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Behavior of *Escherichia coli* O157:H7 during the manufacture and ripening of Fontina Protected Designation of Origin cheese

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ABSTRACT

This study was conducted to describe the cheese-making procedure of Fontina Protected Designation of Origin (PDO) cheese and to evaluate the behavior of *Escherichia coli* O157:H7 during cheese manufacture and ripening. The study was divided into 2 phases: the production of Fontina PDO cheese was monitored at 3 different dairies in the Aosta Valley and an *E. coli* O157 challenge was conducted at a fourth dairy. The dairies employ different commercial starter cultures for cheese making. The growth of lactic acid bacilli (LAB) and the decrease in pH were slower in the first hours and the LAB concentrations were overall higher in dairy A than in the other 2 dairies. The pH remained substantially unchanged during ripening (range 5.2 to 5.4) in all dairies. Water activity remained constant at around 0.98 until d 21, when it decreased to around 0.97 until d 80 in dairies A and B and 0.95 in dairy E. Whole raw cow milk was used for making Fontina cheese according to the standard procedure. For the experimental production, the milk was inoculated with *E. coli* O157:H7 at a concentration of approximately 5 $\log_{10}$ cfu/mL and commercial starter cultures were used according to the Fontina PDO regulation. An increase of 2.0 $\log_{10}$ cfu/g in *E. coli* O157:H7 was observed during the first 9.5 h of cheese making, followed by a decrease at 46 h when pH decreased to 5.4 in all trials. Fresh cheeses were salted and held at 10°C for ripening for 80 d. Water activity was decreased to 0.952 at the end of the ripening stage. The LAB concentrations declined gradually; this trend was more marked for the lactobacilli than either the thermophilic or the mesophilic lactococci. The increase in LAB count and the decrease in pH in the first hours did not seem to affect *E. coli* O157 growth. Ripening was found to inhibit pathogen survival, however, as seen in the reduction of 3 $\log_{10}$ from the maximum concentration measured during the earlier stages of production.

Key words: *Escherichia coli* O157, Fontina Protected Designation of Origin, cheese, cheese making, ripening

INTRODUCTION

Domestic animals for milk production are a known major reservoir of verotoxin-producing *Escherichia coli* (**VTEC**) and are often asymptomatic carriers (Caprioli et al., 2005). Contaminated raw milk and dairy products prepared from contaminated raw milk are a potential source of outbreaks caused by *Escherichia coli* O157:H7 (Honish et al., 2005; Baylis, 2009; Ravel et al., 2009), as also previously reported for Italy (Trevisani et al., 2014; Nobili et al., 2016). The VTEC can cause human infections ranging from uncomplicated diarrhea to such severe diseases as life-threatening hemolytic uremic syndrome and hemorrhagic colitis (Melton-Celsa et al., 2011). In susceptible individuals, the infective dose of VTEC is less than 10 bacteria; to ensure food safety, no VTEC must be present in the cheese (Callon et al., 2016). Behavior of VTEC depends on the temperatures during cheese manufacture and ripening according to the cheese-making method (Farrokh et al., 2013). For these reasons, researchers have examined the behavior of *E. coli* O157:H7 during the manufacture and aging of various cheese varieties such as feta, telemes, Grana Padano Protected Designation of Origin (**PDO**), Cheddar, Camembert, and Gouda cheese (Govaris et al., 2002; Ercolini et al., 2005; Schlesser et al., 2006; Montet et al., 2009; D'Amico et al., 2010). Furthermore, because many regional cheese specialties are made from unpasteurized milk, they may pose a threat to consumer safety by transmitting pathogens such as *E. coli* O157:H7 (Vernozy-Rozand et al., 2005). Contamination by VTEC is also an important microbiological cause of dairy product recalls in Europe (RASFF, 2017). In 2014, the European Food Safety Authority and the European Centre for Disease Prevention and Control reported that 3.6% of raw cow

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milk samples and 1.2% of milk (excluding raw cow milk) and dairy products tested positive for VTEC, with an increase in the proportion of VTEC-positive samples in the European Union over previous years (EFSA and ECDC, 2016).

No studies to date have investigated the behavior of *E. coli* O157 during the production of Fontina. Fontina is a PDO cheese with a semi-cooked paste made from whole raw cow milk collected from a single milking. Produced in the Aosta Valley in northwest Italy, it is obtained from cow milk from the indigenous valdostana (pezzatarossa, pezzatanera, and castana) breed, which has long been reared for milk production. The milk's characteristics derive from feeding with fodder essences typical of the alpine area; the cheese's characteristics reflect the qualities of raw milk. The cheese must be ripened for at least 80 d according to the Fontina PDO regulation.

Raw milk cheese production and commercialization is regulated in Europe under regulation no. 853/2004 (European Union, 2004) which lays down specific hygiene rules for food of animal origin including raw milk cheese. Generally, unsafe food shall not be placed on the European market (European Union, 2002) and microbiological criteria are defined for different kinds of food matrices. Moreover, national laws describe safety criteria related to specific food products: in Italy, VTEC are well regulated in cheese and must be absent in 25 g (Regione Piemonte, 2017).

Fontina was exported to European Union Member States (428 t), the United States (346 t), and Asia (18 t) in 2016 (CLAL, 2017). Some countries pose restrictions on the importation of cheeses made from unpasteurized milk. Food safety regulation requires the demonstration that the level of pathogenic microorganisms in the raw milk cheese does not exceed the level of these microorganisms in the milk from which the cheese was made. Also, it is mandatory that the raw milk cheese does not support the growth of pathogenic microorganisms (Ryser, 2011; Food Standards Australia New Zealand, 2015).

The objectives of this study were to describe Fontina PDO production and to determine the survival of *E. coli* O157:H7 during cheese manufacture and ripening.

MATERIALS AND METHODS

Study Design

The study was divided into a pilot and an experimental phase. The aim of pilot phase was to analyze the entire Fontina PDO production process at 3 dairies. A fourth dairy was selected for the experimental phase in which the microbial challenge with *E. coli* O157:H7 was performed. For the pilot phase, 3 dairies were selected from among the 20 major Fontina PDO producers in the Aosta Valley.

Temperature was monitored with a data logger (FT-800, Maxim Integrated Products, San Jose, CA) during the production process. Three production batches were monitored at each dairy. Milk, curd, and cheese were sampled to enumerate lactic acid bacilli (**LAB**) and measure pH and water activity ($\mathbf{A_w}$) at 0, 2, 2.5, 12, and 24 h and at 7, 14, 21, 56, and 80 d. Milk (100 mL) and curd (100 g) samples were collected with a sterile 25-mL serological pipette and a silver spoon, respectively. Each cheese sample (30 g) was obtained from 3 different wheel sites (10 g each) with a sterile silver corer and then pooled.

The aim of the experimental phase was to study the behavior of *E. coli* O157:H7 during cheese production in a fourth dairy. Three batches of Fontina PDO were made from milk spiked with 3 different *E. coli* O157 strains. For the experimental phase, on the selected dairy characteristics, following a repeated ANOVA design, 3 batches of contaminated *E. coli* O157:H7 samples were collected during the entire production process. The batches were uncontaminated and contaminated milk, curd and whey after cutting, whey and curd after cooking, and curd before pressing, after 6 h of pressing, and before and after salting and ripening (7, 14, 21, 28, 56, and 80 d). The following parameters were measured: LAB, pH, A_w, and *E. coli* O157 concentration.

Microbiological Analysis

From each sample collected as described above, 10 ± 0.5 g was homogenized with 90 mL of buffered peptone water (Oxoid, Milan, Italy) for 2 min in a stomacher (LAB Blender 400, PBI, Milan, Italy). Decimal dilutions in sterile peptone water (Oxoid) were prepared, and aliquots of the dilutions were spread on a specific medium according to ISO (2017). Thermophilic and mesophilic lactococci were enumerated with M17 medium (Oxoid) at 45°C for 48 h and at 30°C for 72 h, respectively. Thermophilic streptococci count was performed using De Man Rogosa and Sharpe agar (MRS, Oxoid) incubated at 45°C for 48 h (Bellio et al., 2016).

The pH was measured by immersing the probe of the pH meter (GLP 22, Crison, Barcelona, Spain) in milk/whey or in a diluted and homogenized sample containing 10 g of curd/cheese sample and 10 mL of distilled water (MFHPB, 2014). Water activity was measured with a calibrated electric hygrometer (Aqualab, Decagon Devices Inc., Pullman, WA) according to ISO (2004).