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Research Note

Microbiological Quality of Burrata Cheese Produced in Puglia Region: Southern Italy

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ABSTRACT

Burrata cheese is a popular typical Italian food product, produced in Puglia (an administrative region of southern Italy), and this study investigated the microbiological quality of 404 samples of this cheese. The samples were analyzed in order to quantify *Escherichia coli* and to detect the presence of *Staphylococcus aureus, Salmonella* spp., and *Listeria monocytogenes*. No sample exceeded the values of *E. coli* set by EC Regulation 1441/07 for some dairy products, while 15 (3.7%) samples tested coagulase-positive staphylococci positive, with values greater than 10^3 CFU/g. One strain of *S. aureus* was identified and characterized from each of these positive samples, and of these strains, 7 (46.6%) produced staphylococcal enterotoxin A, 5 (33.3%) produced staphylococcal enterotoxin C, 2 (13.3%) produced staphylococcal enterotoxin D, and 1 (6.6%) produced both staphylococcal enterotoxins A and D. All strains were *mecA* negative. The 15 *S. aureus* isolates were tested for their antimicrobial resistance patterns, and all analyzed strains showed antimicrobial resistance properties for at least one of the tested antibiotics. Testing for the other pathogens mentioned above gave negative results. The results of our study mean that the microbiological quality of Burrata cheese can be assumed to be good, although care must be taken with raw materials and good hygiene during processing in order to guarantee greater food safety.

''Traditional Italian food products'' are foodstuffs whose processing, preservation, and seasoning methods have been consolidated over time, and are also consistent across an entire region. They have been produced according to traditional Italian regulations for 25 years *(6)*. According to the Ministry of Agriculture and Forestry list (updated in November 2012), of Italy's 4,000 traditional products, 245 are certified as ''Protected Designation of Origin'' and ''Protected Geographical Indication.'' The Puglia region's most recent (June 2011) official list of traditional products contains 231 products including branded, regulated products and foodstuffs. Burrata cheese is one of the most popular of these traditional products, both in Italy and abroad, and the Burrata made in Bari and the Barletta, Andria, and Trani Provinces is a fresh cheese made from cow's milk. Technically, it is a dual structure, i.e., it has a bag-shaped outer part (pouch) consisting of soft, supple spun paste, and an inner part of spun dough cuttings and cream *(8)*. The finished product is spherical, with a ''head'' and a short ''neck,'' and weighs between 100 g and 1 kg. Burrata has a rindless, milky white, smooth, shiny surface made up of fibrous elastic overlapping layers; its average pH values range between 6.1 and 6.2, and water activity values between 0.95 and 0.97 *(12, 30)*. As with all dairy products, the consumption of Burrata presents potential microbiological risks, because pathogenic microorganisms can contaminate the product during different production stages, involving different sources such as primary production, food operators, and the environment. For this reason, we examined Burrata cheese samples produced in the Puglia region to assess their microbiological quality by evaluating processing hygiene criteria (coagulase-positive staphylococci [CPS] and *Escherichia coli*) and food safety criteria (*Salmonella* spp. and *Listeria monocytogenes*), according to EC Regulation 1441/07 *(3)*. This survey also aimed to characterize the *Staphylococcus aureus* isolates in order to detect their enterotoxigenic and antimicrobial resistance characteristics, and to better define the potential risk of foodborne infection for consumers.

MATERIALS AND METHODS

The survey periods were April through July 2009 and February through June 2010. We analyzed 404 samples of Burrata cheese from 12 different dairies, which all have a European Union approval number and are situated in Bari and the Barletta, Andria, and Trani Provinces. Two plants process about 4,730 to 9,460 liters of milk per day (64 samples), two plants process more than 9,460 liters of milk per day (68 samples), and eight plants process up to 473 liters of milk per day (272 samples). Each Burrata sample was

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aseptically collected and placed in a refrigerated container (0 to 4°C). Within 4 h of collection, samples were transferred to the laboratory that carried out *E. coli* quantitative assessment according to International Organization for Standardization (ISO) method 16649-2:2010 and CPS microbiological analysis according to European Norm (EN) ISO method 6888-2:2004 *(1, 5)*. Samples were also analyzed according to EN ISO method 6579:2008 in order to isolate and identify *Salmonella* spp., and according to EN ISO method 11290-1:2005 to isolate and identify *L. monocytogenes (2, 4)*. Furthermore, one colony from each plate with CPS values higher than 10^3 CFU/g was examined in order to identify *S. aureus (1)*. The identified strains were subjected to enterotoxic characterization to detect staphylococcal enterotoxins A (SEA), B (SEB), C (SEC), and D (SED) by reverse passive latex agglutination with the SET-RPLA test kit (Oxoid, Ltd., Basingstoke, UK). The following internal controls were used: *S. aureus* ATCC 13565 (SEA producer), *S. aureus* ATCC 14458 (SEB producer), *S. aureus* ATCC 19095 (SEC producer), and *S. aureus* ATCC 23235 (SED producer). In addition, *S. aureus* strains were PCR tested to detect the *mecA* gene by using the protocol suggested by Araj et al. *(7)*, and also tested by agar diffusion, according to Clinical and Laboratory Standards Institute guidelines *(24)*, in order to evaluate their antibiotic resistance. The following antibiotic disks were used for the susceptibility test: ampicillin (25 μg), tetracycline (30 μg), gentamicin (10 μg), erythromycin (15 μg), enrofloxacin (5 μg), trimethoprim-sulfamethoxazole (25 μg), teicoplanin (30 μg), and vancomycin (30 μg) (Liofilchem, Teramo, Italy). The strains were classified as susceptible, intermediate, or resistant according to the manufacturer's instructions.

CPS levels in relation to the amount of processed milk were statistically analyzed with one-way variance analysis. Statistics were elaborated with StatView, version 5.0 (SAS Institute Inc., Cary, NC), with statistical significance set at $P < 0.05$.

RESULTS AND DISCUSSION

Burrata cheese is one of the best known and most popular traditional Italian food products, and average annual production from 2003 to 2005 was 4,314,250 kg, for a total value of €28,042,625.00 (roughly $36,606,858.00 as of month of article publication) *(25)*. The microbiological risk associated with this product is limited, because it is made mainly from pasteurized milk, and because it undergoes a high-temperature (85 to 90°C) curd-stretching process. However, Burrata is a ready food that is eaten without any other heat treatment, so that the greatest care must be taken with hygiene during production and in order to avoid postprocess contamination. Moreover, the presence of typical foodborne pathogens must be considered when Burrata cheese is made from raw milk. Although this dairy product is widely consumed, no data about microbiological quality are available. In our survey involving 404 samples, we considered both the microbiological parameters according to EC Regulation 1441/07 for dairy products (food safety criteria and process hygiene criteria) and the presence of foodborne pathogens that could be present in raw milk or that could contaminate Burrata cheese during the production process (enterotoxigenic *S. aureus*). Overall, the microbiological quality of all tested samples was good, and all samples were within the legal limits. In more detail, the *E. coli* values in all tested samples were below the microbiological limits set by EC Regulations, with a mean value of 4.4×10^2 CFU/g. This means that all the production plants involved in this study can be considered as having good manufacturing and hygiene practices in terms of raw materials, equipment, and food operators.

On the other hand, CPS testing gave positive results for 15 (3.7%) samples, with mean values of 10^3 CFU/g. Of these samples, 5 (33.3%) were collected in dairy plants that processed more than 9,460 liters of milk per day, 3 (20%) in dairy plants processing 4,730 to 9,460 liters of milk per day, and 7 (46.6%) in dairy plants processing up to 473 liters of milk per day. Statistical analysis showed no correlation ($P > 0.05$) between the CPS values and the daily quantity of milk produced in the sampled dairy plants.

According to EC Regulation 1441/07, since CPS values were less than 10^5 CFU/g, there was no need to test for the presence of staphylococcal enterotoxins directly in the sample. Nonetheless, CPS testing could provide important information for improving hygiene and safety during production. Undoubtedly, plants where CPS-positive samples were found need to improve staff hygiene and product handling procedures, because humans are the main source of CPS contamination *(11)*. Another potential source of CPS could be the raw milk used to make Burrata *(27)*. It is necessary to carry out systematic evaluation of the microbiological quality of raw materials, which should preferably be supplied by referenced farms *(13)*. However, it is important to highlight that the CPS-positive samples had values below the prescribed 10^5 CFU/g limit enforced by EC Regulation 1441/07.

One strain of *S. aureus* was isolated from each CPS-positive sample and was then subjected to enterotoxic characterization and typing to detect the *mecA* gene, coding for PBP2a, a protein that gives methicillin resistance *(10)*. In fact, some authors have stated that the risk of methicillin-resistant *S. aureus* (MRSA) transmission to humans through handling and/or consumption of contaminated foods should not be underestimated *(14, 17, 26)*.

Seven (46%) of the 15 isolates were able to synthesize SEA, 5 (33.3%) SEC, 2 (13.3%) SED, and 1 (6.6%) both SEA and SED. These data are comparable to previous studies reporting similar results about the isolation of enterotoxigenic strains of *S. aureus* from dairy products *(28)*, and SEA and SED are described as the SEs more frequently involved in staphylococcal food poisoning (SFP) outbreaks. Nevertheless, other ''new'' SEs not investigated in our study could be involved in SFP occurrence *(23)*. Not surprisingly, there is a higher incidence of strains that produce SEA and SEC in dairy products. SEA is often associated with contamination by food handling, while SEC is the most frequent enterotoxin produced by biotypes of *S. aureus* associated with ruminants *(15, 16, 32)*. These results are very important in terms of food safety; although the SEs most commonly involved in SFP outbreaks are SEA and SED, SEC has often been identified as a major cause of SFP outbreaks associated with the consumption of dairy products made from raw milk *(31)*. The presence of MRSA in animal-sourced food is increasingly common, and some authors consider this *S. aureus* variant as a potential zoonotic foodborne pathogen *(11)* that can often cause

serious infections *(18, 19)*. The source of food contamination by MRSA can be due both to animals (ruminants with subclinical mastitis) and humans *(9, 20, 22)*. Although all the analyzed *S. aureus* strains in this study were methicillin susceptible, it is necessary to monitor raw materials and food processing workers for MRSA clones. In the present study, 11 of 15 *S. aureus* isolates were resistant to at least one (5 [33.3%] of 15), two (5 [33.3%] of 15), and three (1 [6.7%] of 15) of the antibiotics tested, while no strain was resistant to vancomycin and teicoplanin. The resistance to several antibiotics (such as ampicillin, tetracycline, erythromycin, and trimethoprim-sulfamethoxazole) observed in the enterotoxigenic *S. aureus* strains analyzed in this survey could pose the risk of transmission of this resistance to other microorganisms, and could reduce the therapeutic choices available in the case of human infection.

All the samples examined tested negative for *L. monocytogenes* and *Salmonella* spp. The absence of these microorganisms could indicate the good microbiological quality of the raw milk used to make Burrata cheese, or the reason could be the effects of the high temperatures used to pasteurize the milk and to stretch the curd. Furthermore, it is known that the contamination of dairy products by *L. monocytogenes* is frequently a postprocess contamination *(21, 29)*, which mainly occurs before packaging. The systematic absence of this microorganism in the examined samples of Burrata could indicate good environmental hygiene and good postprocess procedures.

In light of these results demonstrating the absence of foodborne pathogens, Burrata cheese can be considered microbiologically safe. However, some samples tested CPS positive, and this means that food manufacturers should improve processing hygiene via staff training, sanitation standard operating procedures, and thorough selection of raw material suppliers. Enterotoxigenic *S. aureus* is a potential hazard for consumers, but adherence to the correct refrigeration temperature (0 to 4°C) during storage, transport, and retail sale can significantly slow down microorganism replication and the consequent release of SEs in food. For this reason, it is also important to improve consumer awareness and knowledge about the correct storage of perishable foods in the home.

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