Acta Univ. Sapientiae, Alimentaria, **2,** 2 (2009) 287–293

Increase of conjugated linoleic acid content of dairy products by adding sunflower oil

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Abstract. 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 with 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 the increment percent 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.

Key words and phrases: dairy products, sunflower oil, conjugated linoleic acid

1 Introduction

Fatty acid composition of milk fat, especially due to short chain fatty acids present in a relative high amount, is ideal to the human organism as triglycerides containing short chain fatty acids can be attacked more easily by digestive enzymes. Unsaturated fatty acid content of milk fat is relatively low, despite this 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 [2]. The milk fat can contain considerable amount of conjugated linoleic acids (CLA) that have many useful physiological effect according to the references. Their antioxidant properties were also proved, protect cell membranes from the attack of free radicals. Due to this feature, they can have a significant biological role [3, 5].

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 [8, 9]. According to some studies the starter cultures can produces considerable amount of CLA, while others could not establish such a relationship. As until now there has been no unequivocal answer to what effect the microorganisms have on the CLA content on the product, therefore in an earlier research we examined fatty acid composition and CLA content of dairy products produced from cow's milk.

We found several studies in the literature in which the CLA producing capability of different bacterium species was examined [1, 6, 10]. Some researchers reported that the examined bacterium species were able to produce CLA from linoleic acid during the souring [4, 6]. 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 [7] 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.

2 Material and methods

In the course of the examinations the sunflower oil was added before the fermentation. We used a sunflower oil with linoleic acid content of 62.7 relative weight% of fatty acid methyl esters as source of linoleic acid. The starter cultures were obtained from the Corvinus University, Budapest, as aslant 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 incubed at 38 °C for 24 h. From the mother souring mixture obtained 1.0 ml was used for each samples. 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 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 in case of all the three pure cultures. The samples and the blanks were incubed at 38 °C for 24 h, and stored in a deep-freeze until the analysis of the CLA content.

2.1 Lipid extraction

A milk sample amount containing approx. 0.3 g fat was pipetted into a 100 ml beaker, and 80 ml of organic solvent mixture (3:2 mixture of hexane and isopropanol, HIP) was added. The sample was dispergated 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 ml Erlenmeyer flask. The paper filter was washed three times with 10 ml 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 ml measuring flask (hexane solution).

2.2 Methylation

0.5 ml of the hexane solution was taken into a 4 ml capped vial and 0.5 ml 4 M sodium methylate methanol solution was added, it was shaken up and kept at 50 °C for 30 min. Subsequently, 1 ml of hexane and 1 ml of water were added, it was shaken up, and after the layers have separated, 1 ml of the organic layer was pipetted into a 5 ml volumetric flask, to the aqueous layer 1.2 ml of hexane was added, it was shaken up and 1 ml of the hexanic layer was taken 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 ml with hexane, and the obtained solution was stored in a screw capped vial refrigerated until the analysis.

2.3 Conditions of the gas chromatographic analysis

The apparatus was a Chrompack CP 9000 gas chromatograph. The dimension of the column were: 100 m×0.25 mm the stationary phase was CP-Sil 88 (FAME). The detector was a FID at 270 °C, the injector was 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.

3 Results

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

The c9,t11-CLA content of the raw milk was as a mean value of five measurements 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 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 was continued when 200–400 and 600–1500 µl 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 increases 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 shows a little different tendency in 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.

Table 1: Change of CLA content of milk produced by cutures as a function of added sunflower oil content

Sample		CLA-content mg/100 g fat		
		Lactobacillus acidophilus	***Lactobacillus casei***	***Lactobacillus plantarum***
Raw milk		117.92±0.17^{a}	117.92±0.17^{a}	117.92±0.17^{a}
Pasteurized milk		116.54±0.42^{a}	116.54±0.42^{a}	116.54±0.42^{a}
Amount of sunflower oil (µl/100 ml)	50	140.17±2.25^{b}	139.46±1.62^{b}	147.65±1.59^{b}
	100	178.64±2.32^{c}	135.42±1.37^{b}	188.64±3.39^{c}
	15	179.86±1.37^{c}	135.94±1.85^{b}	148.94±1.99^{b}
	200	110.75±4.03^{a}	141.17±2.62^{b}	129.60±2.48^{d}
	300	111.45±4.28^{a}	138.85±3.30^{b}	128.87±4.38^{d}
	400	102.67±3.85^{a}	142.22±2.59^{b}	120.97±2.09^{a}
	600	90.30±3.10^{d}	141.92±6.42^{b}	117.17±4.41^{a}
	1000	84.05±3.74^{d}	137.28±2.25^{b}	115.32±2.92^{a}
	1500	87.58±2.57^{d}	139.69±7.61^{b}	117.67±2.57^{a}

Even at 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 established 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 experienced. In the case of this latter bacterium in the 50–1500 µl/100 ml range the amount of CLA remained almost unchanged, whereas in 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 examinations it can be said that in the case of pure cultures applied in the 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 recom-

mend to make the above trial with each lactic acid bacteria used in the practice in order to obtain the optimal CLA production. In case of the cultures we used the favourable effects reported in the literature, that is, the microbes can convert the added linoleic acid into CLA in 20 to 60% [3, 9, 10], could not be reached , which can be explained by the difference between the bacterium species. It cannot be found in the literature, 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.

4 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 with 100 µl/100 ml sunflower oil with high linoleic acid content increased the CLA content of the sour final product, from 116 mg/100 g fat to 178 for *Lactobacillus acidophilus,* while for *Lactobacillus plantarum* to 187 mg/100 g fat. Supplementation with more than 100 µl sunflower oil reduced the CLA content. In the case of *Lactobacillus casei* the CLA content increment percent is 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.

5 Acknowledgements

The authors gratefully thank to OTKA for their financial support of the researches (Contract No.: 49405). The Sapientia–Hungarian University of Transylvania, Institute of Research Programs, also granted the research. Their financial assistance is gratefully acknowledged.

References

[1] L. Alonso, E.P. Cuesta, S.E. Gilliland, Production of free conjugated linoleic acid by Lactobacillus acidophilus and Lactobacillus casei of human intestinal origin, *Journal of Dairy Science,* 86 (2003) 1941-1946.

[2] J. Csapó, Zs. Csapó-Kiss, *Tej és tejtermékek a táplálkozásban. (Milk and milk products in human nutrition),* Mezőgazda Kiadó, Budapest 2002. 1-464.

[3] Y.L. Ha, N.K. Grimm, M.W. Pariza, M.W. Anticarcinogens from fried ground beef: Heat-altered derivatives of linoleic acid, *Carcinogenesis,* 8 (1987) 1881-1887.

[4] S. Kishino, J. Ogawa, Y. Omura, K. Matsumura, S. Shimizu, Conjugated linoleic acid production from linoleic acid by lactic acid bacteria, *Journal of American Oil Chemist's Society,* 79 (2002) 159-163.

[5] K.N. Lee, D. Kritchevsky, M.W. Pariza, Conjugated linoleic acid and atherosclerosis in rabbits, *Atherosclerosis,* 108 (1994) 19-25.

[6] T.Y. Lin, Conjugated linoleic acid production by cells and enzyme extract of *Lactobacillus delbrueckii ssp. bulgaricus* with additions of different fatty acid, *Food Chemistry,* 94 (2006) 437-441.

[7] D. Ming, Q. Shuting, Conjugated linoleic acid production by fermentation, *International Journal of Food Engineering,* 2, 5 (2006) 1-12.

[8] R.V. Salamon, S. Szakály, Z. Szakály, J. Csapó, Conjugated linoleic acid (CLA) - dairy products - human health. 1. Basic knowledge and CLA in milk, *Journal of the Academic Hungarian Dairying,* 65 (2005a) 2-12.

[9] R.V. Salamon, S. Szakály, Z. Szakály, J. Csapó, Conjugated linoleic acid (CLA) - dairy products - human health. 2. CLA in diary products and different foods, *The Journal of the Academic Hungarian Dairying,* 65 (2005b) 14-21.

[10] R. Sieber, M. Collomb, A. Aeschlimann, P. Jelen, H. Eyer, Impact of microbial cultures on conjugated linoleic acid in dairy products – a review, *International Dairy Journal,* 14 (2004) 1-15.

Received: August, 2009