumen, while the major amount is converted to 11E-18:1 (vaccenic acid) in a rapid reaction catalysed by D⁹ desaturase enzyme. The 11E-18:1 accumulates partially as an

intermediate and is absorbed from the rumen, while the other part is further hydrogenated to C18:0 fatty acid. The vaccenic acid can be desaturated to form 9Z,11E-18:2 in tissues by a D⁹ desaturase enzyme.

The CLA content in cow's milk can be influenced by the diet, and there is also a seasonal variation. The differences in the conjugated linoleic acid content in cheese can be explained by its different origin, processing conditions and ripening time.

Our results showed, that the 9Z,11E-18:2 is the main CLA isomer in these dairy products, and traces of E,E isomers were also detected in milk and in cheese. Cheese having goat's milk origin had the highest level of CLA, and the Trappista cheese produced from Hungarian cow's milk had the lowest. Butter had higher conjugated linoleic acid content than milk or cheese.

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KÖNYVISMERTETÉS

K.Hill, W. von Rybinski, G.Stoll: Alkil-poliglüközidök, Technológia, tulajdonságok és alkalmazások. VCH Verlagsgesellschaft mbH, Weinheim, 1997. ISBN 3-527-29451-1

Az alkil-poliglüközidokat először Emil Fischer szintetizálta és azonosította a laboratóriumában több mint 100 éve. 40 évvel később először szabadalmazták Németországban az alkil-glüközidok mosószerben való alkalmazását. Ezt követő 40-50 évben már kidolgozták az alkil-poliglüközidök gyártásának technológiáját a Fischer által felfedezett szintézis alapján. Rohm és Heas volt az első, akik először hoztak kereskedelmi mennyiségben oktil/decil-poliglüközidot forgalomba. Később a BASF, SEPPIC, az Akzo Nobel, ICI és a Henkel volt a legnagyobb gyártója. Jelenleg a Henkel cég az egyik legnagyobb termelője és forgalmazója. A cégen belül egy egész iskola foglalkozik az alkil-poliglüközidök mosószer, tisztítószer és kozmetikai alkalmazásának kutatásával és a termékek gyártásával. Fontos, hogy oleokémiai alapon, növényolajokból kiindulva készítik termékeiket.

A könyvet a szakma legjobb szakemberei állították össze, akiknek nagy része a Henkel cégehez kötődik.

Rövid történeti áttekintés után részletesen leírják az alkil-poliglüközidok kémiáját, gyártási technológiáját és termelését. Részletesen tárgyalják az alkil-poliglüközidok fiziko-kémiai tulajdonságait (fázis viselkedés, reológiai tulajdonságok, határfelületi viselkedés, mikroemulzió fázis, szilárd fázis adszorpciója), továbbá a testápolókban, a kemény felületek tisztítására és mosászerekhez való felhasználásukat.

Újabban mezőgazdasági célra gyártanak belőlük egy sor fontos terméket.

Külön fejezet foglalkozik a termékek vizsgálatával, fogyasztói cikkekből és környezeti mintákból való meghatározásával.

Fontos helyet foglal el az alkil-poliglüközidök dermatológiai tulajdonságainak, toxikológiájának ismertetése. Kutatási eredmények alapján ismertetik a környezetvédelmi értékelést.

Végül a termékek jelenlegi helyzetét és perspektíváját értékelik.

A könyv összeállítását igen mélyreható szabadalmi kutatás, cikkek, előadások, kutatási eredmények számbavétele és feldolgozása előzte meg. Igen hasznos a hasonló termékeket gyártók, forgalmazók, felhasználók (kozmetikusok, bőrgyógyászok, környezetvédelmi, mezőgazdasági szakemberek, tenzideket felhasználó szakemberek) számára. Nagy haszonnal forgathatják az elméleti kutatással foglalkozó szakemberek is. A maga nemében párrát ritkító munka.

Kiss Béla