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Trends in the quality and hygiene parameters of bulk Italian Mediterranean buffalo (*Bubalus bubalis*) milk: a three year study

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Original

Trends in the quality and hygiene parameters of bulk Italian Mediterranean buffalo (*Bubalus bubalis*) milk: a three year study / Pasquini, M.; Osimani, A.; Tavoletti, S.; Moreno, I.; Clementi, F.; Trombetta, M. F.. - In: ANIMAL SCIENCE JOURNAL. - ISSN 1740-0929. - ELETTRONICO. - 89:1(2018), pp. 176-185. [10.1111/asj.12916]

Availability:

This version is available at: 11566/252482 since: 2022-05-25T17:33:06Z

Publisher:

Published

DOI:10.1111/asj.12916

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Trends in the quality and hygiene parameters of bulk Italian Mediterranean buffalo (*Bubalus bubalis*) milk: a three year study

Journal:	<i>Animal Science Journal</i>
Manuscript ID	ASJ-2016-0668.R1
Wiley - Manuscript type:	Original Article
Date Submitted by the Author:	n/a
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Keywords:	buffalo milk quality, chemical characterization, somatic cell counts, total bacterial counts
Abstract:	<p>Buffalo milk represents an indispensable source of nourishment in many parts of the world and it is the second most consumed milk worldwide. Buffalo milk is actually used for the production of many dairy products such as pasteurized or concentrated milk, butter, yogurt, ice-cream, dehydrated milk products, and cheeses. Due to its high nutritional value and the presence of natural bioactive substances, buffalo milk can also provide health benefits to consumers. In Italy, buffalo milk is used only for cheese making, mainly mozzarella PDO (Protected Designation of Origin), which is a highly valued dairy product. This three-year study, carried out between 2011 and 2013, was aimed at evaluating the quality of bulk Italian Mediterranean buffalo milk by monitoring physico-chemical parameters, somatic cell and total bacterial counts. A total of 51 samples of bulk milk were collected from one herd throughout the monitored period. Analysis of variance, carried out to test month, season, and year main effects, highlighted remarkable seasonal effects for fat, protein, and lactose content, as well as for predicted Mozzarella cheese yield, and somatic cell counts. The calculation of simple correlations allowed the identification of positive correlations between estimated cheese yield and fat and protein content.</p>

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1 Trends in the quality and hygiene parameters of bulk Italian Mediterranean buffalo (*Bubalus*
2 *bubalis*) milk: a three year study

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4 **Running head:** Quality of Mediterranean buffalo milk

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32 **ABSTRACT**

33 Buffalo milk represents an indispensable source of nourishment in many parts of the world and it is the
34 second most consumed milk worldwide. Buffalo milk is actually used for the production of many dairy
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47 **Key words:** buffalo milk quality, chemical characterization, somatic cell counts, total bacterial counts.

INTRODUCTION

Buffalo (*Bubalus bubalis*) milk represents an indispensable source of nourishment in many parts of the world. India and Pakistan produce more than 91% of buffalo milk, which is the second most consumed milk worldwide. As recently reviewed by Cazacu *et al.* (2014), the buffalo milk market is still considered an emerging sector. Actually, the dairy industry produces many products based on the use of buffalo milk; among these are pasteurized or concentrated milk, butter, heat-desiccated dairy products, heat-acid coagulated dairy products, yogurt, ice-cream, dehydrated milk products, and cheeses (Cazacu *et al.* 2014). Due to the high nutritional value of buffalo milk, the demand for such products is increasing; however, there is still a shortage of scientific literature on buffalo milk's physico-chemical and hygienic parameters (Zotos & Bampidis 2014).

In Europe, the countries that produce the largest quantities of buffalo milk are Italy, Turkey, Bulgaria, and Greece (FAOSTAT 2014). In accordance with a recent report from the European Food Safety Authority (EFSA), Italy is the largest producer (88%) of buffalo milk in the EU (EFSA BIOHAZ Panel 2015); in the period 1990-2012, the Italian production of buffalo milk increased from 43,000 to 192,455 t, allowing in 2012 the production (FAOSTAT 2014) of 51,910 and 37,122 t of mozzarella and mozzarella PDO (Protected Designation of Origin), respectively (INEA 2012; 2013). The rearing of buffalo in Italy constitutes an important reality, not only regarding the number of animals bred but also for the popularity of Italian bubaline dairy products. Buffaloes reared in Italy stand out in the world for their genetics, the applied technologies, the monitoring of pathologies, and the hygiene and quality of the end products (Borghese 2005). In Italy, buffalo milk is used only for cheese making, mainly mozzarella PDO, which is a highly valued product especially in the USA, Germany, France, UK, and Japan (Borghese 2005).

Buffalo milk is usually characterized by a rich composition with high content of fat, which constitutes the main fraction, moreover, it is a good source of vitamins A, D, C, and B6; minerals such as Ca and P; and conjugated linoleic acid (Ahmad 2013; Khedkar *et al.* 2016; Simoes *et al.* 2014; Zotos & Bampidis 2014). The presence of trace elements such as boron, cobalt, copper, iron, manganese, sulfur, and zinc has also been ascertained (Ahmad 2013).

The consumption of buffalo milk can provide benefits to people who suffer from hypertension, dental decay, dehydration, respiratory problems, obesity, osteoporosis, and some forms of cancer (Ahmad 2013). Furthermore, a recent study carried out by Kapila *et al.* (2013) highlighted that buffalo milk proteins (β -lactoglobulin and casein) are less allergenic than cow milk proteins, which can significantly increase protein-specific IgE sensitization and lymphocyte proliferation index causing allergies in consumers.

Many parameters can be used to assess the quality of milk, among these are compositional, physical-chemical, hygienic, and sanitary characteristics. The main traits that are usually considered as milk quality parameters are defined as proteins, fats, lactose, and solids-not-fat content. All of these parameters, which can affect the quality of the end products, can vary depending on several factors, including genetic, age, parity, lactation stage, season, feeding, and geographical area of rearing (Gürler *et al.* 2013). Moreover, microbiological contamination and the presence of somatic cells can worsen the quality of milk.

The aim of this study was to evaluate the quality of bulk Italian Mediterranean buffalo milk through monitoring physico-chemical parameters, somatic cell counts and total bacterial counts over a three-year period (2011–2013) in one farm. The effects of month, season, and year on the monitored variables were also evaluated through statistical analyses.

MATERIALS AND METHODS

Description of the Farm

The monitoring of bulk buffalo milk characteristics was performed on a farm located in the Marche region (central Italy) over a three-year period (2011-2013). The farm, in the year 2012 produced 184.8 t of buffalo milk over a total national production of 192,455.3 t; whereas in 2013 the production increased, reaching 422.7 t of buffalo milk over a national production of 194,892.8 t (Istat, 2014; 2015). The animals were housed in free stalls with a concrete paddock and a permanent straw bedding area; the paddocks were equipped with a sprinkler system and fans. The herd was organized in two feeding groups: milking and dry buffalo cows. During the monitored period, all milking buffalo cows were fed a total mixed ratio consisting of: corn silage (10.0 kg/head/day), first cut hay (5.0 kg/head/day), alfa-alfa hay (3.5 kg/head/day), maize flour (3.0 kg/head/day), soybean meal (1.0 kg/head/day), cottonseed (1.0 kg/head/day), and mineral-vitamin pre-mix (0.25 kg/head/day).

The milking area was arranged as a double-9 herringbone-milking parlor where buffaloes were placed in two rows back to back. The herd size of the milking buffalo cows, the average age of lactating animals, and their average days in milking are reported in Table 1. Buffalo cows were milked twice a day (morning and afternoon) and the milk daily stored in a refrigerated tank (+4°C). The milk was delivered within the same day to the dairy plant for cheese making.

Sampling

The milk subjected to analyses was obtained by pooling afternoon and morning milkings, stored at +4°C. Before sampling the raw bulk whole milk was mixed in the farm's tank to ensure homogeneity and then five aliquots of 100 mL were manually collected and pooled into a sterilized baker. Approximately 40 mL of bulk buffalo milk was collected from the pooled milk using sterile conical tubes (BD Falcon, Franklin Lakes, NJ, USA). The samples were transferred to the laboratory under refrigerated conditions (+4°C) and subjected to analyses during the same day. A total of 51 samples of bulk milk were collected throughout the monitored period (Table 1).

Physico-chemical, Microbiological Analyses and Somatic Cell Counts

All of the analyses were carried out in the same accredited laboratory (ACCREDIA, accreditation No. 1239).

Milk constituents (fat %, protein %, anhydrous lactose %, expressed as w/w) of bulk buffalo milk samples were quantified via Fourier Transform Infrared (FTIR) spectroscopy using a CombiFoss FT+ composed by Milkoscan FT Plus – 300 and Fossomatic FC (Foss Electric, Hillerød, DK).

Somatic cell counts (SCC) were determined in accordance with the ISO 13366:2008 standard by flow cytometry, using a high-capacity somatic cell counter CombiFoss FT+ (Foss Electric). Before analysis, each sample was heated to 40-42°C and subjected to counting within 15 minutes. The results were expressed as number of cells mL⁻¹.

Total bacterial counts (TBC) were determined using a fluorimeter (BactoScan FC, Foss Electric) in accordance with Regulation (EC) No 1664/2006, which allows the use of alternative methods for counts at 30 °C when these methods are validated according to the reference horizontal method for the enumeration of microorganisms ISO 4833-1:2013 and the protocol set by the ISO 16140 standards. The results were expressed as colony forming units (cfu) mL⁻¹.

The cheese yield formula for estimating the production of mozzarella cheese (kg) was calculated in accordance with the formula proposed by Altiero et al. (1989) as follows: 3.50 (protein %) + 1.23 (fat %) – 0.88. This formula corresponds to a multiple regression equation that attributes 93% of yield variability to fat and protein content as expressly calculated by Altiero et al. (1989) for buffalo mozzarella cheese production. Estimated cheese yields were expressed as kg of mozzarella produced per 100 kg of processed milk (kg 100 kg⁻¹).

Statistical Analyses

Before statistical analysis, the values of SCC and TBC were first log-transformed. Descriptive statistics, calculated on the whole data set (51 samples), were carried out on fat, protein, lactose, solids-not-fat, cheese yield formula, SCC, and TBC, computing means \pm standard deviation. The values recorded for all of the variables were checked for conformance to a normal distribution and then processed by analysis of variance (ANOVA) carried out using JMP statistical software version 11.0.0 (SAS Institute Inc., Cary, NC, USA). The following main effects were tested: month, season, and year.

The one way ANOVA model was $y_{ij} = \mu + \alpha_i + \epsilon_{ij}$, where μ is the overall mean, α_i is the effect of the i th level of factor (i = January,..., December; i = winter, spring, summer, autumn; i = 2011, 2012, 2013), and ϵ_{ij} is the random error.

Simple correlation among variables was evaluated by the Pearson product-moment correlation coefficient (r).

Cluster analysis of bulk milk parameters was carried out using the Wards' minimum variance method.

RESULTS AND DISCUSSION

The monitoring of bulk buffalo milk composition can reflect the combined effect of environment and genetics on the final quality of milk. It is known that the composition of buffalo milk can be influenced by breed, age, parity, stage of lactation, seasonality, feeding, udder disorders such as mastitis, and genetic polymorphism of milk proteins (Abd El-Salam & El-Shibini 2011). The above-mentioned parameters can affect the composition of milk and, in turn, the quality and process yield of dairy products (Gürler *et al.* 2013).

Descriptive statistics for quality parameters of bulk buffaloes' milk analyzed between 2011 and 2013 are shown in Table 2. The results of ANOVA are shown in Table 3. Multiple comparisons among Least Square Means (LSM) using the Tukey HSD test for quality parameters of bulk buffaloes' milk according to month, season, and year are shown in Table 4.

Regarding fat content, the average fat value reported in the present study (7.13%) was slightly lower than that reported by Zotos & Bampidis (2014) in Greek buffaloes' milk. The monitored fat values were also lower than those reported by Rosati & Van Vleck (2002) for individual milk samples produced by buffaloes reared mainly in the South of Italy (average value $8.59 \pm 0.85\%$); additionally, Di Francia *et al.* (2007a) found higher fat content in milk produced by Italian buffaloes fed a ration containing extruded peas or soybean cake as concentrate components (7.84 and 7.56%, respectively). Finally, the fat values we observed in the present study were slightly higher than the values reported by Cunha Neto *et al.*

(2005) for Brazilian buffalo milk used for the production of yogurt and by Enb *et al.* (2009) for milk produced by Egyptian buffaloes and used for cheese manufacturing. Moreover, we observed a progressive lowering of measured values during the warmer months with respect to the coldest ones. The highest mean value of fat ($8.54 \pm 0.27\%$) was observed during January, whereas the lowest mean contents were detected during May, June, and July ($6.50 \pm 0.27\%$, $6.27 \pm 0.24\%$, and $6.54 \pm 0.24\%$, respectively) without significant differences among these three months. The data trend reported in the present study for fat content is also in agreement with data reported by other authors in Greek and Italian buffaloes that describe peaks of fat content during December, January, and February (Ahmad *et al.* 2008; Bartocci *et al.* 2002; Zotos & Bampidis 2014).

The ANOVA according to year did not show any significant differences, while as expected, according to season, winter and autumn showed the highest mean levels of fat ($7.81 \pm 0.20\%$ and $7.76 \pm 0.16\%$, respectively). Han *et al.* (2012) found a similar fat content trend in buffalo milk samples collected in the United States; in more detail, the level of fat was the highest in January (7.63%), then a drop to a minimum was observed in July (6.57%), and a further increase was recorded in November (7.97%).

Regarding the seasonal effect, the highest fat values were observed during winter and autumn; these results almost overlap the seasonal trend reported by Gürlér *et al.* (2013) for Anatolian buffaloes milk. Interestingly, this latter breed, represents a sub-group of Mediterranean buffaloes as well as Italian Mediterranean ones, and this could explain similar trends in milk compositional parameters.

Moreover, Simoes *et al.* (2014) observed that the fat content of Brazilian buffalo milk was lower during warm and humid seasons (rainy season) with a value of 5.53% (w/w) and higher during cold seasons (dry season), reaching a value of 6.74% (w/w). This difference in fat content may be due to greater energy loss by buffaloes for the maintenance of their homeostasis during the warm and humid season associated with their modest physiological sweating due to their low number of sweat glands. It is worth noting that lactation stage, season, and animal diet can also strongly influence lipid synthesis, which in turn can affect the quality of milk and dairy products (Ménard *et al.* 2010; Yadav *et al.* 2015).

The physiology of lactation coordinates the production of milk and determines its final composition. However, this mechanism still represents a challenge for the current research. The stage of lactation influences the composition of the milk and the amount and quality of fat. Furthermore, the environmental temperature can also exert an effect on the profile of the fatty acids that compose the fat molecules. Buffalo milk presents the highest fat content with respect to other dairy animals, although the factors that influence its concentration in milk are still under study (Yadav *et al.* 2015; Zotos & Bampidis 2014).

204 Given the high importance of fat in milk's composition, new methods to maximize its content are still
205 under study. To this aim, Shelke *et al.* (2012) showed that buffaloes fed with rumen-protected fat (Ca
206 salts of palm fatty acids) have benefited from greater caloric intake during lactation, leading to a higher
207 yield of cheese and to an increase of unsaturated fatty acids in the fat.

208 As for protein content, the ANOVA according to month and year did not show significant differences,
209 whereas a seasonal effect was discovered. In more detail, we measured the highest mean values
210 ($4.48\pm0.05\%$ and $4.50\pm0.05\%$, respectively) during autumn and winter, while we observed the lowest
211 mean value ($4.27\pm0.05\%$) in samples collected during spring, findings similar to those reported for
212 Anatolian bubaline milk (Gürler *et al.* 2013). As reported by Gürler *et al.* (2013), milk protein, as well as
213 fat, may be inversely related with environment temperature; moreover different level of protein and fat
214 may be ascribed to the lactation period of the majority of milking buffalo cows.

215 The mean values of protein content detected in the present one-farm study were lower than those reported
216 by Bonfatti *et al.* (2013a) for Mediterranean buffaloes reared in the Campania region (Italy), which tested
217 at approximately 6%.

218 It is worth noting that Bonfatti *et al.* (2013b) have recently highlighted that the relative amount of the
219 α S1-casein fraction in buffalo milk can heavily affect its behavior during cheese making, although to
220 clarify the effects of buffalo whey and casein fractions on cheese yield further studies are still needed.

221 Regarding lactose, no significant differences were discovered among months. We observed a progressive
222 lowering of lactose mean value among the years. The mean value dropped from $4.95\pm0.07\%$ in 2011 to
223 $4.69\pm0.07\%$ in 2013. We suppose that the decrease of lactose content can be related to the variation of
224 somatic cell counts which reached the highest mean value, although not statistically significant, in 2013
225 when lactose was at minimum. It is worth noting that the increase in SCC lead to a rise of enzymatic
226 activities in mammary tissue, thus reducing lactose synthetic activity (Sharif *et al.* 2007).

227 As for season, we recorded the highest mean value ($4.91\pm0.06\%$) during summer, whereas we detected
228 the lowest mean value of lactose content during winter ($4.59\pm0.07\%$). Large differences in lactose mean
229 values are often reported for milks from different parts of the world, although some of these differences
230 could be due to different method of expression (as monohydrate or anhydrous lactose). The recorded
231 values of lactose showed very low variation similar to the monthly variation reported by Han *et al.* (2012)
232 for buffalo species ($4.49\text{--}4.73\%$), in a commercial water buffalo dairy farm. Lactose content in buffalo
233 milk and dairy products can be a quality indicator used as a marker of mammary inflammation because its
234 low levels can be the expression of reduced synthetic activity of secretory cells in mammary gland tissue,

thus suggesting mastitis in progress (Sharif *et al.* 2007). Indeed, Tripaldi *et al.* (2010) found that lactose content decreased in animals affected by mastitis, this being negatively correlated with somatic cells and contaminating bacteria. Hence, though still controversial, a threshold value of 4.7% lactose as a marker of mammary inflammation has been proposed by different researchers (Tripaldi *et al.* 2010).

Solids not-fat (SNF) of milk consists of protein, lactose and mineral matter content. The SNF residue, after the complete evaporation of water, is a fundamental quality parameter of raw milk that provides useful information about the overall suitability of milk for cheese-making (Bassbasi *et al.* 2014).

Regarding SNF content, the ANOVA according to season and year did not show any significant differences. However, according to month, we measured the lowest and the highest mean SNF values during February ($9.51 \pm 0.07\%$) and September ($10.12 \pm 0.11\%$), respectively. The average values of SNF content measured in the present study were slightly lower than those reported by Hussain *et al.* (2012) and Zicarelli *et al.* (2007) in the same matrix, which were 10.40% and 10.61-10.43%, respectively. A lower mean value of SNF was reported by Gürler *et al.* (2013) for milk produced by Anatolian buffaloes and by Simoes *et al.* (2014) for bubaline milk collected during the dry season and used for production of Marajó cheeses typical of the homonymous Brazilian island.

Cheese yield represents a parameter with heavy economic repercussions. The theoretical value of cheese yield is conditioned by many factors such as the fat and casein content of the milk used, the production technology, and moisture and salt content (Melilli *et al.* 2002). Regarding Mozzarella yield prediction, as estimated with the formula proposed by Altiero *et al.* (1989), no significant differences were discovered according to year, whereas according to month, we recorded the highest and the lowest mean values during January (25.84 ± 0.57 kg 100 kg⁻¹) and June (21.80 ± 0.51 kg 100 kg⁻¹), respectively. Cheese yield being strictly correlated with both fat and protein content, the trend of the cheese yield formula was almost overlapping with that observed for the two parameters. Moreover, we discovered a seasonal effect with the lowest average values of theoretical cheese production recorded in spring and summer; this finding can be due to the lowest levels of both proteins and fats in milk recorded during these two seasons.

Among the few scientific papers that address the estimation of Mozzarella cheese production according to the formula proposed by Altiero *et al.* (1989), Masucci *et al.* (2008) reported higher values of estimated Mozzarella production than that observed in the present study. The values observed by Masucci *et al.* (2008) of 27.2 and 28.0 kg 100 kg⁻¹ were likely due to the superior characteristics of organic buffalo milk, which resulted particularly high in fat and proteins. Our findings for theoretic cheese yield were also

265 lower than those published by Zicarelli *et al.* (2007) who reported an estimation of Mozzarella production
266 of approximately 25 kg per 100 kg of processed buffalo milk.

267 While the international literature has mainly provided data on the chemical composition of buffalo milk,
268 few investigations concerning the trends of the main sanitary (Somatic Cell Count, SCC) and hygienic
269 (Total Bacterial Counts, TBC) buffalo milk parameters have been carried out to date.

270 Regarding SCC, we did not discover significant differences among years. We recorded mean SCC values
271 ranging between 152.84 ± 25.22 and $199.73 \pm 23.43 \times 1,000 \text{ cell mL}^{-1}$ among the three years; these counts,
272 were always lower than the average value ($314,000 \text{ cell mL}^{-1}$) reported by Tripaldi *et al.* (2010) for
273 individual buffalo milk samples collected in the Latium region (Italy). Significant differences were
274 discovered among seasons; in more detail, we recorded the highest mean SCC value in the winter period
275 ($269.44 \pm 34.54 \times 1,000 \text{ cell mL}^{-1}$), whereas we observed the lowest mean value in samples collected
276 during summer ($147.00 \pm 27.69 \times 1,000 \text{ cell mL}^{-1}$). Regarding SCC trends across months, we observed the
277 highest mean value during January ($299.00 \pm 47.34 \times 1,000 \text{ cell mL}^{-1}$) and the lowest one during May
278 ($87.25 \pm 47.34 \times 1,000 \text{ cell mL}^{-1}$).

279 Actually, Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004,
280 laying down specific hygiene rules for food of animal origin, set only a threshold SCC value for bovine
281 raw milk. The threshold SCC value for bovine raw milk is set at $400,000 \text{ cells mL}^{-1}$, while no limits are to
282 date provided for buffalo raw milk, even though this parameter represents the most reliable indicator of
283 inflammatory status of mammalian udders, which may also cause a reduction in protein (casein) content
284 of milk. Somatic cell counts could be used as a quality parameter because, in buffalo milk, they increase
285 with days in lactation and show the highest values in milk from buffaloes with mastitis (Moroni *et al.*
286 2006).

287 The bulk buffalo milk samples under study showed the highest levels of SCC in winter, whereas the
288 lowest values were recorded during summer. These findings are in agreement with SCC observed by
289 Simoes *et al.* (2014) for Brazilian milk, which was assessed at 290,000 and 240,000 cells mL^{-1} during the
290 rainy and dry seasons, respectively. The annual trend of microbiological buffalo milk contamination, as
291 monitored in the present study, revealed SCC levels always lower than the limit of $200,000 \text{ cell mL}^{-1}$ that
292 in cows is usually considered a cut-off point for mastitis diagnosis (Tripaldi *et al.* 2010). As reported by
293 Smith (2002), SCC measurement, which includes polymorphonuclear leukocytes (PMN), macrophages,
294 and lymphocytes, is commonly used to evaluate the health of mammary glands. Furthermore, Kelly *et al.*
295 (2000) highlighted that the increase of SCC during milk secretion usually reflects an increase in PMN

296 whose function is the ingestion and destruction of invading microorganisms as well as secretion of
297 inflammatory regulators. High SCC in milk can exert adverse effects during cheese making, with a direct
298 negative impact on curd firmness, cheese yield, and sensory characteristics; moreover, the presence of
299 somatic cells can enhance the quantity of fat loss in whey (More *et al.* 2013). Furthermore, as reported by
300 Pasquini *et al.* (2003), the lower the number of somatic cells in buffalo milk is, the higher is the quantity
301 of casein and whey proteins in cheese. Therefore, further investigations on the somatic cell populations in
302 milk should be carried out to better explain the relationships among them and buffalo milk's aptness for
303 cheese manufacturing.

304 Finally, as for TBC, multiple comparisons among least square means (LSM) showed no significant
305 differences for the monitored bulk bubaline milk according to month, season, or year.

306 TBC are commonly used as indicators to evaluate the hygiene of the entire production process. The limit
307 values for TBC at 30°C established by Regulation (EC) No 853/2004 are: 100,000 cfu mL⁻¹ for raw cows'
308 milk; 1,500,000 cfu mL⁻¹ for raw milk from other species; in addition, 500,000 cfu mL⁻¹ limit for raw milk
309 from species other than cows used for manufacturing cheese products without heat treatments is also
310 provided.

311 TCB counts for the three-year period showed very low levels of raw milk environmental contaminating
312 microorganisms ranging from 6.46 to 23.41 x 10¹ cfu mL⁻¹. The TBC levels also showed low variability
313 through months, ranging from 3.66 x 10¹ in June to 9.85 x 10¹ cfu mL⁻¹ in March. Interestingly, the low
314 microbiological contamination found during the summer months (17.66 x 10¹ cfu mL⁻¹) could reflect
315 good hygiene practices performed during milking and handling of the raw milk, especially in summer
316 when warm temperatures could easily increase milk's microorganism content. Although the TBC values
317 reported in this study are lower than those reported by other authors (Gürler *et al.* 2013; Simoes *et al.*
318 2014; Tripaldi *et al.* 2010), it is worth noting that sufficiently high TBC could represent a risk for the
319 consumer because, pathogens can constitute a fraction of this hygiene indicator (Brown *et al.* 2000).
320 Hence the monitoring of TBC can provide a record of hygiene performance over time.

321 Important correlations among variables were identified (Table 5), and only those showing high significant
322 *P* values (< 0.01) are discussed. Significant positive correlations were found between protein and fat
323 percentage content ($r=0.64$; $P<0.01$) and between predicted cheese yield and both fat ($r=0.94$; $P<0.01$)
324 and protein ($r=0.87$; $P<0.01$) contents. Our results showed slightly higher correlation than the results
325 reported by Napolano *et al.* (2007). Moreover, fat and protein content are the most important parameters
326 determining cheese yield, which is the most important technological trait in the dairy industry (Bonfatti *et*

327 *al.* 2013b). Proteins, and in particular the content of casein, represent determining factors in the
328 organization of the cheese matrix because they are capable of retaining fat and moisture. Furthermore,
329 milk fat determines the amount of whey that drains from the curd. As reported by Mateo *et al.* (2009)
330 higher fat milks, with constant protein, resulted in lower yield of whey associated with higher reduction in
331 curd moisture content. As expected, SNF (%) was positively correlated with lactose content (Table 5).
332 The cluster analysis (Figure 1) reflected the different trend of milk compositional parameters among
333 seasons, and confirmed the results of ANOVA showing the lack of a significant year effect on
334 compositional milk parameters related to cheese yield production.

335

336 **CONCLUSIONS**

337

338 The trends in the compositional and sanitary parameters considered highlighted the high quality of the
339 Italian Mediterranean bulk buffalo milk under study. Due to the remarkable season effects discovered for
340 many of the monitored parameters, we can conclude that a seasonal adjustment of the buffaloes in terms
341 of delivery scheduling should be desirable for maintaining minimum oscillation of milk components
342 throughout the four seasons, thus resulting in bulk milk standardization and constant production of
343 buffalo dairy products. As is well known, milk testing and quality control represent pivotal activities of
344 any milk processing industry; for buffalo milk, such activities can provide important information for the
345 exploitation of this poorly utilized food matrix. Given the richness in composition of buffalo milk, its
346 health properties are supposed to be superior to those of cow milk, thus an increase of knowledge
347 regarding this matrix could be useful to drive the dairy industry toward the development of new buffalo
348 milk-based products with high nutritional properties.

349

350 **ACKNOWLEDGMENTS**

351

352 The authors wish to thank the *Associazione Regionale Allevatori* of *Marche Region* (ARAM), via
353 Clementina, Falconara Marittima (Italy), for its technical support. The authors also wish to thank the
354 laboratory staff at *Centro Agrochimico Regionale, Qualità delle Produzioni* of *Agenzia per i Servizi nel*
355 *Settore Agroalimentare delle Marche (ASSAM)* for analytical support.

356

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Table 1 Herd characteristics and sampling plan of bulk buffalo milk (n=51) analyzed in the period 2011-2013.

Herd characteristics	Years		
	2011	2012	2013
	#	#	#
Lactating Cows	74	80	87
Herd average age (years)	5	4.8	5
Milk yield (kg/head)	1,804	1,662	1,745
Days in milking	208	207	216
Daily milk yield (kg/day)	8.67	8.02	8.07
Sampling plan	Years		
	2011	2012	2013
	#	#	#
Winter	2	2	5
Spring	3	5	6
Summer	3	5	6
Autumn	2	7	5
Total	10	19	22

Table 2 Descriptive statistics for quality parameters of bulk buffaloes' milk analyzed between 2011 and 2013.

	Fat (% w/w)	Protein (TN) (% w/w)	Lactose (% w/w)	SNF (%)	CYF (kg 100 kg ⁻¹)	SCC (x 1,000 cells mL ⁻¹)	TBC (x 10 ¹ cfu mL ⁻¹)
Mean	7.13	4.39	4.77	9.86	23.27	175.57	9.91
SD	0.85	0.20	0.24	0.28	1.60	109.87	26.11
Minimum	5.36	4.02	3.75	8.63	20.45	42.00	0.40
Maximum	9.99	4.94	5.12	10.39	27.75	566.00	190.40

TN Total Nitrogen; SNF Solids-not-fat; CYF Cheese yield formula; SCC Somatic cell counts; TBC Total bacterial counts.
cfu colony forming units.
SD standard deviation.

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Table 3 ANOVA results for quality parameters of bulk buffaloes’ milk according to month, season and year.

Source of variation	Fat (% w/w)		Protein (TN) (% w/w)		Lactose (% w/w)		SNF (%)		CYF (kg 100 kg ⁻¹)		SCC (x 1,000 cells mL ⁻¹)		TBC (x 10 ¹ cfu mL ⁻¹)	
	df	Variance	df	Variance	df	Variance	df	Variance	df	Variance	df	Variance	df	Variance
Month	11	2.267 ***	11	0.059 n.s.	11	0.107 *	11	0.116 n.s.	11	7.017 ***	11	23,079.5 *	11	47,458.4 n.s.
Error	39	0.285	39	0.037	39	0.042	39	0.067	39	1.312	39	8,965.8	39	74,047.1
Season	3	6.258 ***	3	0.145 *	3	0.189 *	3	0.164 n.s.	3	19.389 ***	3	32,984.8 *	3	41,797.9 n.s.
Error	47	0.368	47	0.035	47	0.048	47	0.072	47	1.493	47	10,735.9	47	69,882.7
Year	2	0.858 n.s.	2	0.075 n.s.	2	0.226 *	2	0.254 *	2	4.085 n.s.	2	11,823.6 n.s.	2	113,462.0 n.s.
Error	48	0.715	48	0.040	48	0.049	48	0.070	48	2.504	48	12,081.1	48	66,312.0

TN Total Nitrogen; SNF Solids-not-fat; CYF Cheese yield formula; SCC Somatic cell counts; TBC Total bacterial counts.
df degrees of freedom.
* Significant at $P \leq 0.05$
** Significant at $P \leq 0.01$
*** Significant at $P \leq 0.001$
n.s. not significant

Table 4 Quality parameters of bulk Mediterranean buffaloes' milk according to month, season and year.

Effect	Fat (% w/w)	Protein (TN) (% w/w)	Lactose (% w/w)	SNF (%)	CYF (kg 100 kg ⁻¹)	SCC (x 1,000 cells mL ⁻¹)	TBC (x 10 ¹ cfu mL ⁻¹)
Month							
January	8.54±0.27 ^a	4.63±0.10 ^a	4.61±0.10 ^a	9.93±0.13 ^{ab}	25.84±0.57 ^a	299.00±47.34 ^a	6.68±13.61 ^a
February	7.02±0.27 ^{bcd}	4.26±0.10 ^a	4.56±0.10 ^a	9.51±0.13 ^b	22.68±0.57 ^{bcd}	274.25±47.34 ^{ab}	7.32±13.61 ^a
March	6.80±0.27 ^{cd}	4.39±0.10 ^a	4.51±0.10 ^a	9.60±0.13 ^{ab}	22.87±0.57 ^{bcd}	127.50±47.34 ^{ab}	9.85±13.61 ^a
April	6.68±0.27 ^{cd}	4.35±0.10 ^a	4.70±0.10 ^a	9.76±0.13 ^{ab}	22.58±0.57 ^{bcd}	293.50±47.34 ^{ab}	3.90±13.61 ^a
May	6.50±0.27 ^d	4.25±0.10 ^a	4.84±0.10 ^a	9.79±0.13 ^{ab}	21.98±0.57 ^{cd}	87.25±47.34 ^b	6.65±13.61 ^a
June	6.27±0.24 ^d	4.28±0.09 ^a	4.94±0.09 ^a	9.91±0.12 ^{ab}	21.80±0.51 ^d	121.80±42.35 ^{ab}	3.66±12.17 ^a
July	6.54±0.24 ^d	4.33±0.09 ^a	4.89±0.09 ^a	9.91±0.12 ^{ab}	22.31±0.51 ^{cd}	125.40±42.35 ^{ab}	4.02±12.17 ^a
August	6.64±0.31 ^{cd}	4.30±0.11 ^a	4.98±0.12 ^a	9.97±0.15 ^{ab}	22.35±0.66 ^{bcd}	116.33±54.67 ^{ab}	5.37±15.71 ^a
September	7.25±0.24 ^{bcd}	4.49±0.09 ^a	4.94±0.09 ^a	10.12±0.12 ^a	23.75±0.51 ^{abcd}	174.00±42.35 ^{ab}	6.74±12.17 ^a
October	7.30±0.24 ^{bcd}	4.42±0.09 ^a	4.84±0.09 ^a	9.95±0.12 ^{ab}	23.56±0.51 ^{abcd}	124.20±42.35 ^{ab}	7.22±12.17 ^a
November	8.04±0.31 ^{abc}	4.50±0.11 ^a	4.71±0.12 ^a	9.90±0.15 ^{ab}	24.77±0.66 ^{abc}	224.33±54.67 ^{ab}	6.83±15.71 ^a
December	8.15±0.24 ^{ab}	4.51±0.09 ^a	4.68±0.09 ^a	9.88±0.12 ^{ab}	24.94±0.51 ^{ab}	175.80±42.35 ^{ab}	8.40±12.17 ^a
Season							
Winter	7.81±0.20 ^a	4.50±0.06 ^a	4.59±0.07 ^b	9.78±0.09 ^a	24.49±0.41 ^a	269.44±34.54 ^a	8.91±8.81 ^a
Spring	6.40±0.16 ^b	4.27±0.05 ^b	4.76±0.06 ^{ab}	9.73±0.07 ^a	21.96±0.33 ^b	153.29±27.69 ^b	5.04±7.06 ^a
Summer	6.79±0.16 ^b	4.36±0.05 ^{ab}	4.91±0.06 ^a	9.95±0.07 ^a	22.73±0.33 ^b	147.00±27.69 ^{ab}	17.66±7.06 ^a
Autumn	7.76±0.16 ^a	4.48±0.05 ^a	4.77±0.06 ^{ab}	9.94±0.07 ^a	24.36±0.33 ^a	166.07±27.69 ^{ab}	7.65±7.06 ^a
Year							
2011	7.09±0.27 ^a	4.33±0.06 ^a	4.95±0.07 ^a	9.97±0.08 ^a	22.99±0.50 ^a	165.60±34.76 ^a	23.41±8.14 ^a
2012	7.36±0.19 ^a	4.46±0.05 ^a	4.78±0.05 ^{ab}	9.93±0.06 ^a	23.79±0.36 ^a	152.84±25.22 ^a	6.46±5.91 ^a
2013	6.95±0.18 ^a	4.37±0.04 ^a	4.69±0.05 ^b	9.74±0.06 ^a	22.95±0.34 ^a	199.73±23.43 ^a	6.74±5.49 ^a

Multiple Comparisons among Least Square Means (LSM) using the Tukey HSD test according to month, season and year (LSM± mean std. err.).

TN Total Nitrogen; SNF Solids-not-fat; CYF Cheese yield formula; SCC Somatic cell counts; TBC Total bacterial counts.

Within each effect, for each variable, means with different letters are significantly different ($P \leq 0.05$).

Table 5 Simple correlation among variables evaluated by the Pearson correlation coefficient (51 bulk milk samples).

	Fat (% w/w)	Protein (TN) (% w/w)	Lactose (% w/w)	SNF (%)	CYF (kg 100 kg ⁻¹)	SCC (x 1,000 cells mL ⁻¹)
Protein (%)	0.6444 **					
Lactose (%)	-0.2777 *	-0.2068 n.s.				
SNF (%)	0.2346 n.s.	0.5514 **	0.7017 **			
CYF (kg 100 kg ⁻¹)	0.9398 **	0.8669 **	-0.2735 n.s.	0.3993 **		
SCC (x 1,000 cells mL ⁻¹)	0.2684 n.s.	0.3113 *	-0.3579 n.s.	-0.0839 n.s.	0.3141 *	
TBC (x 10 ¹ cfu mL ⁻¹)	-0.0255 n.s.	0.0373 **	0.1775 n.s.	0.1724 n.s.	-0.0005 n.s.	0.0202 n.s.

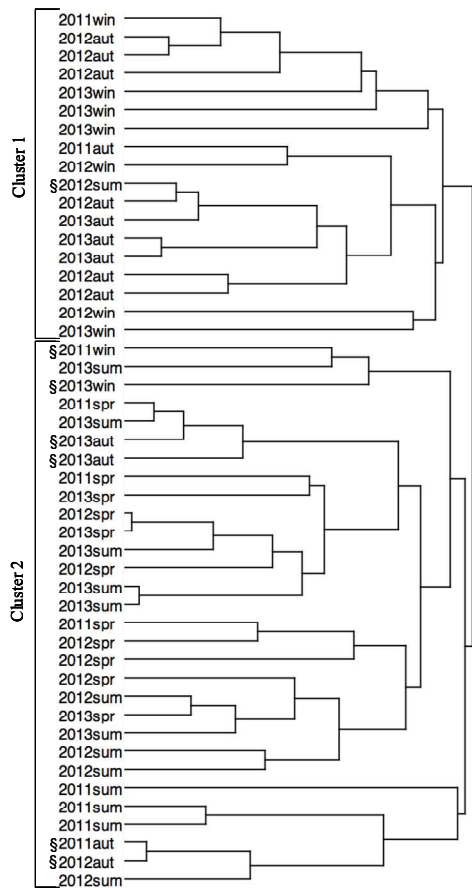
TN Total Nitrogen; SNF Solids-not-fat; CYF Cheese yield formula; SCC Somatic cell counts; TBC Total bacterial counts.

n.s. not significant

* Significant at $P \leq 0.05$

** Significant at $P \leq 0.01$

Fig. 1 Dendrogram of buffaloes' bulk milk parameters analyzed between 2011 and 2013 according to season resulting from Cluster Analysis carried out using the Wards' minimum variance method.



win winter; spr spring; sum summer; aut autumn
\$ mis-assigned records