

INCREASE OF CONJUGATED LINOLEIC ACID CONTENT OF DAIRY PRODUCTS BY ADDING SUNFLOWER OIL

POVEĆANJE SADRŽAJA KONJUGIRANE LINOLNE KISELINE MLIJEČNIH PROIZVODA DODAVANJEM SUNCOKRETOVOG ULJA

Rozália Veronika Salamon, Katalin Lóki, Éva Varga-Visi, Z. Mándoki, J. Csapó

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SUMMARY

In our experiments we investigated the effect of linoleic acid supplementation on the CLA production of *Lactobacillus acidophilus*, *Lactobacillus plantarum* and *Lactobacillus casei*. We established that a supplementation of 100 µl/100 ml sunflower oil with high linoleic acid content increased CLA content of the sour final product, from 116 to 178 for *Lactobacillus acidophilus*, while for *Lactobacillus plantarum* from 116 to 187 mg/100 g fat (about 40%). Supplementation with amounts higher than 100 µl sunflower oil reduced the CLA content. In the case of *Lactobacillus casei* the increment of CLA was only 20%, and it appears that in the range of 100–1500 µl/100 ml sunflower oil supplementation the amount of linoleic acid does not affect the CLA content.

Keywords: dairy products, sunflower oil, conjugated linoleic acid

INTRODUCTION

Fatty acid composition of milk fat, especially due to short chain fatty acids present in a relative high amount, is ideal for the human organism as triglycerides containing short chain fatty acids can be attacked more easily by digestive enzymes. Although unsaturated fatty acid content of milk fat is relatively low, it can contain considerable amount of the necessary essential fatty acids to satisfy the human needs, and due to its animal origin it also contains the essential arachidonic acid (Csapó and Csapóné Kiss Zs., 2002). Milk fat can contain considerable amount of conjugated linoleic acids (CLA) that have a considerable useful physiological effect according to references. Their antioxidant properties have also been proved, they protect cell

membranes from the attack of free radicals. Due to this feature, they can have a significant biological role (Ha et al, 1987; Lee et al, 1994).

The composition of dairy products produced by addition of bacterial cultures is mainly determined by the composition of the starting milk, since the cultures produce mainly aroma substances, and they have less influence on the fatty acid composition, the technological processes, however, can considerably affect the CLA content of the final product (Salamon et al, 2005 a,b). According to some studies the

Rozália Veronika Salamon, János Csapó, Sapientia Hungarian University of Transylvania, Csíkszereda Campus, Department of Food Sciences; Katalin Lóki, Éva Varga-Visi, Zsolt Mándoki, János Csapó, University of Kaposvár, Faculty of Animal Sciences, Department of Chemistry-Biochemistry.

starter cultures can produce considerable amount of CLA, while others cannot establish such a relationship. As until now there has been no unequivocal answer to what effect the microorganisms have on the CLA content of the product, therefore in an earlier research we examined fatty acid composition and CLA content of dairy products produced from cow milk.

We found several studies in the literature in which the CLA producing capability of different bacterium species was examined (Alonso et al., 2003; Lin, 1999; Lin, 2006; Pariza and Yang, 2000; Sieber et al., 2004). Some researchers reported that the examined bacterium species were able to produce CLA from linoleic acid during the souring (Kishino, 2002; Lin, 2006). On the basis of the results the pure cultures *Lactobacillus plantarum*, *Lactobacillus casei*, and *Lactobacillus acidophilus* were found to be the most suitable for increasing the CLA content of dairy products produced by fermentation.

There are relatively few experiments where linoleic acid is added to the milk before the souring in the pure form of a vegetable oil. Ming and Shuting, 2006 examined the CLA producing capability of *Lactobacillus acidophilus* in milk containing lucerne seed oil (the lucerne seed oil contained approx. 40% linoleic acid). In case of the other two bacterium species no studies were found, therefore our aim was to examine the CLA producing capability of the pure cultures *Lactobacillus plantarum*, *Lactobacillus casei*, and *Lactobacillus acidophilus* when sunflower oil with high linoleic content was added in various doses.

MATERIALS AND METHODS

In the course of the investigations sunflower oil was added before the fermentation. We used sunflower oil with linoleic acid content of 62.7 relative weight of fatty acid methyl esters as a source of linoleic acid. The starter cultures were obtained from the Corvinus University, Budapest, as a slant agar. The temperature of the cultures was 4 °C and they were covered with paraffin oil. From the bacteria a mother souring mixture was prepared by adding the pure cultures to 50 ml of pasteurized milk, then the mixture was incubated at 38 °C for 24 h. For each

samples obtained from the mother souring mixture 1.0 ml was used.

For the sample preparation 100 ml of freshly pasteurized, cooled milk with a fat content of 3.2% was used. To the pasteurized milk 1.0 ml of the mother souring mixture and 50, 100, 150, 200, 300, 400, 600, 1000 and 1500 µl of sunflower oil was added. Blank samples were also prepared for all the three pure cultures. The samples and the blanks were incubated at 38 °C for 24 h, and stored in a deep-freeze until the CLA content was analysed.

Lipid extraction

A milk sample amount containing approx. 0.3 g fat was pipetted into a 100 cm³ beaker, and 80 cm³ of organic solvent mixture (3:2 mixture of hexane and isopropanol, HIP) was added. The sample was dispersed in the solvent mixture (IKA Ultra-turrax T25 basic dispersion apparatus, 9,500 RPM, 2 min). The emulsion was filtrated on a paper filter (MN640W, 90 mm diameter) into a 250 cm³ Erlenmeyer flask. The paper filter was washed three times with 10 cm³ of HIP mixture, the organic layers were combined. 5 g of waterfree sodium sulfate was added and the liquid was shaken up in order to eliminate water. The organic layer was decanted from the salt and evaporated under reduced pressure at 80 °C. The residue was washed with n-hexane into a 10 cm³ measuring flask (hexane solution).

Methylation

0.5 cm³ of the hexane solution was taken into a 4 cm³ capped vial and 0.5 cm³ 4 M sodium methylate methanol solution was added, it was shaken and kept at 50 °C for 30 min. Subsequently, 1 cm³ of hexane and 1 cm³ of water were added, shaken, and after the layers had separated, 1 cm³ of the organic layer was pipetted into a 5 cm³ volumetric flask, 1.2 cm³ of hexane was added to the aqueous layer, it was shaken up and 1 cm³ of the hexanic layer was put into the volumetric flask. This extraction with hexane was repeated twice more, the last time as far as it was possible, the whole hexanic layer was collected, and the volumetric flask was filled up to 5 cm³ with hexane, and the obtained solution was stored in a screw capped vial refrigerated until analysed.

Conditions of the gas chromatographic analysis

The apparatus was a Chrompack CP 9000 gas chromatograph. The dimension of the column was: 100 m×0,25 mm the stationary phase was CP-Sil 88 (FAME). The detector was a FID at 270 °C, the injector was a splitter at 270 °C. The carrier gas was helium at 235 kPa.

The column temperature was programmed: 140 °C for 10 min; at 5 °C/min up to 235 °C, isotherm for 30 min. The injected volume was 2 µl.

For the preparation of the CLA standards, CLA mix obtained from Sigma was used.

The c9,t11-CLA content of the raw milk was as a mean value of five measurements of 117.92 mg/100 g milk which changed to an average value of 116.43 mg/100 g fat in the pasteurized milk. This, however, does not mean a decrease due to the pasteurization since this decrease is minimal, and it is within the error limit of the measurement. In the case of samples with *Lactobacillus acidophilus* the highest c9,t11-CLA content was measured when 100 µl sunflower oil was added (178.37 mg/100 g fat). This maximal value decreased to 141.82 µg/100 g fat when 150 µl sunflower oil was added. The decrease continued when 200- 400 and 600–1500µl was added from 105.91–112.42 mg/100 g fat, to 90.30–91.30 µg/100 g fat.

Almost the same tendency can be observed in case of *Lactobacillus plantarum* when the CLA content increased to 147.85 mg/100 g fat when 50 µl sunflower oil was added, and to 186.88 mg/100 g fat when 100 µl of sunflower oil was added. After that, the decrease showed a little different tendency in

RESULTS AND DISCUSSION

Table 1 shows the change in the CLA content of milk with the pure cultures and with increasing volume of sunflower oil.

Table 1. Change of CLA content of milk produced by cultures as a function of added sunflower oil content
Tablica 1. Promjena sadržaja KLK mlijeka proizvedenog kulturama kao djelovanje dodanog sadržaja suncokretovog ulja

Samples - Uzorci		CLA-content mg/100 g fat - Sadržaj KLK mg/100 g masnoće		
		<i>Lactobacillus acidophilus</i> ^a	<i>Lactobacillus casei</i> ^b	<i>Lactobacillus plantarum</i> ^b
Raw milk ^a - Sirovo mlijeko		117,92±0,17	117,92±0,17	117,92±0,17
Pasteurized milk ^a - Pasterizirano mlijeko		116,54±0,42	116,54±0,42	116,54±0,42
Amount of sunflower oil Količina suncokretovog ulja (ml/100ml)	50 ^b	140,17±2,25	139,46±1,62	147,65±1,59
	100 ^c	178,64±2,32	135,42±1,37	188,64±3,39
	150 ^d	179,86±1,37	135,94±1,85	148,94±1,99
	200 ^a	110,75±4,03	141,17±2,62	129,60±2,48
	300 ^a	111,45±4,28	138,85±3,30	128,87±4,38
	400 ^a	102,67±3,85	142,22±2,59	120,97±2,09
	600 ^a	90,30±3,10	141,92±6,42	117,17±4,41
	1000 ^a	84,05±3,74	137,28±2,25	115,32±2,92
1500 ^a	87,58±2,57	139,69±7,61	117,67±2,57	

^a Values in the same columns or rows without a different letter are significantly different: p ≤ 0,05.

comparison with *Lactobacillus acidophilus* since when 150–1500 μl of sunflower oil was added the CLA content decreased from 148.81 to 117.29 mg/100 g fat. *Lactobacillus casei* exhibits a different tendency than the other two bacteria, it appears that the sunflower oil supplementation in the range of 50–1500 μl does not affect the CLA content. When 50 μl of sunflower oil was added the CLA content increased to 139.11 mg/100 g fat, then it reached its maximum with 142.94 mg/100 g fat when 400 μl of sunflower oil was added, and even with the addition of 1500 μl of sunflower oil the CLA content was 135.65 mg/100 g fat.

Comparing the response of the three lactobacilli to the addition of linoleic acid it can be said that in the case of *Lactobacillus acidophilus* and *Lactobacillus plantarum* when 100 μl /100 ml of sunflower oil was added the amount of CLA increases by 35–40%, while in the case of *Lactobacillus casei* only an increase of 20% was observed. In the case of the latter bacterium in the 50–1500 μl /100 ml range the amount of CLA remained almost unchanged, whereas in the case of *Lactobacillus acidophilus* and *Lactobacillus plantarum* a definite maximum was found at the addition level of 100 μl /100 ml sunflower oil.

Based upon our investigations it can be said that in the case of pure cultures applied in practice certain caution should be exercised when adding sunflower oil prior to the fermentation since there are pure cultures that are nearly indifferent to the amount of added linoleic acid (*Lactobacillus casei*), and there are others that react with maximal production of CLA upon addition of optimal amount of linoleic acid (*Lactobacillus acidophilus*, *Lactobacillus plantarum*), and there can be such pure cultures where the addition of linoleic acid can decrease the CLA content of the soured final product. We recommend the above trial with each lactic acid bacteria used in practice in order to obtain the optimal CLA production.

In the case of the cultures we used the favourable effects reported in the literature, that, the microbes can convert the added linoleic acid into CLA in 20 to 60% (Ha et al, 1987; Salamon, 2005b; Sieber et al, 2004), could not be achieved, which can be explained by the difference between the bacterium species. It cannot be found in the lite-

ature, however, that there can be an optimal linoleic acid intake for each bacterium (in our case in 100 μl sunflower oil /100 ml milk), above which the linoleic acid can act as growth inhibitor, reducing the amount of CLA, in fact, the CLA content can decrease even below the value of the starting milk.

SUMMARY

In this research the effect of linoleic acid supplementation on the CLA production of *Lactobacillus acidophilus*, *Lactobacillus plantarum* and *Lactobacillus casei* was examined. It was established that a supplementation of 100 μl /100 ml sunflower oil with high linoleic acid content increased the CLA content of the final sour product, from 116 mg/100 g fat to 178 for *Lactobacillus acidophilus*, while for *Lactobacillus plantarum* to 187 mg/100 g fat. Supplementation of more than 100 μl sunflower oil reduced the CLA content. In the case of *Lactobacillus casei* the CLA content increment was only 20% (from 116 mg/100 g fat to 143 mg/100 g fat), and it appears that in the range of 100–1500 μl /100 ml sunflower oil supplementation the amount of linoleic acid does not affect the CLA content.

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SAŽETAK

U pokusima smo istraživali djelovanje dodavanja linolne kiseline na proizvodnju KLK *Lactobacillus acidophilus*, *Lactobacillus plantarum* i *Lactobacillus casei*. Ustanovili smo da je dodavanje 100 ml/100 ml suncokretovog ulja s visokim sadržajem linolne kiseline povećalo sadržaj KLK konačnog kiselog proizvoda od 116 na 178 za *Lactobacillus acidophilus* a za *Lactobacillus plantarum* od 116 na 187 mg/100 g masnoće (oko 40%). Dodavanje količina većih od 100 ml suncokretovog ulja smanjilo je sadržaj KLK. U slučaju *Lactobacillus casei* postotak povećanja KLK bio je samo 20%, pa izgleda da dodavanjem suncokretovog ulja u rasponu od 100 do 1500 ml količina linolne kiseline ne djeluje na sadržaj KLK.

Ključne riječi: mliječni proizvodi, suncokretovo ulje, konjugirana linolna kiselina (KLK)