

# Differences in chemical, physical and microbiological characteristics of Italian *burrata* cheeses made in artisanal and industrial plants of Apulia Region

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## Abstract

The *burrata* cheese is a traditional product from Southern Italy, consisting of an envelope of *pasta filata* (stretched curd) filled with cream and *pasta filata* strips (usually leftovers from mozzarella production). Physical [water activity ( $a_w$ ), pH], chemical (moisture, NaCl content) and microbiological [total viable count (TVC), *Listeria monocytogenes*, *Salmonella* spp., *Yersinia enterocolitica*, *Bacillus cereus*, *Escherichia coli*, *Enterobacteriaceae*, coagulase-positive staphylococci] characteristics of *burrata* cheeses manufactured in artisanal and industrial plants were evaluated. The artisanal *burrata* showed lower  $a_w$  values in the filling and the final product. The same was recorded in the filling for the moisture, probably due to differences between the types of cream used in the artisanal and the industrial cheesemaking. The pH value of the filling differed between the two groups but no difference was recorded in the final product. Microbiological differences were also recorded, with higher values for TVC and *E. coli* in artisanal than industrial *burrata*. All samples were negative for the other microbial determinations, with the exception of coagulase-positive staphylococci and *Y. enterocolitica*, which were detected in artisanal *burrata*. Differences in cheesemaking process were probably responsible for the strong variability of the physical and chemical data between the two cheeses; furthermore, differences in the hygienic features were also recorded. Even though artisanal products showed lower  $a_w$  and pH values and higher NaCl concentration, the higher *E. coli* loads highlighted the need for a more accurate compliance with hygienic procedures along the artisanal cheesemaking process.

## Introduction

*Burrata* is a traditional cheese produced in the Apulia Region (Southern Italy), particularly in Bari, Barletta-Andria-Trani and Foggia Provinces, as well as in Martina Franca, a town in the Province of Taranto. The product is a *pasta filata* (stretched curd) cheese consisting of a filling made of cream mixed with mozzarella cheese strips (usually leftovers from mozzarella production) inside an envelope made of mozzarella. This product came probably from the idea of reusing the scrap from mozzarella cheese production, and mixing it with cream to be used as filling of a cheese envelope shaped like a *caciocavallo* cheese (Salvadori del Prato, 2001). Although *burrata* cheese is not included in the list of EU protected agricultural products, it is listed in the register of traditional foodstuffs of the Apulia Region and is known worldwide (Lo Russo, 2008). Its production technology is far from being standardised, having not yet achieved a legal protection. This allows manufacturers to use production techniques that are sometimes very different. Moreover, different raw materials can be used depending on the manufacturer [*i.e.* whey cream obtained by spontaneous rising or ultra high temperature (UHT) centrifuged cream for the stuffing]. These could result in products different in shape, sensory traits, or safety aspects.

The absence of product and production standards in *burrata* cheesemaking and consequently the presence of different products on the market (Tantillo, 2007) can also influence the duration of their shelf life. For these reasons data on microbial, physical and chemical characteristics of *burrata* cheese are necessary for dairy producers to achieve a sort of standardisation that may contribute to improve the quality and safety of such a product.

The aim of the present study was to evaluate the physical, chemical and hygienic characteristics of *burrata* cheese manufactured in two dairy plants that used different raw materials and production techniques.

## Materials and Methods

### Cheesemaking process and sample collection

Samples of milk, intermediate products (cream, *pasta filata* strips, and envelope) throughout the cheesemaking process, and final products (*burrata* cheeses) were collected in two different sessions from an artisanal (AP) and an industrial (IP) dairy plant, both located in the Foggia Province (Apulia Region, Southern Italy). The *burrata* cheeses were produced according to two different production

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techniques. According to the tradition, the artisanal process was entirely carried out manually starting from raw milk and whey cream obtained by spontaneous rising from the water used for the curd stretching, collected from the previous production cycles. The industrial cheesemaking used pasteurised milk and UHT cream obtained by centrifugation and all the steps were performed using specific equipment, typical of most mozzarella production lines. Briefly, for both productions, the milk was added with natural whey starter (3/100 L) and, after a partial acidification (pH 6.1), heated to 37°C. Then, calf rennet was added (15-30 mL/100 L) and the coagulation took place after 30 min. The curd was cut and kept under whey for maturation (acidification) for 30 min. Later the curd was kneaded under hot salted water (85-90°C) and stretched to produce *pasta filata* strips and envelope. The cream was added to the strips to assemble the filling. Finally, it was used to fill the mozzarella envelope that was closed thereafter.

Two sampling sessions were performed in each plant. In each sampling session from the same batch, 5 samples were collected at the IP and 5 samples at the AP: the raw material (milk), the semi-finished (cream, *pasta filata* used for the filling and the envelope, and whole filling made with cheese strips and cream), and the finished product (*burrata* ready for consumption). The collection was performed under sterile conditions: samples were packaged in sterile plastic bags, refrigerated and sent to the laboratory of the School of Biosciences and Veterinary Medicine,

University of Camerino [Matelica (MC), Italy].

### Analytical determination

For the physical and chemical traits, activity water [ $a_w$ , using a BTRS1 Rotronic hygroscope (PBI International, Milan, Italy)], pH [according to Bendall (1975) measured using a Beckman 310 pHmeter (Beckman, Galway, Ireland)], moisture, and NaCl content (AOAC, 1990) were determined. Determinations were performed on all the samples in duplicate. Milk samples were analysed only for pH and protein content (AOAC, 1990).

As for the microbiological evaluation, total viable count (TVC), different foodborne pathogens (*Listeria monocytogenes*, *Salmonella* spp., *Yersinia enterocolitica*, *Bacillus cereus*) and process hygiene criteria (*Escherichia coli*, coagulase-positive staphylococci) were considered. These analyses were performed on all samples in duplicate except for the filling, whose overall microbiological characteristics were obtained from the analysis of the cream and the *pasta filata* strips used for its preparation.

The quantitative determination of microbiological parameters was carried out by inoculating the media with specific scalar dilutions of 10 g (or mL) of sample homogenised in 90 mL

of saline solution with peptone, while for the qualitative evidence, 25 g (or mL) of the sample were diluted 1:10 in specific enrichment broths, properly incubated and then inoculated on specific media. The following determinations were performed.

TVC on Plate Count Agar (PCA) (Biolife, Milan, Italy) and only milk samples on Plate Count Agar Wide Skim Milk (Biolife), in both cases incubated at 30°C for 72 h. *E. coli* on selective *E. coli*/Coliform Medium (Oxoid, Basingstoke, UK), incubated at 37°C for 24 h, and subsequently in TBX Medium (Oxoid) at 44°C for 18-24 h for confirmation. Coagulase-positive staphylococci on Baird Parker Medium (Biolife), incubated at 37°C for 24-48 h, followed by confirmation of the typical colonies using Staphylase test (Oxoid). *B. cereus* on *B. cereus* Agar Base (PEMBA) (Biolife), added with *B. cereus* Antimicrobial Supplement (Biolife), incubated at 30°C for 24-48 h, and subsequently on Blood Agar at 30°C for additional 24 h. *Salmonella* spp. with a pre-enrichment in Buffered Peptone Water (Oxoid), followed by selective enrichment on Rappaport Vassiliadis Soya Peptone (RVS) Broth (Oxoid) at 42°C for 24 h, and then on Chromogenic *Salmonella* Agar Base (Biolife) and Brilliant Green Agar (Biolife), at 37°C for 24 h. The

identification was performed using the API 20E biochemical test (bioMérieux, Italy), as specified in the manufacturer's guide. *L. monocytogenes* on *Listeria* Enrichment Broth Low Acriflavine (LEBB) (Biolife) incubated at 30°C for 48 h, and subsequently on *Listeria* Oxford Agar Base (Biolife) and ALOA Agar Ottaviani Agosti (Biolife) at 37°C for 24-48 h. The identification was made through the biochemical test API *Listeria* (bioMérieux, Marcy l'Etoile, France), as specified in the manufacturer's guide. *Y. enterocolitica* on selective enrichment medium *Yersinia* PSB Broth (Biolife) incubated at 25°C for 5 days, and subsequently on *Yersinia* Selective Agar Base (Oxoid), supplemented with *Yersinia* Selective Supplement (Oxoid) incubated at 25°C for 24-48 h, after treatment in a solution of KOH 0.5%. The suspect colonies were identified using the API 20E biochemical test (bioMérieux), according to the manufacturer's guide, except for the incubation temperature, which was 30°C instead of 37°C.

All quantitative microbiological data were reported as colony forming unit (CFU)/g or mL and converted to  $\log_{10}$ .

### Statistical analyses

Data derived from the different sampling

**Table 1. Physical and chemical data of samples collected during the artisanal and industrial production of burrata cheese (n=10).**

Sample	Parameter	AP	IP	P*
Cream	$a_w$	0.953±0.014	0.964±0.007	0.267
	pH	4.96±0.43	7.22±0.02	<0.001
	Moisture (%)	43.63±0.28	61.89±1.49	<0.001
	NaCl (%)	1.84±0.67	0.22±0.11	0.003
Mozzarella strips	$a_w$	0.961±0.002	0.963±0.003	0.459
	pH	6.16±0.07	6.68±0.12	<0.001
	Moisture (%)	70.72±0.70	69.96±1.16	0.304
	NaCl (%)	0.41±0.11	0.80±0.33	0.069
Filling	$a_w$	0.957±0.005	0.967±0.001	0.016
	pH	6.21±0.10	6.73±0.11	<0.001
	Moisture (%)	52.41±1.85	65.82±0.74	<0.001
	NaCl (%)	1.07±0.44	0.22±0.11	0.009
Burrata cheese	$a_w$	0.963±0.003	0.969±0.001	0.010
	pH	6.35±0.14	6.51±0.15	0.189
	Moisture (%)	59.46±2.62	62.59±0.67	0.060
	NaCl (%)	0.99±0.56	0.12±0.01	0.020

AP, artisanal production; IP, industrial production;  $a_w$ , water activity. Values are expressed as mean±standard deviation. \*Significant for P<0.05.

**Table 2. Microbiological data ( $\log_{10}$  colony forming unit/g or mL) of samples collected during the artisanal and industrial production of burrata cheese (n=10).**

Sample	Parameter	AP	IP	P*
Cream	TVC	8.35±0.11	2.09±0.93	<0.001
	<i>E. coli</i>	4.51±0.33	1.13±0.25	<0.001
Mozzarella strips	TVC	6.93±0.04	5.51±0.53	0.006
	<i>E. coli</i>	5.30±0.07	1.85±0.40	<0.001
Burrata cheese	TVC	7.85±0.28	7.11±0.17	0.017
	<i>E. coli</i>	5.35±0.11	3.62±0.24	<0.001

AP, artisanal production; IP, industrial production; TVC, total viable count; *E. coli*, *Escherichia coli*. Values are expressed as mean±standard deviation. \*Significant for P<0.05.

sessions were aggregated for statistical analyses. The physical, chemical and microbiological data obtained in all the different samples were analysed with an unpaired *T* test (Statview SAS, Cary, NC, USA) to detect differences between products from AP and IP. The significance level was set at  $P < 0.05$ .

## Results

As for the milk, no significant difference ( $P = 0.872$ ) was recorded for pH values between AP ( $6.75 \pm 0.06$ ) and IP ( $6.70 \pm 0.01$ ); protein content differed significantly ( $P = 0.002$ ) between AP ( $3.00 \pm 0.06$ ) and IP ( $3.36 \pm 0.06$ ) (data not shown).

As for the semifinished and finished products, the physical and chemical characteristics are reported in Table 1 and the microbiological data are shown in Table 2.

Differences in pH values were recorded in all the semi-finished samples, with a constantly lower value in AP than in IP, but not in the final products. Differences in the cream used, and therefore in the filling, were recorded also for moisture content (higher in industrial than in artisanal cream) and for NaCl concentration (lower in industrial than in artisanal cream).

The microbiological counts revealed higher values of TVC and *E. coli* in cream, mozzarella strips and final products from AP than those from IP. Coagulase-positive staphylococci were detected in all the artisanal samples collected but the counts were always well below the limit set by the European Commission Regulation n. 1441/2007 (European Commission, 2007). No coagulase-positive staphylococci were detected in IP samples. No *Salmonella* spp., *B. cereus* and *L. monocytogenes* were detected in both AP and IP samples. *Y. enterocolitica* was qualitatively detected in samples obtained in the second sampling session from AP mozzarella strips and *burrata* cheese and from IP pasteurised milk (data not shown).

## Discussion

The different cheesemaking technologies, mainly consisting in the cream used but also in the *pasta filata* envelope and strips, could be responsible for the chemical and physical differences recorded between AP and IP samples. The values of NaCl detected in the cream are quite interesting: the significantly ( $P = 0.003$ ) higher value in AP samples is strictly related to the traditional cheesemaking technology that

provides for the addition of hot water with 10% NaCl used for curd stretching, from which the artisanal cream is recovered by spontaneous rising, as previously described in the cheesemaking process. The spontaneous rising can even explain the much lower ( $P < 0.001$ ) pH observed in the artisanal cream. Moreover, the proportion of the cream and mozzarella strips used for preparing the filling (generally not standardised in the artisanal cheesemaking) could have affected the final products that differed only for  $a_w$  and NaCl values. The  $a_w$  parameter is relevant for microbial growth and the values recorded in both types of *burrata* cheese were high enough to allow almost all pathogenic microorganisms to grow if other hurdles are not provided in the production process. Furthermore, in these fresh cheeses the salt content has to be low for improving the taste perception by the consumer. The effect of salt in prolonging the shelf life of mozzarella cheese is almost contradictory, as longer durations were obtained for mozzarella without or with low (0.23/100 g) salt concentration (Faccia *et al.*, 2012).

From a microbiological perspective the AP samples showed higher TVC and *E. coli* values than IP. Moreover, coagulase-positive staphylococci and *Y. enterocolitica* were found in samples from AP, even though at low concentration or as not pathogenic strains, respectively (data not reported). The TVC, considered as a process hygiene parameter in some foods, could be strongly affected by the number of *Lactobacillus* and *Lactococcus* (LAB) in cheeses, where they play an important role as hurdle against microbial growth. Higher TVC values were constantly recorded in all products from AP, which showed lower pH values in the intermediate samples (cream, *pasta filata* strips and filling). The production procedure of whey cream obtained by spontaneous rising (24 h at room temperature) used in the AP can explain the growth of a much higher number of LAB, as well as other bacteria, in products from AP than those from IP. As emphasised by other authors (de Candia *et al.*, 2007), the LAB present in the whey used as starter for making *pasta filata* (strips and envelope) may also have influenced the characteristics of the products. Nonetheless, much higher values of *E. coli* were recorded in cream, mozzarella strips and final products from AP than those from IP. This parameter highlights deficiencies especially in the hygiene process. The whey cream obtained by spontaneous rising and the whey used as starter may be responsible for the high amount of *E. coli* in the products, and even the high temperature (85–90°C) used in the kneading/stretching step of *pasta filata*

production is not sufficient to reduce the *E. coli* load to an acceptable level. Indeed, Spano *et al.* (2003) reported that the stretching of curd at temperatures over 80°C should be able to eliminate up to 5 log of *E. coli* O157:H7, but time/temperature curve has to be carefully taken into consideration to obtain effective results (Trevisani *et al.*, 2014). Also, in IP the trend of *E. coli* increase in the final product was observed, with average values higher than the legal limit of acceptance set by the European Commission Regulation n. 1441/2007 (European Commission, 2007). This suggests that *E. coli* contamination and/or growth during the last phases of the cheesemaking could occur, *i.e.* during filling/closing, immersion for hardening, and packaging steps. The reported data disagree with Dambrosio *et al.* (2013), who did not observe *burrata* samples exceeding the limit of *E. coli* set by the EC Regulation, while 3.7% of samples were positive for coagulase-positive staphylococci with values greater than 3 log CFU/g. No relevant pathogens were recorded in products from AP and IP, and the level of coagulase-positive staphylococci was almost low, in contrast with other studies on mozzarella cheese, where high levels of such bacteria were reported (De Luca *et al.*, 1997). The presence of virulent *Y. enterocolitica* is reported in different cheeses (Hanifian and Khani, 2012; Kowalich and Lobacz, 2015) but no data on mozzarella or *burrata* cheese is available from the literature. The milk is reported as the major source of such a contamination (EFSA, 2013) and even pasteurised milk was found to be positive to *Y. enterocolitica* (Gupta *et al.*, 2015). Even if not detected in pathogenic form, the necessity of improving the control on this microorganism should be emphasised.

## Conclusions

The differences observed in *burrata* cheesemaking have to be taken into consideration when a proper risk analysis is performed. The bacterial contamination of the product could be reduced only if an appropriate temperature/time ratio, ensured by the curd kneading at 85–90°C during the stretching step, is reached. Control strategies to avoid *E. coli* proliferation are further needed, especially in the artisanal process and in particular during the preparation of whey cream obtained by spontaneous rising and used in the filling.

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