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Fatty acid composition of the bovine milk fat globules obtained by gravity separation

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Abstract

Gravity separation of milk fat is the process by which fat globules tend to gather on the surface and form a layer of cream. The aim of this study was to assess the differences in the lipid quality of cow milk fractions after gravity separation. Bulk cow milk samples were taken from an individual farm and subjected to gravity separation for 24 h at 4°C. Three fractions were separated. The diameter (μm), the number of fat globules per ml and fatty acid profile of each fraction were determined. The top fraction showed a significantly higher average diameter and number of globules/ml than the middle and lower fractions. In addition, the bottom fraction showed higher amounts of oleic acid, linolenic acid, arachidonic acid and CLA. The interest in gravity separation is due both to its technological applications the possibility of obtaining dairy products with different physical characteristics also in terms of fat nutritional value.

Introduction

The gravity separation of milk fat is the process by which fat globules, with a lower density than the liquid in which they are scattered, tend to gather on the surface and form a layer of cream (Ma and Barbano, 2000). The speed of the separation of the fat globules is connected to Stokes' law. This law expresses the force of viscous friction acting on a sphere in laminar motion in a fluid. Therefore, the speed and extent of gravity separation of fat is linked to intrinsic and extrinsic factors. Intrinsic factors are related to the milk quality which, as is known, depends on several physiological and environmental parameters. Extrinsic factors are linked to technological treatments. For example, given the same duration of milk storage, the temperature affects the speed of separation. In fact, the tendency of the fat globules to aggregate increases at refrigeration temperatures. Globules then emerge faster, forming large aggregates, which increase the volume of the top fat layer and retain more liquid. This phenomenon is called "cold agglutination" and is due to the action of agglutinins, which are immunoglobulins (IgM) some of which are derived from blood, and others are secreted by the mammary gland (Walstra, 1994). The increase in the temperature causes the clusters to become smaller, the layer of the cream has a smaller volume, and the milk fat globules retain less water. Furthermore, at higher temperatures the same volume of cream has a higher fat content (Bortolazzo *et al.*, 2010).

Gravity separation is still used in the manufacture of some aged cheeses of a protected designation of origin (PDO) (Mona *et al.*, 2011) such as Parmigiano Reggiano and Grana Padano that are made using partially skimmed milk. The cream that is obtained is used for the production of butter (Gori *et al.*, 2011). In the cheese manufacture, gravity separation is used in order to optimize the protein: fat in the boiler that affect the cheese yield. Furthermore, it could be applied to the production of other products such as yogurt and fermented milk. This type of fat standardization is different from industrial practices, where the partially skimmed milk is mostly prepared by means of centrifugal separators.

The natural creaming also separates different sized milk fat globules. In addition, during gravity separation, the milk does not undergo intense physical treatments, unlike other processes used to separate

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the cream, such as microfiltration and centrifugation, which may cause alterations in the membrane, thus changing the physico-chemical, nutritional and technological properties of the native fat globules. In addition, according to Ma and Barbano (2000), the natural creaming process produces a semi-skimmed milk with a higher proportion of small-sized milk fat globules compared to centrifugation. The aim of this study was to assess the differences in the lipid quality of cow milk fractions after gravity separation.

Materials and Methods

Sampling and milk analysis

A total of 16 bulk cow milk samples were taken during a month from a single farm. The cows were reared intensively and all the cows were fed with the same diet, consisting in a mixed ration formulated according to NRC (2001) requirements for dairy cattle. All the samples were refrigerated at 4°C before being taken to the laboratory for analysis.

Gravity separation

An aliquot of each fresh raw milk sample was placed in 60 ml cylinders (height: 10.5; internal diameter: 2.7) as described by Ma and Barbano (2000) and underwent gravity separation for 24 h at 4°C in double. Milk fractions were then drained from the bottom of the plastic cylinder and were collected separately in three fractions: bottom (5 ml) (B), middle (M) (50 ml), and top (T) (5 ml).

Morphometric analysis of milk fat globules

A direct method (Martini *et al*., 2013) was used to determine the diameter (μm), and the number of fat globules per ml of each fraction by a fluorescence microscope (Leica Ortomat Microsystem, Milan, Italy) equipped with a camera and an image analysis software. The globules were grouped into three sizes: small globules (SG) with a diameter <2 μm, medium-sized globules (MG) with a diameter from 2 to 5 μm, and large globules (LG) with a diameter >5 μm.

Fatty acid analysis

Fat extraction of whole milk and each fraction was performed using hexane and ethanol, according to Rose Gottlieb's method. Methyl esters of fatty acids (FAME) were obtained after transesterification with sodium methoxide (AOAC, 1995). The composition of total FAs was determined by gas chromatography using a Perkin Elmer Auto System (Norwalk, CT, USA) equipped with a flame ionization detector (FID) and a capillary column (ThermoScientific TR-FAME 60 m x 0.25 mm ID; film thickness 0.25 μm, Fisher Scientific UK). Helium was used as a carrier gas with a flow of 1 ml min^{-1}. The initial oven temperature was set at 50ºC, after 2 min the temperature was increased at a rate of 2ºC min^{-1} to 180ºC and held for 20 min; then increased at 1ºC min^{-1} to 200ºC and held for 15 min; finally the temperature was increased at a rate of 1ºC min^{-1} to 220ºC and held for 30 min. Injector and detector temperatures were 270ºC and 300ºC, respectively. The peak areas of individual FAs were identified by comparison with a fatty acid standard injection (Food Industry FAME Mix – Restek Corporation • 110 Benner Circle • Bellefonte, PA 16823) and quantified as a percentage of the total fatty acids. In addition, nonadecanoic acid methyl ester (C19:0 Restek Corporation • 110 Benner Circle • Bellefonte, PA 16823) was also used as an internal standard.

Statistical analysis

The results of the fatty acid composition and of the morphometric characteristics of the MGFs were analyzed by ANOVA for repeated measurements, where sampling time and fat fractions- bottom (B), middle (M), and top fractions (T)- were fixed effects. Mean were compared by Tukey test. Significant differences were considered at the level $P < 0.05$. The statistical analysis was carried out using JMP (2002) software.

Results and Discussion

The three fractions T, M and B had 17.55%±2.786, 2.18%±0.40 and 0.55%±0.385 of fat respectively. Table 1 shows the morphometric characteristics of milk fat globules in the three fractions of milk obtained as a result of gravity separation. The T fraction showed a significantly higher average diameter and number of globules / ml than both the M and B. This result is due to the fact that the larger sized globules tend to emerge and concentrate on the surface of the liquid, while the smaller globules tend to stay in emulsion. Consequently the M and B fractions were characterized by an average diameter of fat globules which was approximately half that of the cream (Figure 1). This result agrees with findings reported by Ma and Barbano (2000).

The different sizes of globules in the different fractions obtained, could lead to products with different technological characteristics. For example, it is known that the average diameter of milk fat globules is related both to the cheese yield and the water content of the cheeses (Martini *et al.*, 2008). Analysis of the fatty acid profile of the different fractions (Table 2) showed differences in some fatty

Table 1. Morphometric characteristics of cow milk fat globules in the three fractions (T= top fraction; M=middle fraction; B= bottom fraction) obtained by gravity separation

		T	M	B	SEM
Average diameter	µm	5.16A	2.94B	2.42B	0.975
Globules/ml	N*10^10	1.83A	1.43B	1.13C	0.475
Small globules (<2 µm)	%	13.75B	45.70A	50.14A	10.058
Medium globules (between 2 and 5 µm)	%	45.51	38.25	41.13	11.248
Large globules (>5 µm)	%	40.74A	16.06B	8.73B	9.002

A, B, C: Within a row means without a common superscript differ at P<0.05

acids. This is interesting since individual fatty acids in food could have different effects on human health and on the risk of developing chronic diseases such as obesity, diabetes and cardiovascular diseases (Vannice and Rasmussen, 2014).

The differences found in this study, are linked to the fact that the size of the globules affects the milk fatty acid profile, due to contribute of the fatty acids from the membrane, which has a different composition from whole milk and from the core of the globules and which has a greater incidence in the small globules (Martini *et al.*, 2013). In addition, the B fraction (average diameter 2.42 µm) showed a tendency to contain lower short chain fatty acids (SCFAs). The lower SCFAs in the milk, containing smaller globules, is related to the greater amount of membrane per unit volume of the fat, since the bovine and ovine MFGMs are made up above all of medium and long chain fatty acids (Jensen and Nielsen, 1996; Martini *et al.*, 2012; 2013).

In the B fraction, significantly lower amounts of C10:0, C13:0 and C15:0 were found, compared to the T fraction. Whereas the C16:1 was higher in the B fraction , as also observed by Briard et al. (2003) in small cow milk fat globules separated by microfiltration. C17:1 was significantly less in the M fraction. In addition, in the B fraction, higher percentages of C18 unsaturated fatty acids were present, which is interesting from a nutritional point of view. These were C18:3 n3 (α-linoleic acid-ALA) (+ 8% compared to the T fraction), CLA cis 9 trans 11 (rumenic acid) (+ 10% compared to the T fraction) and C18:1 cis 9 (oleic acid) (+ 4% compared to the T fraction).

Our results seem to be confirmed by studies previously carried out in cows (Jensen and Nielsen, 1996; Lopez *et al.*, 2011) which showed higher

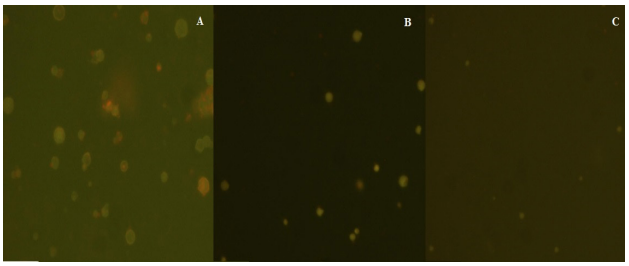

Figure 1. Milk fat globules of the top (A) middle (B) and bottom fractions (C) stained with Acridine Orange and observed by fluorescent microscopy at magnification 40X (Top fraction was diluted 1:200 in double-distilled water before the staining, middle and bottom fraction were diluted 1:100)

C18 unsaturated fatty acids, and rumenic acids in the smaller globules and in the milk fat globule membranes. Higher percentages of CLA cis 9 trans 11 and C18:3 n3 were also observed in the sheep milk membranes (Martini *et al.*, 2013).

Oleic acid is the most important representative of monounsaturated fatty acids in the diet and the isocaloric replacement of about 5% of energy from saturated fatty acids by this fatty acid has been reported as having a beneficial effect on risk factors for CVD (Lopez-Huertas, 2010). In terms of rumenic acid, the main form of CLA in ruminants, anti-obesity, anti-diabetic and antiatherogenic effects have been reported in animals. Studies on humans still have conflicting results (Aro *et al.*, 2000; Melanson *et al.*, 2009), which is why the recommended intake of CLA in humans has not yet been established.

C18:3 n3 (α-linolenic acid ALA) is a fatty acid precursor of omega 3 fatty acids. It cannot be synthesized by humans and must be supplied by the diet. FAO-WHO (2010) suggests minimum intake levels for ALA of 0.5% of energy in adults to prevent deficiency symptoms. Although clinical benefits have not been observed across all studies, several experimental and prospective observational studies support the evidence that ALA consumption reduces the incidence of cardiovascular disease (Mozaffarian, 2005).

Similarly to the findings of Martini *et al.* (2012) for ewes and of Briard *et al.* (2003) for large cow milk globules, in our study the T fraction (with a globule average diameter larger than 5 µm) showed a higher content of C18:1 trans 11 (vaccenic acid). In addition, in B fraction higher percentages of C20:4 n6 (arachidonic acid) (+ 300% compared to T fraction) were present, and of other long chain acids such as C22:0, C22:5 and C24:0. C20:4 is synthesised by the body from LA, and the dietary reference value for arachidonic acid has not been established (EFSA. 2010). Arachidonic acid is also

Table 2. Fatty acid composition of the three cow's milk fractions (T=top fraction; M=middle fraction; B= bottom fraction) obtained by gravity separation

FAME	T	M	B	SEM
C4:0	2.45	2.41	2.38	0.217
C6:0	1.86	1.80	1.78	0.140
C8:0	1.14	1.14	1.10	0.085
C10:0	2.76^{a}	2.65^{ab}	2.59^{b}	0.184
C12:0	3.21	3.10	3.08	0.200
C13:0	0.16^{A}	0.15^{B}	0.14^{B}	0.018
C14:0	10.96	10.90	11.14	0.511
C14:1	1.06	1.14	1.15	0.149
C15:0	1.26^{A}	1.19^{B}	1.19^{B}	0.063
C16:0	34.09	33.53	34.12	0.605
C16:1	1.42^{b}	1.46^{ab}	1.55^{a}	0.118
C17:0	0.53	0.56	0.57	0.065
C17:1	0.23^{A}	0.20^{B}	0.26^{A}	0.045
C18:0	10.75	10.40	10.42	0.617
C18:1 trans 9	0.71	0.63	0.68	0.245
C18:1 trans 11	1.18^{A}	0.81^{B}	0.86^{B}	0.233
C18:1 cis 9	19.97^{b}	20.00^{b}	20.84^{a}	0.962
C18:2 trans 9,12	0.26	0.27	0.29	0.041
C18:2 cis 9,12	3.78	3.22	3.33	0.675
C18:3 n3	0.25^{b}	0.27^{a}	0.27^{a}	0.017
C18:3 n6	0.10	0.12	0.13	0.034
C20:0	0.08^{B}	0.10^{A}	0.10^{A}	0.019
CLA cis 9, trans 11	0.51^{b}	0.52^{b}	0.56^{a}	0.061
C20:1	0.05	0.05	0.05	0.027
C21:0	0.08	0.10	0.09	0.084
C20:2	0.03	0.03	0.03	0.016
C20:3 n3	0.03	0.04	0.05	0.049
C20:3 n6	0.11	0.14	0.13	0.044
C22:0	0.04^{B}	0.05^{B}	0.06^{A}	0.014
C22:1	0.14	0.13	0.12	0.028
C20:4 n6	0.02^{b}	0.03^{ab}	0.06^{a}	0.032
C23:0	0.04	0.03	0.03	0.019
C22:2	0.03	0.03	0.03	0.018
C20:5	0.04	0.04	0.05	0.040
C24:0	0.03^{B}	0.05^{AB}	0.08^{A}	0.037
C24:1	0.03	0.05	0.04	0.023
C22:5	0.06^{b}	0.06^{b}	0.08^{a}	0.027
C22:6	0.06	0.06	0.06	0.039
SCFA (≤C10)	8.23	7.99	7.84	0.531
MCFA(≥C11≤C17)	53.42	52.74	53.70	1.176
LCFA(≥C18)	38.39	37.21	38.45	1.505
SFA	69.72	68.39	69.11	0.936
MUFA	25.04	24.73	25.82	0.958
PUFA	5.28	4.82	5.07	0.167
C18:3n3/C18:2n6	0.07^{b}	0.09^{a}	0.08^{a}	0.018

A, B: Within a row, means without a common superscript differ at P<0.05

a,b: Within a row, means without a common superscript differ at P<0.01

FAME: fatty acid methyl ester; SCFA: short chain fatty acids; MCFA: medium chain fatty acids; LCFA: long chain fatty acids; SFA: saturated fatty acids; MUFA: mono unsaturated fatty acids; PUFA: poly unsaturated fatty acids.

considered an essential fatty acid above all for the development of children. Thus FAO-WHO (2010) suggest in children aged 0-6 months a minimum intake of 0.2-0.3% energy. An excessive synthesis or ingestion of C20:4 influences adipose tissue development, and increases the synthesis of pro inflammatory prostaglandins and leukotrienes, as well as the synthesis of endocannabinoids (Massiera *et al*., 2003).

A C18:3/C18:2 ratio is generally considered in the diet as indicative of a balanced intake between the omega 3 and omega families, of which ALA and LA are precursors. The interest in this ratio derives from the fact that antagonistic effects between the two families of fatty acids have been observed. The higher intake of n-6 fatty acids may reduce the formation of anti-inflammatory mediators from n3 fatty acids. However, this hypothesis is not supported by studies in humans (Simopoulos, 1999; 2001), for which an optimal ratio of these fatty acids in the diet has not yet been established (EFSA, 2010). Furthermore, in our study this ratio was significantly lower in the T phase, with a larger diameter of the globules.

Conclusions

Milk gravity separation resulted in three fractions with different morphometries of milk fat globules and with some differences in the fatty acid profile. In particular, the bottom fraction presented higher amounts of oleic acid, linolenic acid, arachidonic acid, and CLA.

The interest in gravity separation is due to both its technological applications for cheese making, and the possibility of obtaining dairy products (milk and yogurt) with different characteristics, also in terms of different fat nutritional values.

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