Short Communication: Concentration of Conjugated Linoleic Acid from Milk Fat with a Continuous Supercritical Fluid Processing System

P. Romero K.,* S.S.H. Rizvi,* M. L. Kelly,† and D. E. Bauman†
*Institute of Food Science, Stocking Hall
†Department of Animal Science, Morrison Hall,
Cornell University, Ithaca, NY 14853

ABSTRACT

A continuous pilot-scale supercritical carbon dioxide system was utilized for the concentration of conjugated linoleic acids (cis-9, trans-11 C18:2) from anhydrous milk fat, which was separated into five fractions (S1 to S5) in the pressure and temperature range of 2.4 to 24.1 MPa (350 to 3500 psi) and 40 to 60°C, respectively. The highest concentration of CLA attained showed an increase of about 89% and occurred in the raffinate fraction (S1) when the solvent to feed ratio was 65. This was followed by a gradual decrease in the concentration of this fatty acid from S2 to S5. This study shows the feasibility of selectively enhancing the CLA concentration in one of the fractions of milk fat with a benign solvent in a one-step process. Other unique attributes of the CLA-rich fraction are also listed.

(**Key words:** milk fat, conjugated linoleic acid)

Abbreviation key: AMF = Anhydrous milk fat, **CLA** = conjugated linoleic acid, **SC** = supercritical.

INTRODUCTION

Conjugated linoleic acids (**CLA**) are a family of octadecadienoic acids, which have been reported to have a range of positive health effects in experimental animal models (2). In particular, CLA are a potent cancer preventive agent in animal models. Dairy products are the major source of CLA in human diets, and enhancing the milk fat concentration of CLA is of interest because of its beneficial health effects. The presence of CLA in milk fat relates to the pathways of biohydrogenation of unsaturated fatty acids in the rumen (5). One means to increase CLA concentration in milk fat is to manipulate the diet to increase the rumen escape of CLA or trans-11 C18:1. A number of factors have been established to alter the rumen environment in a manner to cause an increase in the CLA content in milk fat (2). Another means to alter the milk fat content of CLA is to selectively fractionate milk fat during the manufacturing processes.

Supercritical fluid processing is one method of fat fractionation, and several studies have described this technique (1, 7, 8). The supercritical (**SC**)-CO_2 fractions of milk fat are unique in their physical and chemical properties, and this procedure has been used to obtain milk fat fractions suitable for applications in different food formulations and nutritional supplements. The SC-CO_2 solid fraction obtained from anhydrous milk fat (**AMF**) is the most desirable and unique in that it is lower in cholesterol, is richer in β-carotene, and has a higher concentration of long-chain, unsaturated fatty acid-containing triacylglycerols in a very narrow range of high molecular weight (3, 7). Our objective was to utilize SC-CO_2 in the fractionation of milk fat and to examine the CLA content of various fractions as a means of selectively enriching it in one of the fractions.

The feedstock used for this process was AMF, which was derived from commercially available milk fat; the milk fat fractions were obtained with a continuous pilot-scale SC-CO_2 system. It consisted of a column (1.8 m long and 4.9 cm in internal diameter) packed with stainless steel 304 Goodloe knitted mesh packing and five separation vessels. The AMF feedstock was fed on top of the column, and SC-CO_2 at the desired pressure and temperature conditions was fed from the bottom in a countercurrent mode. The extraction was conducted over a pressure range of 24.1 to 2.4 MPa and temperature of 40 to 60°C, as described elsewhere (4). The CLA content of the milk fat fractions was determined by gas chromatography. Milk fatty acids were transesterified, and fatty acid methyl esters were separated with a gas chromatograph (Hewlett Packard GCD system HPG1800A; Avondale, PA). Methods and gas chromatographic conditions were as described previously (6). CLA peaks were identified using pure standards (Nu Check-Prep, Inc; Elysian, MN). All the samples were produced at least twice using the SC-CO_2 processing system, and the analysis was done in duplicate.

Received June 1, 1999.
Accepted September 7, 1999.
Corresponding author S.S.H. Rizvi; e-mail: ssr3@cornell.edu.

Table 1. Operating conditions for supercritical (SC)-CO_2 fractionation of anhydrous milk fat (AMF) and conjugated linoleic acid (CLA) content of various fractions (S1 to S5) at solvent-to-feed ratio of 65.

Sample	°C	Pressure MPa (psi)	Fat yield (wt %)	CLA (mg/g)	CLA change (wt %)
AMF	. . .	. . .	100.0	4.2	. . .
S1	40	24.1 (3500)	8.8	7.8	+88.9
S2	50	17.2 (2500)	31.6	4.5	+7.4
S3	40	10.3 (1500)	41.8	4.2	−1.0
S4	60	7.9 (1150)	12.0	2.8	−32.5
S5	40	2.4 (350)	5.0	3.1	−25.3

Table 1 shows the details of the fractionation conditions along with the yields for the five fractions (S1 to S5) obtained and their respective CLA contents. The highest concentration of CLA attained showed an increase of 89% and occurred in fraction S1 at a solvent to feed ratio of 65. This finding was followed by a slight increase in CLA concentration of S2, and then a steady decline was observed in the remaining fractions (S3, S4, and S5). The increase in CLA content in S1 followed the same trend as the long-chain (C14 to C18), unsaturated fatty acid-containing triacylglycerols, which tended to selectively concentrate in this fraction (3, 4) and then decrease proportionately in the remaining fractions. This finding was expected because SC-CO_2 fractionation occurs primarily on the basis of molecular weight and dielectric properties of fatty acids and glycerides.

Experiments were also performed to further evaluate the effect of solvent-to-feed ratio (rate of flow of SC-CO_2 and AMF) on the extractability of AMF fractions and their CLA enrichment. With the same extraction conditions (pressure and temperature) as shown in Table 1, the solvent-to-feed ratio varied from 40 to 65. The maximum CLA concentration was obtained at the highest solvent-to-feed ratio (Figure 1).

Generally, as the ratio of solvent flow rate to feed flow rate increases, the total extracted quantity of solute also increases, which leads to a lower yield of higher molecular weight raffinate with higher CLA. At a solvent-to-feed ratio of 65, the yield of S1 (raffinate) was 8.8 wt % (Table 1) for the highest CLA content compared with other operating conditions (Figure 1). Therefore, it would be possible to incrementally increase the CLA content in S1 further, but the cost would be prohibitively high, so higher solvent-to-feed ratios were not investigated.

A comparison of some of the other attributes of AMF with its supercritical fractions is shown in Table 2.

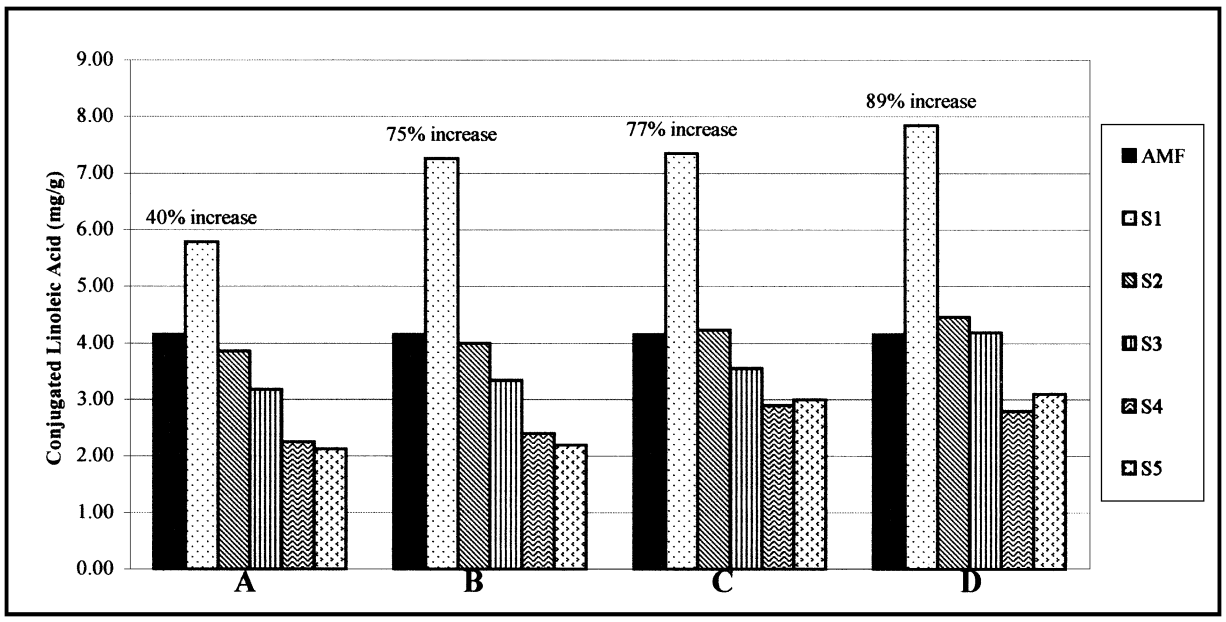

Figure 1. Concentration of conjugated linoleic acid (milligrams per gram fat) in anhydrous milk fat (AMF) and its supercritical (SC) CO_2 milk fat fractions obtained at different solvent-to-feed ratios: A = 40, B = 56, C = 60, D = 65, S1 to S5 = separated milk fractions 1 to 5.

Table 2. Fatty acids, cholesterol, β-carotene, and conjugated linoleic acid (CLA) distribution in supercritical (SC)-CO_2 milk fat fractions (S1 to S5).

Components	AMF	S1	S2	S3	S4 + S5
Saturated fatty acids (wt %)	64	51	63	67	71
Unsaturated fatty acids (wt %)	29	39	29	26	23
Unsaturated/saturated	0.45	0.76	0.47	0.39	0.33
Cholesterol (mg/100 g)	273	110	252	307	345
β-Carotene (mg/kg)	2.15	8.74	1.06	0.52	0.48
CLA (mg/g)	4.2	7.8	4.5	4.2	2.8

Results demonstrate the unique properties of S1, which has the highest unsaturated to saturated fatty acid ratio, β-carotene, and CLA content and the lowest cholesterol level among the AMF and the remaining fractions. The melting point of this fraction has been previously reported to be above 30°C (3) and it, therefore, exists as semisolid at room temperatures.

The S1 fraction, obtained by processing milk fat with SC-CO_2 could be added back into dairy products like skim milk, low-fat yogurt, cheese, ice cream, and butter to make a nutritionally improved product with unique chemopreventive properties. The uniqueness of this fraction would make it very appealing to be used as a nutraceutical to fortify dairy products that could easily be incorporated as part of a healthy diet. Another possibility would be to use this product to manufacture specialty butter with unique nutritional and anticarcinogenic properties with the added advantage that it would not melt at room temperatures and will thus not require refrigerated storage.

Further sensory testing, nutritional evaluation, and rheological analysis need to be performed on products obtained with fraction S1 to establish a complete profile of its unique properties and consumer acceptability.

REFERENCES

1 Arul, J. B., J. Makhlouf, R. Tardif, and M. R. Sahasrabudhe. 1987. Fractionation of AMF by supercritical carbon dioxide. J. Food Sci. 52:1231–1236.

2 Banni, S., and J. C. Martin. 1998. Conjugated linoleic acids and metabolites. Pages 261–302 *in* Trans Fatty Acids in Human Nutrition. J. L. Sebedio and W. W. Christie, ed. The Oily Press, Dundee, Scotland.

3 Bhaskar, A. R., S.S.H. Rizvi, C. Bertoli, L. B. Fay, and B. Hug. 1998. A comparison of physical and chemical properties of milk fat fractions obtained by two processing technologies. J. Am. Oil Chem. Soc. 75:1249–1264.

4 Bhaskar, A. R., S.S.H. Rizvi, and J. W. Sherbon. 1993. Anhydrous milk fat fractionation with continuous countercurrent supercritical carbon dioxide. J. Food Sci. 58:748–752.

5 Griinari, J. M., and D. E. Bauman. Biosynthesis of CLA and incorporation into milk fat. Conjugated Linoleic Acid: Biochemical and Nutritional, Clinical, Cancer, and Methodological Aspects. M. P. Yuraweca, M. M. Mossoba, J.K.G. Kramer, G. Nelson, and M. W. Pariza, ed. AOCS Press, XXX, XX. In press.

6 Kelly, M. L., J. R. Berry, D. A. Dwyer, J. M. Griinari, P. Y. Chouinard, M. E. Van Amburgh, and D. E. Bauman. 1998. Dietary fatty acid sources affect conjugated linoleic acid concentrations in milk from lactating dairy cows. J. Nutr. 128:881–885.

7 Rizvi, S.S.H., and A. R. Bhaskar. 1995. Supercritical fluid processing of milk fat: fractionation, scale-up, and economics. Food Technol. 49:90–98.

8 Singh, B., and S.S.H. Rizvi. 1994. Design and economic analysis for continuous countercurrent processing of milk fat with SC-CO_2. J. Dairy Sci. 77:1731–1745.