NATIVE MILK FAT GLOBULE SIZE AND ITS INFLUENCE ON THE NATURAL CREAMING PROPERTIES OF BUFFALO MILK

Mina Martini[1,2,*], Iolanda Altomonte[2] and Federica Salari[2]

ABSTRACT

We investigated the influence of the physical characteristics of fat globules on the creaming properties of buffalo milk and on the fatty acid profile of the various fractions separated by natural creaming. A total of six bulk buffalo milk samples were taken from one individual farm in central Italy. An aliquot of each fresh raw milk sample underwent gravity separation and three fractions were separately collected: the bottom, middle, and top. The top and medium fractions showed a significantly ($P<0.01$) higher average diameter of the milk fat globules and a higher percentage of large globules. The top fraction was also made up of more densely packed globules as revealed by the higher ($P<0.01$) number of globules per ml. The smallest globules however tended to remain in emulsion, by virtue of the greater amount of membrane per unit volume, which makes them compatible with the aqueous phase. As a result the highest percentages of small globules were found in the bottom phase. The creaming capacity of buffalo milk was lower compared to cow milk. Despite the higher contribution of lipids in the top fraction, there were more fatty acids that are considered beneficial to human health, such as C18:0 ($P<0.01$), C18:2 cis9, 12; C18:2 cis9, t11 (rumenic acid) and C20:3 n6. In conclusion, natural creaming can act on the quality of the products by selecting globules with different diameters and nutritional quality, thus increasing the nutritional value of dairy products.

Keywords: *Bubalus bubalis*, buffalo, Italian Mediterranean buffalo, buffalo milk creaming, native milk fat globules, fat globule size, fatty acids

INTRODUCTION

Buffalo milk is the second largest volume of milk produced globally after bovine milk, with more than 102 million tons produced each year (FAOSTAT, 2013). In Italy, the most commonly breed reared is the Italian Mediterranean Buffalo. This is a breed of water buffalo whose selection and genetic improvement is controlled by the Italian Buffalo Breeders Association (ANASB). Buffalo livestock in Italy is small scale in comparison with the large numbers bred in the Far countries, however it is important in economic terms (Borghese and Mazzi, 2005).

Buffalo milk is also rich in terms of its components, and the high fat and protein content

[1]Department of Veterinary Sciences, University of Pisa, Pisa, Italy, *E-mail: mina.martini@unipi.it
[2]Interdepartmental Research Centre Nutraceuticals and Food for Health, Pisa, Italy

makes it particularly suitable for cheese-making (Ménard *et al*., 2010). Italian Mediterranean buffalo milk is primarily intended for the production of mozzarella, whose market is still expanding. In addition, other types of products such as fresh and aged cheeses, yogurt, and butter have been increasing on the market.

Natural creaming by gravity separation is used to obtain partially skimmed milk and in order to optimize the protein fat:ratio in some cheese manufacturing processes. In fact, changes in the concentrations of protein and fat in milk significantly influence the composition of the cheese and its yield (Bojanić Rašović *et al*., 2013). Natural creaming traditionally occurs in bovine milk devoted to the production of Parmigiano Reggiano cheese. Partially skimmed milk is used for the cheese-making, whereas the cream that naturally rises during the milk storage is used for producing butter. Since milk fat globules (MFGs) can influence the renneting behaviour, water content and the texture of manufactured dairy products (Logan *et al*., 2014; Dimitreli *et al*., 2014), natural creaming separates different sized milk fat globules (Ma and Barbano, 2000; Martini *et al*., 2017), which in turn may have an impact on the processing and on the nutritional value of products, thus leading to the production of new types of dairy products. This work evaluated the influence of the physical characteristics of the fat globules on the creaming properties of buffalo milk and on the fatty acid profile of the various fractions separated by natural creaming.

MATERIALS AND METHODS

Sampling and milk analysis

A total of six bulk buffalo milk samples were taken during a 45 days period from one individual farm in central Italy. The buffaloes were reared intensively and all the animals 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 duplicate. Milk fractions were then drained from the bottom of the plastic cylinder and were collected separately in three fractions: the bottom (5 ml) (B), middle (M) (50 ml), and top (T) (5 ml).

Creaming capacity was calculated according Ma and Barbano (2000) as follows:

$$CC = 100\% \times \left[\frac{\text{(total grams of fat in T fraction)}}{\text{(total grams of fat in the whole milk column)}}\right]$$

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 equipped with a camera and 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 (AOAC, 2000). Methyl esters of fatty acids (FAME) were obtained after transesterification with sodium methoxide (Christie, 1989). The composition of total FAs was determined by gas chromatography and identified as described in Martini *et al.* (2017).

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: B, M, and T were fixed effects. Means were compared by the Tukey test. Significant differences were considered at the level P<0.05. The statistical analysis was carried out using JMP (2002) software. The morphometric characteristics of milk fat globules in the three fractions obtained as a result of gravity separation are reported in Table 1.

RESULTS AND DISCUSSION

The T and M fractions showed a higher (P<0.01) average diameter of the globules than the B fraction. This separation is directly related to the fat globule size: in fact, small sized particles tend to stay in stable emulsion because of a reduced flotation speed (Truong *et al*., 2016). Consequently, the B fraction was characterized by an average globule diameter that was approximately half of the other two fractions. The smaller globule size in the bottom fractions agrees with the findings reported by Ma and Barbano (2000); Martini *et al.* (2017) in bovine milk. Despite the T and M fractions showing a similar average globules diameter, the cream layer (T fraction) was made up of more densely packed fat globules, as revealed by the higher (P<0.01) number of globules per ml (more than double). A significant difference in fat percentages among the phases was registered, as a consequence of fat concentration on the milk surface (Table 1). A comparison with bovine milk (Martini *et al*., 2017)

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

Morphometric characteristics	T	M	B	SEM
Fat (%)	16.66^{A}	6.35^{B}	1.16^{C}	1.888
Average diameter (μm)	6.43^{A}	6.05^{A}	2.69^{B}	2.144
Globules/ml (N*10^{10})	1.81^{A}	0.69^{B}	0.75^{B}	0.564
Small globules (<2 μm) (%)	13.51^{B}	26.55^{B}	52.68^{A}	14.994
Medium globules (between 2 and 5 μm) (%)	44.96^{a}	23.61^{b}	33.89^{ab}	13.110
Large globules (>5 μm) (%)	41.53^{a}	49.85^{a}	13.44^{b}	22.531

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

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

revealed a similar concentration of globules (n/ml) in the T phase between the two species, however different distributions of the categories of globules were registered in the three phases. In fact, the highest percentages of large globules were present both in the T and M phases of the buffalo milk, whereas large globule percentages were higher solely in the T phase of the bovine milk. In addition the smallest globules, by virtue of the greater amount of membrane per unit of volume, which makes them compatible with the aqueous phase (Truong *et al.*, 2016), are more stable and tend to remain in emulsion. This feature was evident from noting that the highest ($P<0.01$) percentage of small globules was in the B phase.

The differences found in the distribution of globules between bovine and buffalo milk are related to the different creaming capacity. In fact, we found a creaming capacity of 20.39%, about a half than reported for cattle (Ma and Barbano, 2000). The creaming capacity depends not only on the diameter of the fat globules (larger in buffalo compared to bovine milk) (Menard *et al*., 2010), but also on the density of the medium in which they are scattered (whey), which in turn depends on the water and total solids content. Buffalo milk is higher in density than cow milk and its creaming capacity is lower. In addition, the gravity separation is influenced by the structure and composition of the fat globule membrane (MFGM). The MFGM acts on the physical stability of the globules and on the coalescence and aggregation (Nguyen *et al*., 2015). The presence of agglutinins in bovine MFGM leads to the formation of clusters of globules which increase the speed of creaming. The buffalo MFGM contains fewer agglutinins (Pandya and Khan, 2006), which may also contribute to a lower cream separation.

The three phases had a different fatty acid composition (Table 2), related to the different average diameter and the percentage of different categories of globules. Research on bovine milk confirms the different fatty acid composition of globules with a different average diameter (Martini *et al*., 2016). The T phase contained higher percentages of long chain ($P<0.01$) and polyunsaturated fatty acids ($P<0.05$), and lower short ($P<0.05$) and medium chain ($P<0.01$) percentages than the other two phases.

In fact, although the T fraction had a larger average diameter, it showed a number of globules/ml that were approximately three times higher. The number of globules probably contributes to a greater total amount of membrane compared to the other fractions. It is well known that the phospholipids of the MFGM are made up of polyunsaturated and long chain fatty acids (Fong, 2007; Martini *et al*., 2013; Islam *et al*., 2014). The buffalo T fraction showed significantly lower percentages of C12:0 ($P<0.05$), C14:0 ($P<0.01$), higher percentages of C18:0 ($P<0.01$), C18:2 cis9,12, C18:2 cis9, t11 (rumenic acid), and C20:3 n6 ($P<0.05$). The higher membrane uptake in the T phase appears to be confirmed, albeit indirectly, by studies reporting high percentages of C18:0, C18:2 cis-9, 12 in the phospholipids of buffalo membranes (Islam *et al*., 2014).

CONCLUSIONS

Our study of fat stratification as a result of natural creaming revealed that although the level of lipids in the cream was higher, there were more fatty acids, which are considered beneficial for human health. In conclusion, natural creaming can act on the quality of the products through the selection of globules with different diameters and

Table 2. Fatty acid composition of the three buffalo milk fractions obtained by gravity separation (T = top fraction; M = middle fraction; B = bottom fraction).

FAME (g/100 g of fat)	T	M	B	SEM
C4:0	2.63^{b}	2.96^{a}	2.94^{a}	0.201
C6:0	1.76^{b}	1.88^{a}	1.91^{a}	0.088
C8:0	0.92^{b}	0.98^{a}	0.99^{a}	0.041
C10:0	1.93^{b}	2.02^{ab}	2.07^{a}	0.101
C11:0	0.09	0.05	0.09	0.044
C12:0	2.50^{b}	2.63^{a}	2.67^{a}	0.113
C13:0	0.12	0.12	0.12	0.018
C14:0	11.37^{B}	11.87^{A}	12.00^{A}	0.314
C14:1	0.58	0.72	0.68	0.164
C15:0	1.23	1.26	1.22	0.042
C15:1	0.34	0.34	0.32	0.027
C16:0	31.82^{B}	32.64^{AB}	33.49^{A}	0.578
C16:1	1.16	1.16	1.31	0.145
C17:0	0.61^{A}	0.61^{A}	0.57^{B}	0.015
C17:1	0.22^{a}	0.09^{b}	0.17^{a}	0.070
C18:0	15.27^{A}	14.56^{B}	13.07^{C}	0.298
C18:1 *trans-9*	1.00	0.95	0.90	0.111
C18:1 *trans-11*	0.49	0.47	0.46	0.099
C18:1 cis-9	19.39	19.14	19.16	0.695
C18:2 trans-9,12	0.29	0.29	0.29	0.031
C18:2 cis-9,12	4.21^{a}	3.53^{b}	3.69^{b}	0.470
C18:3n3	0.24	0.24	0.24	0.026
C18:3 n6	0.15	0.11	0.14	0.052
C20:0	0.08	0.08	0.10	0.016
C18:2 cis-9, trans11	0.62^{a}	0.50^{b}	0.58^{ab}	0.080
C20:1	0.05^{b}	0.05^{b}	0.06^{a}	0.007
C21:0	0.09	0.07	0.07	0.024
C20:2	0.02	0.03	0.03	0.015
C20:3n3	0.02	0.04	0.04	0.020
C20:3 n6	0.15^{a}	0.08^{ab}	0.06^{b}	0.060
C22:0	0.14	0.13	0.14	0.017
C22:1	0.11	0.11	0.12	0.008
C20:4n6	0.04	0.02	0.02	0.018
C23:0	0.03	0.03	0.03	0.013
C22:2	0.07	0.05	0.06	0.014
C20:5	0.03^{a}	0.01^{b}	0.02^{ab}	0.014

Table 2. Fatty acid composition of the three buffalo milk fractions obtained by gravity separation (T = top fraction; M = middle fraction; B = bottom fraction). (Continue)

FAME (g/100 g of fat)	T	M	B	SEM
C24:0	0.06	0.04	0.04	0.020
C24:1	0.04	0.03	0.02	0.022
C22:5	0.07	0.06	0.07	0.016
C22:6	0.05	0.04	0.09	0.039
SCFA (≤C10)	7.23^{b}	7.85^{a}	7.90^{a}	0.403
MCFA(≥C11≤C17)	50.05^{C}	51.49^{B}	52.63^{A}	1.042
LCFA(≥C18)	42.71^{A}	40.66^{AB}	39.47^{B}	1.349
SFA	70.65	71.94	71.51	1.087
MUFA	23.40	23.06	23.18	0.738
PUFA	5.95^{a}	5.00^{b}	5.31^{b}	0.582
n6/n3	8.81	8.58	8.43	1.266
UFA/SFA	0.42	0.39	0.40	0.023
Atherogenic index	3.23	3.45	3.43	0.164
Thrombogenic index	3.69	3.93	3.86	0.171

A,B: Within a row means without a common superscript differ at $P<0.01$

a, b: Within a row means without a common superscript differ at $P<0.05$

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: polyunsaturated fatty acids; UFA: unsaturated fatty acids.

Atherogenic index = [(4 *C14:0) + C16:0 + C18:0] / [ΣMUFA + Σn6 + Σn3]

Thrombogenic index = [C14:0 + C16:0 + C18:0] / [0.5MUFA + 0.5 n6 + 3 n3 + n3/n6]

a different nutritional quality, thus increasing the nutritional value of dairy products.

REFERENCES

AOAC. 2000. Method 905.02 Fat in milk. *In AOAC International Official Method of Analysis*, 17th ed. Gaithersburg, MD, USA.

Bojanić Rašović, M., N. Nikolić, A. Martinović, V. Katić, R. Rašović, M. Walcer and K. Domig. 2013. Correlation between protein to fat ratio of milk and chemical parameters and the yield of semi-hard cheese. *Biotechnology in Animal Husbandry*, **29**: 145-159.

Borghese, A. and M. Mazzi. 2005. *Buffalo Population and Strategies in the World Buffalo Production and Research, Reu Technical Series 67*. Food and Agriculture Organization of the United Nations, Rome, Italy. 316p.

Christie, W.W. 1989. *Gas Chromatography and Lipids: A Practical Guide.* The Oily Press, Scotland, UK. 184p.

Dimitreli, G., D. Petridis, P. Akakiadou and S. Chrysalidou. 2014. Effect of protein supplementation, fat globule size and storage time on the rheological and sensory properties of buffalo milk stirred yogurt. *J. Food Res*., **5**: 31-44

Food and Agriculture Oganization. 2013. http://faostat.fao.org/site/291/default.aspx

Fong, B.Y., C.S. Norris and A.K.H. Mac Gibbon. 2007. Protein and lipid composition of bovine milk-fat globule membrane. *Int. Dairy J*., **17**: 275-288.

Islam, M.A., M.K. Alam, M.N. Islam, M.A.S. Khan, D. Ekeberg, E.O., Rukke and G.E. Vegarud. 2014. Principal milk components in buffalo, Holstein cross, indigenous cattle and Red Chittagong cattle from Bangladesh. *Asian Austral. J. Anim. Sci*., **27**: 886-897.

JMP. 2002. *User's Guide, Version 5.0 SAS*. Cary, NC, USA.

Logan, A., L. Day, A. Pin, M. Auldist, A. Leis, A. Puvanenthiran and M.A. Augustin. 2014. Interactive effects of milk fat globule and casein micelle size on the renneting properties of milk. *Food Bioprocess Tech.*, **7**: 3175-3185.

Ma, Y., and D.M. Barbano. 2000. Gravity separation of raw bovine milk: Fat globule size distribution and fat content of milk fractions. *J. Dairy Sci.*, **83**: 1719-1727.

Martini, M., I. Altomonte, S.A.M. Sant'Ana and F. Salari. 2017. Fatty acid composition of the bovine milk fat globules obtained by gravity separation. *Int. Food Res. J.*, **24**: 148-152.

Martini, M., F. Salari and I. Altomonte. 2016. The macrostructure of milk lipids: The fat globules. *Crit. Rev. Food Sci. Nutr*., **56**: 1209-1221.

Martini, M., I. Altomonte and F. Salari. 2013. Evaluation of the fatty acid profile from the core and membrane of fat globules in ewe's milk during lactation. *LWT-Food Sci. Technol*., **50**: 253-258.

Menard, O., S. Ahmad, F. Rousseau, V. Briard-Bion, F. Gaucheron and C. Lopez. 2010. Buffalo vs cow milk fat globules: Size distribution, zetapotential, compositions in total fatty acids and in polar lipids from the milk fat globule membrane. *Food Chem*., **120**: 544-551.

Nguyen, H.T.H., L. Ong, E. Beaucher, M.N. Madec, S.E. Kentish, S.L. Gras and C. Lopez. 2015. Buffalo milk fat globules and their biological membrane: In situ structural

investigations. *Food Res. Int*., **67**: 35-43.

National Research Council, NRC. 2001. *Subcommittee on Dairy Cattle Nutrition, Committee on Animal Nutrition, Board on Agriculture and Natural Resources*, 7th ed. National Academy Press, Washington, D.C., USA. 381p.

Pandya, A.J. and M.H. Khan. 2006. Buffalo milk utilization for dairy products. *In* Park, Y.W. and G.F.W. Haenlein. (ed.) *Handbook of Milk of Nonbovine Mammals.* Blackwell Publishing Professional, Oxford, England, UK, and Ames, Iowa, USA.

Truong, T., M. Palmer, N. Bansal and B. Bhandari. 2016. *Effect of Milk Fat Globule Size on the Physical Unctionality of Dairy Products.* Springer Cham Heidelberg (ed.) New York, Dordrecht, London, UK.