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Nutritional composition of four commercial cheeses made with buffalo milk

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Summary

This paper compares the nutritional characteristics of four commercial cheeses made from the same buffalo milk using different production processes. Four subsequent cheese-making processes were made over a period of one month by a cheese company in Italy. For each cheese-making from the same bulk buffalo milk, four different cheeses were made: mozzarella, caciotta, capriccio and Blu del Granduca. All the samples were analysed in terms of determination of chemical composition and fatty acid profile. The manufacturing process affected the nutritional and health characteristics of the cheeses. The cheese-making process led to a lower retention of calcium in mozzarella, and a higher nitrogen content in dry matter. In addition, the cheese-making process and ripening influenced the fatty acid profiles, and modified various atherogenic (C12:0 and C14:0) and beneficial fatty acids (C18:3 *n*-3, *cis*-9,*trans*-11 conjugated linoleic acid, C20:5 and C20:6). Despite the higher fat on wet basis, the ripened cheeses, in particular the Blu cheese, showed a healthier fatty acid profile than mozzarella.

Keywords

buffalo cheese; nutritional composition; fatty acids

Cheese is a popular food, a good source of nutrients and is generally considered as part of a healthy diet. Cheese is classified according to the type of coagulation, curd consistency, fat content, time of ripening and type of milk used (cows', ewes', buffalo, goats' milk). The type of milk gives the cheese different nutritional and organoleptic properties. In turn, the different levels of susceptibility of these milks to the cheese-making process and the various steps in the manufacturing process (coagulation, acidification, grain draining, forming, pressing, salting and ripening) affect the characteristics of the final products [1].

Buffalo milk makes up over 12 % of milk production worldwide [2]. This milk is rich in fat (about 8 %), which is one of the main milk nutrients and is responsible for the high energy of buffalo milk. Fat is also involved in the cheese yield, firmness and flavour of dairy products.

The protein percentage in buffalo milk is also relatively high compared to cows' milk (4.7 % vs 3.3 %) and makes buffalo milk a source of good quality protein. In Italy, buffaloes are mainly reared for the production of mozzarella cheese [3] and Italy is the world's second largest producer of buffalo cheese [2]. Although traditional mozzarella is well known, hard and semi-hard cheeses made from buffalo milk are not so common.

The aim of this paper was to compare the nutritional characteristics of four commercial cheeses obtained by different manufacturing processes from the same buffalo milk.

MATERIAL AND METHODS

Cheese-making and samplings

Four different subsequent manufacturing

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processes were made over the period of a month by a Tuscan cheese producer (Italy) from the same bulk buffalo milk. Each process gave four different cheeses: mozzarella, caciotta, capriccio, and Blu del Granduca.

Buffalo mozzarella is a fresh, soft, semi-elastic textured cheese belonging to the kneaded curd family. The process of making pasta filata cheese includes adding natural whey as a starter culture and chymosin/pepsin rennet to raw milk. Clotting takes place at 85–95 °C and then it is stretched at 68–70 °C.

Caciotta is a semi-hard semi-cooked cheese, produced from pasteurized milk. Kid goat rennet paste is added as an enzymatic coagulant and commercial trademark starter cultures are also included. After the breaking of the curd, it is partially cooked at 45 °C and then pressed. The cheese is salted in brine and ripened for 25–30 days at temperatures between 12 °C and 14 °C.

Capriccio is a soft, white or yellowish cheese made with pasteurized milk. Calf rennet paste is added as an enzymatic coagulant and commercial trademark starter cultures are also included. It is slow cooked to 42 °C, then salted by brine and aged for 30 days at 6–10 °C to form a white fungus crust composed of *Geotrichum candidum* and *Penicillium candidum*.

Blu del Granduca is a semi-soft cheese made with pasteurized milk and chymosin/pepsin rennet, using a commercial starter culture. In addition, *Penicillium roqueforti* is added on the 10th day and the cheese is ripened for 60 days.

Our samples were taken at the time of marketing for mozzarella, and at the end ripening stage for other three cheeses. For each cheese manufacturing, three loaves were taken and two samplings were carried out on each loaf. A total of 96 samples were taken to the laboratory under refrigeration (below 5 °C) and then frozen (–20 °C) until the analysis.

Chemical analysis and fatty acid profile of cheeses

Each sample was analysed in duplicate in terms of gross composition (total solid, fat, proteins and ash), phosphorus [4, 5], calcium and magnesium contents [6], and fatty acid composition. Cheese fat was extracted following Rose-Gottlieb's reference method [4]. Methyl esters of fatty acids were prepared using methanolic sodium methoxide according to Christie [7]. The composition of total fatty acids was determined by gas chromatography using a Perkin Elmer Auto System (Norwalk, Connecticut, USA) equipped with a flame ionization detector (FID) and a capillary column (FactorFour; Varian, Middelburg, Netherlands; 30 m × 0.25 mm; film thickness 0.25 μm). Helium was used as a carrier gas with a flow rate of 1 ml·min^{-1}. The initial oven temperature was set at 50 °C, after 5 min the temperature was increased at a rate of 3 °C·min^{-1} to 140 °C and held for 2 min; then increased by 1 °C·min^{-1} to 240 °C and held for 20 min. Injector and detector temperatures were 270 °C and 300 °C, respectively. The peak areas of individual fatty acids were calculated by comparison with fatty acid standard injection (Sigma Aldrich Chemical, St. Louis, Missouri, USA) and quantified as a percentage of total fatty acids.

Statistical analysis

The results were analysed by ANOVA using JMP software (SAS, Cary, North Carolina, USA). The model contained the fixed effects of the cheese type. The effect of the sampling time on cheese composition was found to be not significant and, therefore, was excluded from the statistical model. The significance of the differences between means was evaluated by Student's *t*-test considering $P < 0.05$ as the significance level.

RESULTS AND DISCUSSION

Chemical and nutritional composition of the milk and cheeses

There were no significant differences in the quality of bulk milk during the period of the study. Milk composition is reported as mean and standard deviation in Tab. 1.

The moisture in the mozzarella cheese (Tab. 2) was consistent with regulations for protected designation of origin (PDO) buffalo mozzarella with a maximum tolerable humidity of 65 %. Mozzarella had a lower content of fat on a wet matter basis compared to the three ripened cheeses (caciotta, capriccio, Blu del Granduca). Fat on dry matter basis did not significantly differ in the

Tab. 1. Buffalo milk composition.

	Content [%]
Dry matter	19.4 ± 1.4
Fat	7.5 ± 1.0
Total nitrogen	4.5 ± 0.3
Casein nitrogen	3.0 ± 0.5
Lactose	5.0 ± 0.2
Ash	0.8 ± 0.0
Calcium	0.2 ± 0.0
Phosphorus	0.2 ± 0.0

Values are expressed as mean ± standard deviation, $n = 4$.

Tab. 2. Composition of four buffalo cheeses.

Parameters	Mozzarella	Caciotta	Capriccio	Blu del Granduca	*SEM*
Moisture [g·kg^{-1}]	575.51^{A}	236.75^{B}	219.02^{B}	232.11^{B}	37.01
Values expressed on wet basis					
Fat [g·kg^{-1}]	205.53^{B}	385.91^{A}	394.52^{A}	374.31^{A}	29.69
Total nitrogen [g·kg^{-1}]	156.16^{C}	219.76^{AB}	226.04^{A}	206.43^{B}	17.21
Ash [g·kg^{-1}]	13.91^{C}	38.72^{B}	46.14^{B}	61.90^{A}	8.38
Calcium [g·kg^{-1}]	3.12^{C}	8.20^{A}	9.32^{A}	7.05^{B}	0.95
Phosphorus [g·kg^{-1}]	3.65^{C}	7.53^{A}	7.86^{A}	6.47^{B}	0.78
Magnesium [g·kg^{-1}]	0.13^{C}	0.47^{AB}	0.57^{A}	0.32^{B}	0.12
Fat/Protein ratio	1.41^{B}	1.75^{A}	1.76^{A}	1.83^{A}	0.23
Calcium/Phosphorus ratio	0.87^{B}	1.10^{A}	1.20^{A}	1.11^{A}	0.15
Values expressed on dry matter basis					
Fat [g·kg^{-1}]	504.48	506.84	505.45	489.74	56.16
Total nitrogen [g·kg^{-1}]	365.56^{A}	287.82^{B}	288.85^{B}	272.03^{B}	38.58
Ash [g·kg^{-1}]	33.21^{C}	50.73^{B}	58.73^{B}	81.45^{A}	10.89
Calcium [g·kg^{-1}]	7.42^{C}	10.87^{A}	11.95^{A}	9.16^{B}	1.16
Phosphorus [g·kg^{-1}]	8.68^{B}	9.94^{A}	10.04^{A}	8.32^{B}	0.73
Magnesium [g·kg^{-1}]	0.32^{C}	0.61^{AB}	0.75^{A}	0.50^{BC}	0.13
Energy from fat [kJ·kg^{-1}]	19008.49	19096.41	19042.82	18453.32	2115.84
Energy from proteins [kJ·kg^{-1}]	6656.59^{A}	5243.13^{B}	5260.30^{B}	4954.24^{B}	702.67

Different superscript capital letters indicate statistical differences across a row at $P < 0.01$.
Moisture was calculated as the difference in the weight of the sample before and after drying. *SEM* – standard error of the mean.

analysed samples.

Fat has many important functions in food, contributing to the taste, texture and appearance. However, it has been also associated with cardiac illnesses and with changes in the oxidative status in tissues [8].

The caciotta cheese had a similar fat content as cows' milk-based caciotta [9]. The literature reports a wide fat range for blue cheeses (between 48 % and 60 % on dry matter). In any case, Blu del Granduca showed a similar fat content on dry matter basis as Bleu d'Auvergne, which is made from cows' milk [10].

Cheese contains a high biologically valuable protein that is almost 100 % digestible, as the ripening phase of the manufacturing process involves a progressive breakdown of casein to water-soluble peptides and free amino acids [11]. The total nitrogen, on the whole, registered increasing values for mozzarella, Blu del Granduca, caciotta and capriccio. However, regarding the same dry matter weight, the nitrogenous component was significantly higher in mozzarella than the other cheeses, showing a similar average protein content on dry matter basis (375.2 g·kg^{-1}) to that reported for buffalo mozzarella cheese by Sameen et al. [12]. The higher protein on dry matter basis of mozzarella compared to the ripened cheeses was due, in part, to the production process and to the ripening. In fact, during the milk manufacturing process, pre-acidification promotes the reduction in pH and repulsion forces between the micelles, thus speeding up the coagulation process. The expulsion of whey during the maturation contributes to the loss of a part of the nitrogen component [13].

The content of nitrogen compounds in the aged cheese was comparable to those of beef meat [14]. The ash content on dry matter basis was higher in the Blu compared to the other cheeses and similar to values for Roquefort, Cashel blue and Huntsman [10]. A lower calcium content was found in mozzarella, whereas caciotta and capriccio showed significantly higher calcium and phosphorus. The calcium content on dry matter basis in the Blu del Granduca was similar to values reported for other cheeses with a similar manufacturing process, such as Bleu d'Auvergne, Danish blue, and Huntsman [10].

In the literature, inconsistent values were reported for mozzarella cheese in relation to calcium, with values ranging from a minimum of 471 mg (INRAN – National Institute for Food and Nutrition Research, Rome, Italy) to a maximum of 1030 mg on dry matter basis [12]. Calcium is important in the structure, texture and functionality of mozzarella and other cheeses. In fact, it maintains the integrity and strength of the protein matrix. A low calcium content is linked to a softer curd [15]. Calcium in cheese is present as a soluble form, which protects it against precipitation in the intestine, thus facilitating absorption [16]. Calcium safeguards against osteoporosis, particularly in those who consume inadequate quantities at a young age [17], and may protect against arterial hypertension and colon cancer [18]. The average recommended dietary allowance (*RDA*) or adequate intake (*AI*) of calcium is about 900 mg per day (800 mg to 1000 mg, depending on the country) for adults, increasing to 1200 mg per day for adolescents and the elderly. Fifty grams of capriccio (recommended portion of cheese) provide 465 mg of calcium, 52 % of the *RDA* in adults.

Besides calcium, cheese is also a good source of phosphorus and magnesium [19]. The average value of phosphorus in the mozzarella cheese in our study was similar to that found in buffalo milk mozzarella by INRAN, while caciotta and capriccio also had a significantly higher content of magnesium than the other cheeses analysed.

The calcium/phosphorus ratio was statistically different among the four cheeses analysed, but always nutritionally acceptable. All the cheeses with the exception of mozzarella had an average ratio of Ca/P greater than 1.0, whereas in the buffalo milk, the molar ratio was similar (Tab. 1). It is worth noting that the manufacturing technology in the mature cheese maintained a higher content of calcium compared to phosphorus. On the other hand, the mozzarella cheese-making process led to a lower calcium retention in the curd.

The contribution of energy from fat on dry matter was not significantly different among the samples. The energy from proteins was significantly higher in mozzarella cheese.

Fatty acid profile

No differences in saturated (SFAs), monounsaturated (MUFAs) and polyunsaturated (PUFAs) fatty acids were detected among the cheeses. Regarding the short chain fatty acid composition (SCFAs) (Tab. 3), there were decreasing values in the fat of caciotta, capriccio, mozzarella and Blu cheeses. The Blu had a higher content of long chain fatty acids (LCFAs). In addition, n-3/n-6 ratio was significantly lower in mozzarella than in Blu, while the others had intermediate values.

Different types of fats have different health effects, and not all SFAs within foods, such as cheese, lead to an increase in plasma cholesterol to the same extent [20]. Some SFAs play an important role in cell regulation by protein modification (acetylation), in gene expression as well as in the modulation of genetic regulation, in the regulation of bioavailability of PUFAs, and in fat deposition [11]. The beneficial health effects of some saturated SCFAs have been reported. C4:0 (butanoic acid) protects colonocytes from oxidative stress, modulates cell proliferation and differentiation [21].

Short and intermediate-chain, even-numbered fatty acids (C4:0–C12:0) provide characteristic flavour notes [22]. Lower C4:0 ($P \leq 0.01$) and C6:0 (hexanoic acid) ($P \leq 0.05$) were detected in fat of mozzarella and Blu compared to caciotta and capriccio. C4:0 contributes to "cheesy" flavours, whereas C6:0 acid has a "pungent" flavour. Caciotta contained more C8:0 (octanoic acid) compared to Blu, which contributes to a "waxy", "soapy", "goat-like", "musty", "rancid" and "fruity" note [22].

Lower contents ($P \leq 0.01$) of C12:0, C14:0 and C16:1 were found in the fat profile of Blu. This could be linked to the metabolism of fatty acids by *Penicillium* spp. In fact, different *Penicillium* spp. can metabolize free fatty acids produced by lipase in the rennet paste (pregastric esterase) and from the fungi and lactic acid bacteria, to flavour compounds, such as methyl ketones, lactones, esters, alkanes and secondary alcohols [22, 23]. The lower content of C12:0 and C14:0 found in fat of Blu could be interesting from a nutritional point of view, since the total plasma cholesterol-raising effects of SFAs are generally greater with medium chain lengths (C12:0, C14:0, and C16:0) than those with longer chain lengths [24]. Fat of Blu had a significantly higher content ($P \leq 0.01$) of C18:0 (octadecanoic acid) compared to the other cheeses. This fatty acid is rapidly converted by the body to C18:1 (*cis*-9 octadecanoic acid), which makes cheese a healthy source of fat in the diet and is not related to a cardiovascular risk [25].

trans-11 C18:1 (known as vaccenic acid), which is an isomer of *cis*-9 octadecanoic acid, was found mainly in the Blu and caciotta cheese fat. This fatty acid is a precursor of *cis*-9,*trans*-11-octadecadienoic (rumenic) acid, the main form of conjugated linoleic acid (CLA) in ruminants, which is a group of positional and geometric isomers of linoleic acid with conjugated double bonds. Both rumenic and vaccenic acids have several beneficial properties for human health. Similar values for CLA were observed by Van Nieuwenhove et al. [26] in fresh cheese made from buffalo milk. Cheeses have been identified as important sources of CLA, and the role of processing and ripening

Tab. 3. Fatty acid composition of four buffalo cheeses.

Fatty acid methyl ester [g·kg^{-1}]	Mozzarella	Caciotta	Capriccio	Blu del Granduca	*SEM*
C4:0	28.32^{B}	30.83^{A}	31.45^{A}	29.45^{AB}	2.59
C6:0	17.83^{ab}	18.51^{a}	18.51^{a}	17.10^{b}	1.39
C8:0	9.32^{AB}	9.84^{A}	9.34^{AB}	8.62^{B}	0.88
C10:0	19.75^{A}	21.35^{A}	20.55^{A}	18.32^{B}	1.67
C11:0	0.70	1.21	0.92	0.82	0.33
C12:0	25.14^{A}	26.42^{A}	25.30^{A}	23.40^{B}	1.69
C13:0	1.50^{A}	1.00^{B}	0.92^{B}	0.92^{B}	0.16
C14:0	114.13^{A}	117.04^{A}	112.80^{AB}	110.42^{B}	3.60
C14:1	9.75^{A}	8.85^{B}	9.54^{AB}	8.13^{B}	1.17
C15:0	12.92^{A}	12.75^{B}	12.45^{B}	12.24^{B}	0.58
C15:1	3.63^{b}	4.25^{a}	4.23^{a}	3.70^{b}	0.75
C16:0	329.05	338.33	331.53	339.80	7.95
C16:1	15.05^{B}	15.92^{A}	15.31^{A}	13.90^{C}	1.92
C17:0	6.15^{A}	5.04^{C}	4.94^{C}	5.62^{B}	0.28
C17:1	1.83	1.70	2.02	1.90	0.39
C18:0	133.65^{B}	131.65^{B}	141.90^{B}	152.01^{A}	9.24
C18:1 *trans*-9	3.90	5.41	4.22	4.95	1.04
C18:1 *trans*-11	8.65^{C}	12.32^{A}	9.61^{BC}	10.70^{AB}	1.71
C18:1 *cis*-9	193.6	195.8	195.8	196.8	7.24
C18:2 *trans*-9,12	2.11^{b}	2.50^{ab}	3.02^{a}	2.91^{a}	0.74
C18:2 *cis*-9,12	34.72^{a}	30.40^{ab}	30.10^{ab}	25.92^{b}	7.70
C18:3 *n*-3	2.95^{b}	3.84^{a}	3.71^{a}	3.60^{a}	0.48
C18:3 *n*-6	1.02	1.12	1.25	1.25	0.24
C20:0	1.05^{a}	0.70^{ab}	0.71^{ab}	0.64^{b}	0.29
cis-9, *trans*-11 CLA	4.12^{B}	7.13^{A}	7.43^{A}	7.05^{A}	0.83
C20:1	0.40	0.32	0.32	0.31	0.19
C21:0	1.40	1.40	1.32	1.40	0.48
C20:2	0.40^{A}	0.10^{B}	0.10^{B}	0.21^{B}	0.16
C20:3 *n*-3	0.41	0.52	0.52	0.60	0.38
C20:3 *n*-6	0.90^{A}	0.12^{C}	0.54^{BC}	0.62^{AB}	0.31
C22:0	1.32^{a}	0.93^{b}	1.40^{a}	1.22^{ab}	0.28
C22:1	1.20	0.91	1.23	1.13	0.27
C20:4 *n*-6	0.10	0.10	0.22	0.10	0.15
C23:0	0.23	0.23	0.24	0.23	0.14
C22:2	0.72	0.62	0.62	0.73	0.14
C20:5	0.15^{b}	0.35^{a}	0.35^{a}	0.30^{b}	0.20
C24:0	0.70	0.52	0.72	0.83	0.26
C24:1	0.23^{b}	0.15^{b}	0.41^{a}	0.30^{ab}	0.17
C22:5	0.74^{a}	0.53^{ab}	0.53^{ab}	0.40^{b}	0.20
C22:6	0.21^{C}	0.45^{BC}	0.60^{AB}	0.83^{A}	0.28
SCFA (≤ C10)	74.90^{BC}	83.54^{A}	80.70^{AB}	74.10^{C}	5.83
MCFA (≥ C11; ≤ C17)	530.64^{a}	519.00^{ab}	512.71^{b}	511.25^{b}	13.89
LCFA (≥ C18)	394.50^{b}	397.50^{ab}	406.61^{ab}	414.73^{a}	17.32
SFA	712.00	710.74	712.14	717.65	10.07
MUFA	239.61	242.02	239.14	238.05	8.23
PUFA	48.41	47.40	48.83	44.44	7.62
UFA/SFA	0.41	0.41	0.40	0.39	0.02
n-3/*n*-6	0.12^{b}	0.16^{ab}	0.16^{ab}	0.18^{a}	0.05

Values are expressed on the total of milk fatty acids.
Different superscript capital letters indicate statistical differences across a row at $P < 0.01$. Different superscript lowercase letters indicate statistical differences across a row at $P < 0.05$.
SEM – standard error of the mean, CLA – conjugated linoleic acid, SCFA – short chain fatty acids; MCFA – medium chain fatty acids; LCFA – long chain fatty acids; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; UFA/SFA – unsaturated/ saturated fatty acids.

in terms of CLA content is still controversial [27]. Some authors have reported that the effect of ripening on the total CLA content and isomer distribution is negligible [28].

Free *cis*-9,*cis*-12 octadecadienoic acid (linoleic acid) accumulates during the ripening process and it appears likely that bacteria form CLA from free linoleic acid [26, 29]. This could explain why the lipid component of aged cheeses was higher in CLA content, whereas mozzarella had a higher linoleic acid content. Lower contents ($P \leq 0.05$) of C18:3 *n*-3 and C20:5 *n*-3 (eicosapentaenoic acid, EPA) were found in the mozzarella fat. C22:6 (docosahexaenoic acid, DHA) showed an increasing trend in mozzarella, capriccio, caciotta and Blu cheeses. EPA and DHA are beneficial in a number of clinical conditions particularly where there is a predominant inflammatory component, such as rheumatoid arthritis, and inflammatory bowel disease, as well as in certain cardiac conditions associated with inflammation such as after a myocardial infarction [30].

CONCLUSIONS

The manufacturing process affects the nutritional and health characteristics of cheese made with the same milk. In mozzarella, the cheese making process led to a lower retention of calcium in the curd and a higher content of nitrogen in dry matter. The cheese-making process and ripening influenced the fatty acid content, modifying various atherogenic (C12:0 and C14:0), and beneficial fatty acids (C18:3 *n*-3, *cis*-9,*trans*-11 CLA, C20:5 and C20:6). Despite the higher fat content on wet basis, the ripened cheeses and especially the Blu cheese showed a healthier fatty acid profile than mozzarella.

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