# INCREASE OF CONJUGATED LINOLEIC ACID AND VACCENIC ACID IN ANHYDROUS MILK FAT USING DRY FRACTIONATION AND ITS EFFECT ON THE ATHEROGENIC INDEX

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*SUMMARY*

*Cows' feed supplementation and dry fractionation of anhydrous milk fat (AMF) was carried out to increase conjugated linoleic acid (CLA) and vaccenic acid (VA) contents, and to evaluate the resulting atherogenic index (AI). Dairy cows were fed sunflower seeds and pasture. Milking from the starting day was considered as control milk and from the 16th day as enriched milk. Control and enriched AMF were fractionated to obtain fractions at 25, 20, 15, 10, 5, 0 and -5°C. Lipid profiles were determined by gas chromatography and mass spectrometry. Enriched AMF and fractions showed lower levels of saturated fatty acids and higher levels of unsaturated fatty acids when compared to control samples. In particular, VA increased 154% in enriched AMF and 123% after fractionation (liquid fraction at 0ºC), while CLA increased 31% in enriched AMF and 59% after fractionation (liquid fraction at 0ºC). The AI in enriched fractions was lower than in control fractions. The liquid fraction obtained at 15ºC may be the most useful one due to its fairly low AI (0.95), its VA and CLA concentrations (6.52 and 8.0 g/100g, respectively), as well as its fractionation temperature that does not require as much energy as lower-temperature fractions would consume. Dry fractionation is an inexpensive chemical-free process that may be used to produce CLA-rich and low-AI fat that is desirable for healthier food products such as butter, cream, cookies and bread, in a way that would not be possible with conventional dairy processing equipment.*

## Introduction

Conjugated linoleic acid (CLA) is a group of positional and geometric isomers of linoleic acid with conjugated double bonds. Although still controversial, it has been reported that *cis*-9,*trans*-11 CLA may provide beneficial effects on human and animals health such as reduction of body fat, anticarcinogenic, antiatherogenic and hypocholesterolemic effects, as well as inhibition of proinflammatory cytokines, among others (Ha *et al*., 1987; Lee *et al*., 1994; Pariza *et al*., 2001; Wahle *et al*., 2004; Battacharya *et al*., 2006; Park and Pariza, 2007).

Vaccenic acid (18:1-*cis*, *trans*-11 VA) is the main *trans* fatty acid in milk fat (Turpeinen *et al*., 2002) and an alternative source of *cis*-9,*trans*-11 CLA by Δ-9 desaturation (Bauman *et al*., 2000; Park y Pariza, 2007). Although little is known about the effects of VA, some hypolipidemic effects were observed in rats (Wang *et al*., 2008; Tyburczy *et al*., 2009, Wang *et al*., 2009).

The use of milk fat has been diversified by the implementation of different technologies including dry fractionation, which separates fat into fractions that are markedly different in both composition and melting points (Deffense, 1995; O'Shea *et al*., 2000). Dry fractionation is a physical process that overcomes the use of controversial chemicals. Milk fat may be dry fractionated into a solid fraction, called 'stearin' that has a high content of saturated fatty acids, as well as a liquid fraction named 'olein', which is rich in polyunsaturated fatty acids (Arnaud *et al*., 2006). CLA and VA contents may be increased in milk fat by using dry fractionation (O'Shea *et al*., 2000) to produce CLA- and VA-rich dairy products such as butter, cream, ice cream, anhydrous milk fat, among others.

Anhydrous milk fat (AMF) is widely used in the food industry, mainly for developing spreadable butters, chocolate, ice cream and bakery products (Kaylegian and Lindsay, 1995). Significant changes were observed in AMF when dry fractionated. This process increased C14:0 and C16:0 fatty acids in the solid fractions, as well as C18:1 in

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## INCREMENTO DE ÁCIDO LINOLEICO CONJUGADO Y ÁCIDO VACCÉNICO MEDIANTE UN FRACCIONAMIENTO EN SECO DE GRASA BUTÍRICA ANHIDRA Y SU EFECTO SOBRE EL ÍNDICE ATEROGÉNICO

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*RESUMEN*

*El objetivo del estudio fue realizar un fraccionamiento en seco de grasa butiríca anhidra (AMF) para incrementar sus niveles de ácido linoleico conjugado (CLA) y ácido vaccénico (VA) y evaluar sus índices aterogénicos (AI). Las vacas fueron alimentadas con semillas de girasol y pastura. El ordeño del día inicial se consideró leche control y el del día 16 como leche enriquecida. Ambas leches fueron fraccionadas hasta obtener fracciones a 20, 15, 10, 5, 0 y -5°C. El perfil lipídico se determinó por cromatografía de gases acoplada a masas. La AMF enriquecida y las fracciones mostraron disminución de ácidos grasos saturados e incremento de ácidos grasos insaturados, al compararlas con muestras control. Los contenidos de VA aumentaron 154% en las fracciones enriquecidas y 123% después del fraccionamiento en seco (fracción líquida a 0°C). Los niveles de CLA incrementaron 31% en la AMF enriquecida y 59% después del fraccionamiento en seco. El índice aterogénico en las fracciones enriquecidas fue menor que en los controles. La fracción líquida obtenida a 15°C es la más útil, por su bajo índice aterogénico (0,95) y sus contenidos de VA y CLA (6,52 y 8,0 g/100g, respectivamente); además de requerir menor consumo de energía que a menor temperatura. El fraccionamiento en seco es un proceso de bajo costo, libre de químicos, que puede utilizarse para producir grasa láctea rica en CLA con bajos índices aterogénicos, para la preparación de productos alimenticios saludables como mantequilla, cremas, galletas y pan, lo que no sería posible con equipo convencional de procesamiento lácteo.*

## INCREMENTO DE ÁCIDO LINOLÉICO CONJUGADO E ÁCIDO VACÊNICO MEDIANTE UM FRACCIONAMENTO EM SECO DE GORDURA BUTÍRICA ANIDRA E SEU EFEITO SOBRE O ÍNDICE ATEROGÊNICO

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*RESUMO*

*O objetivo do estudo foi realizar um fracionamento em seco de gordura butírica anidra (AMF) para incrementar seus níveis de ácido linoleico conjugado (CLA) e ácido vacênico (VA) e avaliar seus índices aterogênicos (AI). As vacas foram alimentadas com sementes de girassol e pastagem. A ordenha do dia inicial se considerou leite controle e o do dia 16 como leite enriquecido. Ambos os leites foram fraccionadas até obter fracções a 20, 15, 10, 5, 0 e -5°C. O perfil lipídico se determinou por cromatografia de gases acoplada a massas. A AMF enriquecida e as frações mostraram diminuição de ácidos graxos saturados e incremento de ácidos graxos insaturados, ao compara-las com amostras controle. Os conteúdos de VA aumentaram 154% nas frações enriquecidas e 123% depois do fraccionamento em seco (fração líquida a 0°C). Os níveis de CLA incrementaram 31% na AMF enriquecida e 59% depois do fraccionamento em seco. O índice aterogênico nas frações enriquecidas foi menor que nos controles. A fração líquida obtida a 15°C é a mais útil, por seu baixo índice aterogênico (0,95) e seus conteúdos de VA e CLA (6,52 e 8,0 g/100g, respectivamente); além de requerer menor consumo de energia que a menor temperatura. O fraccionamento em seco é um processo de baixo custo, livre de químicos, que pode utilizar-se para produzir gordura láctea rica em CLA com baixos índices aterogênicos, para a preparação de produtos alimentícios saudáveis como manteiga, cremes, bolachas e pão, o que no seria possível com equipamento convencional de processamento lácteo.*

the liquid fractions (Brystom and Hartel, 1994). O`Shea *et al*. (2000), in a study regarding the enrichment of CLA content of AMF by dry fractionation, reported that C16:0 and C18:0 concentrations in solid fractions were increased by 6.7 and 24.7% respectively, and that oleic acid, CLA and VA contents in liquid fractions were also increased by 25.6, 63.2 and 36% respectively, when compared to control AMF.

Studies that aimed enrichment of CLA fractions through dry fractionation have used ordinary AMF as a raw material. Few studies, if any, have reported the dry fractionation of CLA/VA-rich AMF. The present study was undertaken to investigate such a possibility. Dry fractionation of CLA/VA-rich AMF was performed to prepare different fractions, which were analyzed for their CLA and VA contents as well as for their atherogenic index (AI), and the results obtained were compared.

### Materials and Methods

*Milk collection*

This study was approved by the Ethics Committee of the Research Department of the University of Veracruz, Mexico. Four healthy multiparous Holstein cows from Teocelo, Veracruz, México (19º23', 96º58'W, 1186masl), with 126 ±26 days of lactation were fed daily, during 30 days, with 4kg of Api-Aba concentrate (Malta-Cleyton, México) containing 16% of crude protein, 2.5kg of crude sunflower seeds and pasture. Milking from the starting day (day 0) was considered as control milk. During the adaptation period (15 days) milk samples were

collected on the 5th, 10th and 15th days. Starting on the 16th day, samples (enriched milk) were collected daily until the end of the experiment (Cruz-Hernandez *et al*., 2007). Milk was always collected at 18:00, immediately transported to the laboratory in a cooler and processed.

*Anhydrous milk fat (AMF) preparation*

AMF was prepared as indicated in the Dairy Processing Handbook (Tetra Pack, 1995). Control and enriched milk were initially skimmed. The obtained creams were then heated at 60°C for 60min to allow all fat crystals to melt. After, the melted fats were centrifuged at 4000rpm for 60min at 35°C in a Hettich 32R Universal refrigerated centrifuge. Fats (control and enriched AMFs) were decanted and stored at -10°C until further processing.

*AMF dry fractionation*

Dry fractionation was performed in a Cole-Parmer 12101-51 Programmable Polystat Refrigerated Circulating Bath. In order to completely melt any fat crystal, AMF from control and enriched milk was heated at 60°C during 1h. Subsequently, AMF was rapidly cooled down to 30°C and then processed at a cooling rate of 0.3°C/h, with a stirring speed of 16rpm until reaching 25°C. Then, fat was centrifuged to separate the solid fraction from the corresponding liquid fraction. The solid fraction and a 10ml sample of the liquid fraction were stored at -10ºC until further analysis. The remaining liquid fraction was again fractionated. This process was repeated in both control and enriched AMF to obtain liquid and solid fractions at 25, 20, 15 and 10°C, as well as at 5, 0 and -5°C for the enriched AMF samples only.

*Fatty acid analysis*

Fatty acids were methylated in duplicate according to Christie (1982). Fatty acid methyl esters (FAME) were analyzed by gas chromatography (Agilent Technologies Model 6890N equipped with a J & W Scientific 122-5062 5%-phenyl-methylpolysiloxane capillary column DB-5 (60m × 0.25 ml × 0.25µm), a split-splitless injector and an autosampler) and by mass spectrometry (Agilent Technologies 5975 inert XL model). The temperature program was: initial temperature of 40ºC during 4min, increased by 30°C/min to 210ºC, increased by 1ºC/min to 213ºC, increased by 20ºC/min to 225ºC and held for 40min. Mass spectra were obtained by electron-impact ionization at 70eV. The identification of the peaks of each fatty acid was made by comparing the spectra with the library (HP-Chemestation NIST 05 Mass Spectral Research Program Version 2.0d) and was confirmed through their corresponding standards (C:4-C:24 isomers, No.18919 Supelco, México); isomers *cis*-9, *trans*-11 CLA (CLA No. 05632, Sigma-Aldrich, México) and *cis*-18:1, *trans*-11 VA (VA No. 46905-U, Supelco, México). All chemicals and solvents were of analytical grade.

*Atherogenic index calculation*

The atherogenic Index of each obtained fraction was calculated according to Ulbricht and Southgate (1991) as

$$IA = \frac{aS' + bS'' + cS'''}{dP + eM + fM'}$$

where S′: concentration (g/100g) of C12:0; S'', C14:0; S''', C16:0; P: sum of polyunsaturated fatty acids; M: C18:1; M': sum of monounsaturated fatty acids; a-f: empirical constants, where a, c, d, e, f have a value of 1, while b= 4.

TABLE I
MEANS AND STANDARD ERROR OF THE MEAN (SEM) OF FATTY ACIDS CONTENT IN CONTROL AND CLA/VA RICH AMFS (g/100g FAME*)

| Fatty acid (FAME*) | Control AMF | CLA/VA rich AMF |
|---|---|---|
| C 4:0 | 5.10 ±0.47 a | 3.20 ±0.65 a |
| C 6:0 | 2.14 ±0.33 a | 1.26 ±0.09 b |
| C 8:0 | 1.84 ±0.03 a | 0.88 ±0.04 b |
| C 10:0 | 2.79 ±0.01 a | 1.26 ±0.04 b |
| C 12:0 | 2.68 ±0.01 a | 1.51 ±0.16 b |
| C 14:0 | 8.97 ±0.04 a | 6.43 ±0.03 b |
| C 14:1 | 0.76 ±0.01 a | 0.63 ±0.02 a |
| C 15:0 | 0.84 ±0.01 a | 0.73 ±0.03 a |
| C 16:0 | 22.99 ±0.18 a | 19.12 ±0.35 b |
| C 16:1 | 1.16 ±0.05 a | 1.03 ±0.06 b |
| C 17:0 | 0.58 ±0.05 a | 0.44 ±0.01 a |
| C 18:0 | 13.26 ±0.01 a | 14.99 ±0.40 a |
| C 18:1 ω9 | 23.52 ±0.29 a | 29.76 ±0.46 b |
| C 18:1*t* | 1.33 ±0.37 a | 0.39 ±0.05 a |
| C 18:2 ω6 | 3.11 ±0.04 a | 2.66 ±0.01 a |
| VA | 3.21 ±0.04 a | 8.16b ±0.86 |
| CLA | 5.70 ±0.13 a | 7.45b ±0.03 |
| ∑SFA | 61.2 | 49.8 |
| ∑UFA | 38.8 | 50.1 |

* Fatty acid methyl ester
a, b: Values within rows with different superscript letters are significantly different (p≤0.05) C18:1t : elaidic acid, VA: vaccenic acid, CLA: conjugated linoleic acid, SFA: saturated fatty acids, UFA: unsaturated fatty acids.

*Statistical analysis*

Data are presented as means and SEM. Statistical analysis was performed using one-way ANOVA and Tukey's multiple mean comparison test (Statistica 8.0). Statistical significance was declared at p≤0.05.

**Results and Discussion**

Table 1 shows the fatty acids profiles of control and enriched AMF before fractionation. Both AMFs were rich in C16:0, C18:0 and C18:1. Total unsaturated fatty acids (UFA) concentration was notably higher (30%) in the enriched AMF when compared to the control. On the other hand, total saturated fatty acids (SFA) content in the enriched AMF was considerably lower (18%) than in the corresponding control AMF.

VA and CLA contents were found to be increased in enriched AMF (154 and 31%, respectively, p≤0.05) when compared to control AMF. The VA and CLA concentrations, 8.16 and 7.45g/100g of fat, were ≈1.22 and 3.5 fold higher than those previously reported by our group (Silva-Hernandez *et al*., 2007). In that study, VA and CLA concentrations were 6.67 and 2.12g/100g of fat. Other authors have also reported modifications in VA and CLA contents in milk when manipulating cows' feed. For example, supplementation of dairy cows diet with linolenic acid-rich seeds such as peanut, sunflower, flaxseed or canola increased VA and CLA contents in milk fat (Collomb *et al*., 2004, Cruz-Hernández *et al.,* 2007). Also, fish oil supplementation of dairy cows diets also increased VA and CLA contents in milk fat (AbuGhazaleh *et al*., 2003).

In the fractionation procedure, at the end of each fractionation step two frac-

TABLE II
MEANS AND STANDARD ERROR OF THE MEAN (SEM) OF FATTY ACIDS CONTENT IN CONTROL AND ENRICHED FRACTIONS (g/100g FAME*)**

| Fractions | | °C | C 4:0 | C 6:0 | C 8:0 | C 10:0 | C 12:0 | C 14:1 | C 14:0 | C 15:0 | C 16:0 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | Solid | 25 | 4.75 ±0.06 | 1.91 ±0.03 | 1.67 ±0.01 | 2.64 ±0.01 | 2.62 ±0.01 | 9.19 ±0.02 | 0.68 ±0.03 | 0.88 ±0.01 | 1.01 ±0.06 |
| | | 20 | 4.09 ±0.01 | 1.91 ±0.11 | 1.78 ±0.01 | 2.8 ±0.01 | 2.71 ±0.04 | 9.37 ±0.01 | 0.73 ±0.01 | 0.86 ±0.01 | 1.15 ±0.05 |
| | | 15 | 5.49 ±0.58 | 2.2 ±0.29 | 1.84 ±0.03 | 2.73 ±0.04 | 2.58 ±0.06 | 8.61 ±0.16 | 0.73 ±0.01 | 0.81 ±0.01 | 1.12 ±0.31 |
| | | 10 | 5.29 ±0.38 | 2.1 ±0.18 | 2 ±0.03 | 2.97 ±0.06 | 2.75 ±0.04 | 9.18 ±0.1 | 0.79 ±0.03 | 0.83 ±0.01 | 1.22 ±0.18 |
| | Liquid | 25 | 5.23 ±0.53 | 2.22 ±0.43 | 1.9 ±0.04 | 2.84 ±0.01 | 2.7 ±0.01 | 8.88 ±0.05 | 0.78 ±0.01 | 0.82 ±0.01 | 1.21 ±0.22 |
| | | 20 | 4.62 ±0.16 | 1.84 ±0.11 | 1.98 ±0.01 | 2.96 ±0.01 | 2.73 ±0.04 | 9.11 ±0.11 | 0.8 ±0.01 | 0.82 ±0.02 | 1.27 ±0.09 |
| | | 15 | 4.44 ±0.14 | 1.76 ±0.09 | 2.01 ±0.01 | 3.05 ±0.01 | 2.77 ±0.01 | 9.26 ±0.1 | 0.88 ±0.04 | 0.83 ±0.01 | 1.26 ±0.25 |
| Enriched | Solid | 25 | 3.95 ±0.32 | 1.67 ±0.04 | 1.2 ±0.05 | 1.84 ±0.06 | 1.9 ±0.01 | 7.260 ±0.27 | 0.53 ±0.01 | 0.8 ±0.01 | 1 ±0.99 |
| | | 20 | 4.62 ±0.47 | 1.82 ±0.18 | 1.23 ±0.01 | 1.96 ±0.02 | 1.97 ±0.04 | 7.29 ±0.21 | 0.53 ±0.01 | 0.8 ±0.01 | 1.02 ±0.39 |
| | | 15 | 4.04 ±0.81 | 1.58 ±0.33 | 1.32 ±0.01 | 1.98 ±0.05 | 1.93 ±0.06 | 7.12 ±0.22 | 0.58 ±0.07 | 0.8 ±0.01 | 0.89 ±0.42 |
| | | 10 | 4.37 ±0.16 | 1.78 ±0.03 | 1.37 ±0.02 | 2.02 ±0.01 | 1.9 ±0.01 | 7.13 ±0.11 | 0.64 ±0.01 | 0.79 ±0.01 | 1.06 ±0.15 |
| | | 5 | 2.98 ±0.57 | 1.28 ±0.11 | 1.33 ±0.07 | 2.21 ±0.01 | 2.12 ±0.04 | 7.38 ±0.11 | 0.68 ±0.01 | 0.77 ±0.01 | 1.19 ±0.14 |
| | | 0 | 3.78 ±0.13 | 1.36 ±0.01 | 1.53 ±0.01 | 2.28 ±0.01 | 2.16 ±0.01 | 7.21 ±0.01 | 0.68 ±0.01 | 0.74 ±0.01 | 1.4 ±0.01 |
| | | -5 | 4.13 ±0.07 | 1.57 ±0.01 | 1.56 ±0.01 | 2.36 ±0.01 | 2.18 ±0.02 | 6.59 ±0.04 | 0.8 ±0.02 | 0.65 ±0.01 | 1.51 ±0.01 |
| | Liquid | 25 | 4.21 ±0.13 | 1.69 ±0.02 | 1.32 ±0.02 | 1.99 ±0.01 | 1.96 ±0.04 | 7.09 ±0.04 | 0.61 ±0.02 | 0.78 ±0.01 | 1.06 ±0.11 |
| | | 20 | 5.03 ±0.25 | 2.13 ±0.02 | 1.36 ±0.04 | 2.0 ±0.01 | 1.94 ±0.03 | 7.04 ±0.03 | 0.58 ±0.01 | 0.78 ±0.01 | 0.92 ±0.49 |
| | | 15 | 4.56 ±0.44 | 1.75 ±0.24 | 1.39 ±0.01 | 2.09 ±0.01 | 2.02 ±0.01 | 7.03 ±0.06 | 0.65 ±0.01 | 0.74 ±0.01 | 1.24 ±0.2 |
| | | 10 | 3.41 ±0.28 | 1.52 ±0.11 | 1.49 ±0.03 | 2.25 ±0.04 | 2.15 ±0.04 | 7.23 ±0.18 | 0.75 ±0.01 | 0.72 ±0.02 | 1.22 ±0.54 |
| | | 5 | 4.71 ±0.05 | 1.88 ±0.02 | 1.49 ±0.04 | 2.18 ±0.03 | 2.07 ±0.03 | 6.72 ±0.09 | 0.66 ±0.01 | 0.69 ±0.01 | 1.34 ±0.04 |
| | | 0 | 4.68 ±0.49 | 1.8 ±0.3 | 1.55 ±0.04 | 2.29 ±0.06 | 2.16 ±0.03 | 6.52 ±0.2 | 0.76 ±0.07 | 0.64 ±0.01 | 1.47 ±0.35 |

| Fractions | | °C | C 16:1 | C 17:0 | C 18:0 | C 18:1 ω9 | C 18:1*t* | C 18:2 ω6 | VA | CLA | SFA | UFA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | Solid | 25 | 24.12 ±0.01 | 0.63 ±0.01 | 3.04 ±0.07 | 22.21 ±0.18 | 1.4 ±0.08 | 14.38 ±0.03 | 3.44 ±0.04 ab | 5.36 ±0.06 a | 62.79 | 37.14 |
| | | 20 | 23.68 ±0.05 | 0.6 ±0.01 | 3.21 ±0.05 | 22.65 ±0.11 | 1.32 ±0.05 | 13.69 ±0.04 | 3.67 ±0.03 ab | 5.69 ±0.03 a | 61.49 | 38.42 |
| | | 15 | 23.38 ±0.09 | 0.6 ±0.01 | 3.09 ±0.18 | 22.67 ±0.15 | 1.11 ±0.04 | 13.82 ±0.01 | 3.45 ±0.06 ab | 5.69 ±0.12 a | 62.06 | 37.86 |
| | | 10 | 22.26 ±0.06 | 0.53 ±0.01 | 2.99 ±0.13 | 25.14 ±0.18 | 1.16 ±0.09 | 12.07 ±0.1 | 2.82 ±0.01 a | 5.80 ±0.06 ab | 59.98 | 39.92 |
| | Liquid | 25 | 24.12 ±0.01 | 0.56 ±0.01 | 3.13 ±0.37 | 24 ±0.45 | 1.3 ±0.02 | 12.85 ±0.04 | 3.12 ±0.04 ab | 5.81 ±0.15 ab | 60.56 | 39.35 |
| | | 20 | 23.68 ±0.05 | 0.55 ±0.01 | 2.93 ±0.03 | 24.62 ±0.01 | 1.13 ±0.06 | 12.82 ±0.12 | 2.85 ±0.25 a | 5.89 ±0.11 ab | 60.42 | 39.49 |
| | | 15 | 23.38 ±0.09 | 0.54 ±0.01 | 2.94 ±0.04 | 25.23 ±0.01 | 1.02 ±0.11 | 11.97 ±0.11 | 3.92 ±0.05 b | 5.81 ±0.03 ab | 58.85 | 41.06 |
| Enriched | Solid | 25 | 20.16 ±0.02 | 0.53 ±0.01 | 3.04 ±1.16 | 24.64 ±0.96 | 1.86 ±0.13 | 16.31 ±0.22 | 6.55 ±0.28 cdef | 6.68 ±0.37 bc | 55.62 | 44.3 |
| | | 20 | 20.05 ±0.07 | 0.54 ±0.01 | 2.99 ±0.21 | 24.43 ±0.04 | 1.88 ±0.07 | 15.51 ±0.19 | 6.29 ±0.08 cde | 6.98 ±0.02 cd | 55.79 | 44.12 |
| | | 15 | 20.71 ±0.01 | 0.53 ±0.01 | 2.98 ±0.35 | 24.87 ±0.21 | 1.72 ±0.16 | 16.18 ±0.01 | 5.81 ±0.11 c | 6.87 ±0.15 c | 56.19 | 43.72 |
| | | 10 | 19.86 ±0.08 | 0.51 ±0.01 | 3.06 ±0.06 | 25.91 ±0.06 | 1.88 ±0.04 | 14.76 ±0.03 | 5.70 ±0.06 c | 7.15 ±0.06 cde | 54.49 | 45.4 |
| | | 5 | 18.44 ±0.04 | 0.48 ±0.01 | 3.51 ±0.1 | 28.44 ±0.12 | 2.17 ±0.2 | 12.82 ±0.15 | 6.30 ±0.22 cdef | 7.81 ±0.32 def | 49.81 | 50.1 |
| | | 0 | 16.35 ±0.05 | 0.43 ±0.01 | 3.51 ±0.01 | 30.96 ±0.09 | 1.96 ±0.21 | 10.81 ±0.01 | 6.34 ±0.04 cdef | 8.43 ±0.06 fg | 46.65 | 53.28 |
| | | -5 | 14.32 ±0.04 | 0.38 ±0.01 | 3.49 ±0.04 | 32.88 ±0.31 | 2.11 ±0.04 | 9.29 ±0.13 | 7.13 ±0.08 ef | 8.95 ±0.05 g | 43.03 | 56.87 |
| | Liquid | 25 | 20.16 ±0.02 | 0.53 ±0.01 | 3.17 ±0.03 | 25.53 ±0.3 | 1.81 ±0.08 | 15.22 ±0.03 | 5.95 ±0.16 cd | 7.11 ±0.09 cd | 54.68 | 45.24 |
| | | 20 | 20.05 ±0.07 | 0.51 ±0.01 | 2.96 ±0.55 | 25.4 ±0.72 | 2 ±0.03 | 14.72 ±0.06 | 5.96 ±0.18 cd | 7.10 ±0.22 cd | 55.0 | 44.92 |
| | | 15 | 20.71 ±0.01 | 0.47 ±0.01 | 3.42 ±0.17 | 28.29 ±0.07 | 1.95 ±0.15 | 12.31 ±0.01 | 6.52 ±0.03 cdef | 8.00 ±0.06 ef | 49.86 | 50.07 |
| | | 10 | 19.86 ±0.08 | 0.43 ±0.01 | 3.16 ±0.45 | 30.03 ±0.01 | 2.2 ±0.25 | 11.42 ±0.23 | 6.71 ±0.34 def | 8.32 ±0.23 fg | 47.52 | 52.39 |
| | | 5 | 18.44 ±0.04 | 0.43 ±0.01 | 3.7 ±0.01 | 30.67 ±0.2 | 1.92 ±0.02 | 10.25 ±0.01 | 7.01 ±0.06 ef | 8.97 ±0.03 g | 45.76 | 54.27 |
| | | 0 | 16.35 ±0.05 | 0.38 ±0.01 | 3.66 ±0.07 | 32.62 ±0.16 | 1.8 ±0.06 | 9.14 ±0.01 | 7.17 ±0.23 f | 9.08 ±0.13 g | 43.34 | 56.56 |

* Fatty acid methyl ester.
** Only VA and CLA fatty acids contents were statistically compared through the Tukey's test.
*** The number in the title of each column indicate the fractionation temperature.
a-g: Values within rows with different superscript letters are significantly different ($p \leq 0.05$).
18:1*t*: elaidic acid, VA: vaccenic acid, CLA: conjugated linoleic acid, SFA: saturated fatty acids, UFA: unsaturated fatty acids.

tions were obtained: a solid one, higher in saturated fatty acids, and a liquid one, higher in unsaturated fatty acids. Since the fatty acid composition of AMF triglycerides brings about large effects on the overall melting point behavior (Kaleigan, 1999; Peters-Erjawetz *et al*., 1999), liquid fractions of enriched AMF could be remelted and re-fractionated at lower temperatures (5, 0 and -5 ºC) than control AMF liquid fractions which could only be fractionated until 10 ºC. At the lowest temperature (10 ºC for control and -5 ºC

TABLE III
ATHEROGENIC INDEX (AI) OF CONTROL AND ENRICHED FRACTIONS

| Fraction (°C) | Control Fractions | | Enriched Fractions | |
|---|---|---|---|---|
| | Solid | Liquid | Solid | Liquid |
| 25 | 1.71 ±0.01 a | 1.54 ±0.01 ab | 1.16 ±0.14 c | 1.11 ±0.01 cd |
| 20 | 1.66 ±0.01 ab | 1.57 ±0.05 ab | 1.16 ±0.05 c | 1.11 ±0.06 cd |
| 15 | 1.59 ±0.03 ab | 1.51 ±0.03 b | 1.17 ±0.05 c | 0.95 ±0.01 def |
| 10 | 1.55 ±0.02 ab | --- | 1.11 ±0.02 cd | 0.92 ±0.06 efg |
| 5 | --- | --- | 1.00 ±0.05 cde | 0.82 ±0.02 fg |
| 0 | --- | --- | 0.89 ±0.01 efg | 0.75 ±0.03 g |
| -5 | --- | --- | 0.75 ±0.01 g | --- |

[a-g] Values with different superscript letters are significantly different ($p \leq 0.05$).

for enriched AMF) only the solid fraction could be obtained.

After fractionation, VA and CLA contents in control fractions were lower than the corresponding ones in enriched fractions (Table II). Similarly, VA and CLA concentrations were, as a whole, lower in solid fractions than in liquid fractions. The highest amounts of VA and CLA were found in liquid fractions which were obtained from enriched AMF at 15, 10, 5 and 0°C, representing a 123% increase in VA and a 59% increase in CLA contents (liquid fraction at 0°C), when compared with control AMF. Since VA and CLA are unsaturated, their melting points are relatively low; therefore, finding high contents of these fatty acids in liquid fractions that were obtained at the lowest fractionation temperatures is not surprising. Solid and liquid fractions that were obtained at 10°C (Table II) in this study showed, for instance, higher concentrations of VA and CLA when compared to the findings reported by O'Shea *et al*. (2000). These authors carried out a study to evaluate dry fractionation of anhydrous milk fat at 19, 15 and 10°C. However, they only reported VA and CLA contents for the fractions that were obtained at 10°C; the solid fraction presented VA and CLA concentrations of 4.10 and 1.31g/100g of fat, respectively, while liquid fractions showed 5.18 and 2.22g/100g, respectively.

The fractionation process showed relatively constant SFA and UFA concentrations on the solid fractions obtained at 25, 20, 15 and 10°C. However, SFA and UFA contents changed in the fraction acquired at -5°C. SFA concentration was reduced about 10%, while UFA content was increased nearly 10%l (Table II).

The atherogenic index (AI) is an indicator of risk for cardiovascular diseases, and is helpful when comparing fatty acids profiles. In the present study, the AI in control AMF was significantly higher than the AI in enriched AMF (1.60 ±0.02 *vs* 0.93 ±0.03; $p \leq 0.05$). After fractionation, the enriched fractions presented a significantly lower AI ($p \leq 0.05$) than the control fractions (Table III). Ulbricht and Southgate (1991) conducted a study on seven dietary factors associated with cardiovascular diseases, which indicated that dairy products like milk, butter and cheese have a relatively high AI (2.03). However, the enriched fractions showed a much lower AI than those reported by Ulbricht and Southgate (1991). Actually, the highest AI was 1.17 in the solid fraction obtained at 15°C. Therefore, the liquid fraction obtained at 15°C may be the most useful one, due to its relatively low AI, as well as a fractionation temperature that does not require as much energy as the lower-temperature fractions would consume. This liquid fraction had an AI=0.95, significantly lower ($p \leq 0.05$) than the AI of any other fraction's obtained at a higher temperature, and also lower than that obtained for the solid fraction at 15°C. Additionally, the 15°C-liquid fraction's AI was significantly higher ($p \leq 0.05$) than that obtained for the liquid fraction at 0°C, which had an AI= 0.75. However, energy consumption to reduce the temperature from 15 to 0°C might be an important issue to consider when fractionating at an industrial scale.

In conclusion, the dry fractionation of milk fat obtained from dairy cows fed a diet supplemented with sunflower seeds resulted in an anhydrous milk fat with a fatty acid profile that might be beneficial to health: a lower atherogenic index and an increased CLA and VA content.

In consequence dry fractionation may be an alternative process in the food industry to offer chemical-free products, that have better nutritional characteristics and a lower atherogenic index, provididing a benefit for human health.


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