

# Determination of microbial contamination sources for use in quality management of cheese industry: “Dil” cheese as an example

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**Abstract** The microbiological quality, safety and shelf-life of cheeses depend on manufacture and handling in an environment that meets basic standards for hygiene and the management of hygiene in the process. In this research contamination sources of “Dil” cheese during production in a local dairy plant in Bursa, Turkey were determined. Eighteen different control points (raw milk, pasteurized milk, heated curd, molded cheese before kneading, kneaded cheese, brine solution for kneading, thermophilic culture, rennet, calcium chloride solution, brine solution for cheese, cheese vat, workers hands, production room air, production room floor, production room wall, packaging material and packaged cheese) have been examined for the enumeration of total aerobic mesophilic bacteria, *Staphylococci*, *Enterobacteriaceae*, *Salmonella* spp., *Escherichia coli*, lactic acid bacteria, *Pseudomonas* spp. and yeast-moulds. It was determined that viability of lactic acid bacteria in thermophilic culture was not in high numbers and some contaminations to “Dil” cheese were detected from the starter culture. Brine solutions and rennet were contaminated with *Staphylococci*. Yeast and moulds in production room air were the major sources of contamination. Pasteurization and kneading in hot brine solution can eliminate some of the microorganisms but that was not sufficient in the production of Dil cheese. Finish cheese should meet specific hygienic standards, with respect to

regulations post-contaminations to the cheese must be inhibited and a HACCP plan should be established during production.

**Keywords** Dil cheese · Production stages · Microbiological contaminations

## 1 Introduction

Food borne disease and food poisoning are becoming more and more common throughout the world. Both of these public health problems and the microbiological spoilage of foods can be minimized by the careful choice of raw materials, correct production, and storage stages. Especially undesirable microorganisms in milk and dairy products can be very harmful to human health and monitoring microbiological loads or to determine particular microbial types is requiring in dairy industry, occasionally (Mostert and Jooste 2002). The environment of plants, improperly cleaned plants and equipments, food handlers are the main sources of contaminations to the cheese. Consumption of contaminated cheese with pathogenic microorganisms and their toxins can be caused serious food borne problems to threat public health and big economic losses. But the most traditional cheeses are usually produced under poor hygienic conditions with different manufacturing technologies in local dairy plants (Temelli et al. 2006; Aygun et al. 2005).

Types of organisms in milk and cheese may be increased either by contamination or by growth of the microorganisms already present. Methods of production, handling and manufacture should be

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designed to prevent both. The most significant sources of contamination are known milk-contact surfaces and hands of dairy workers but the starter culture, rennet, calcium chloride, brine solution have also some effects on the quality of cheese (Robinson and Tamime 2002).

“Dil” cheese is a pasta filata, string texture semi-hard type cheese and it is consumed as a table cheese or used for pizza production like Mozzarella cheese. Dil cheese is a fresh variety and unripened cheese (Kamber 2005; Kilic and Isin 2004). Although thermal treatment of cheese curd affects the microbial quality of the product, some post-contaminations could be occur in the plants after the productions (Salmeron et al. 2002). During production of Dil cheese, the pasteurized or raw milk is coagulated with calf rennet, acidified using commercial starter culture of *Streptococcus thermophilus* and a thermophilic *Lactobacillus*; the coagulum is cut and the curds/whey mixture cooked to 41°C. The whey is drained off and the curds are held to allow acidification. When the curd pH reaches 5.1–5.3, the curds are heated, kneaded and stretched in hot water or dilute brine (78°C to a curd temperature) mechanically. Dil cheese is consumed as soon as possible after manufacture (Kamber 2005; Ucuncu 2004).

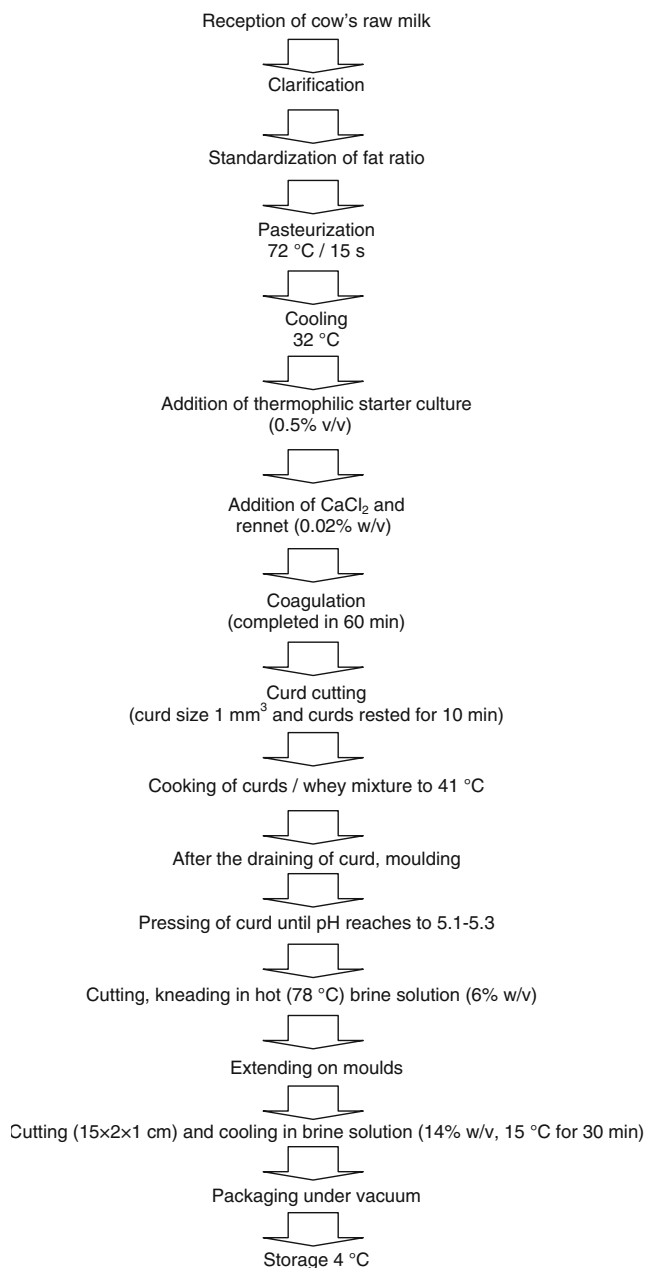
Inhibiting the growth of pathogens is possible to prevent contamination of the milk and cheese. A few cheese types are made in automated systems, but small-scale production involves manual manipulation of the curd during manufacture, molding and ripening. It is stated that good hygiene is critical at each of these steps (Fox et al. 2000). Detailed investigations have demonstrated that the sources of contamination were raw milk, inadequately pasteurized milk, or post-pasteurization contamination with organisms originally derived from raw milk or from processing environments and equipments. It was reported that there have been outbreaks of infection associated with the consumption of cheese, and the predominant organisms responsible included *Salmonella*, *Listeria monocytogenes*, verocytotoxin producing *Escherichia coli* (VTEC) and *Staphylococcus aureus* in the last years (Little et al. 2008; Carvalho et al. 2007).

The aim of this paper was to investigate hygiene conditions and possible contamination sources that cause limiting shelf-life of “Dil” cheese during production and to determine possible risk assessments where special attention or improved cleaning is needed.

## 2 Materials and methods

### 2.1 Sample collection

Samples were taken three times and performed in two parallels ( $n = 3 \times 2$ ) in different periods from a local dairy plant in Bursa, Turkey. A flow diagram of Dil cheese process is shown in Fig. 1. Samples were collected from the raw milk, pasteurized milk, curd, moulded cheese before kneading, kneaded cheese, brine solution for kneading, thermophilic culture,



**Fig. 1** Flow diagram for Dil cheese production

rennet, calcium chloride solution, brine solution for cheese, cheese vat and equipments, workers hands, production room air, floor and wall, packaging material and packaged cheese. All samples were transferred to the laboratory at 4°C and analyzed within 4 h after collection.

## 2.2 Preparation of samples

For microbiological analyses 200 g from solid and 200 ml liquid samples were taken into sterile laboratory bottles with screw caps. 10 cm<sup>2</sup> area from the environment and equipments has been swabbed by a pre-wetted swab in 0.1% (w/v) sterile peptone water and put into the same diluents water and transferred to the laboratory at 4°C (Unluturk and Turantas 2002). Samples from workers hands were taken according to Temelli et al. (2006) in a modified method: workers washed their hands with 20 ml 0.1% sterile peptone water into the separate sterile jars and jars were transferred to the laboratory at 4°C. All taken samples were diluted with 0.1% sterile peptone water and analyzed by using pour plate and spread plate methods. The media used and the incubation conditions for microbiological analyses are presented in Table 1. Environmental air was analyzed with specific agar plates without lids were stayed open in a place for 15 min during normal air circulation in plants (ISO 1986). All count data were written as logarithms (log cfu/g).

## 3 Results and discussion

All the average microbial counts of Dil cheese can be seen in Table 2. Total mesophilic bacteria and

*Enterobacteriaceae*, *E. coli* and *Staphylococci* counts in raw milk were determined as 7.8, 4.83, 3 and 3.2 log cfu/ml, respectively, and it is shown that the raw milk did not comply with the limits stated by Turkish Food Codex and pre-pasteurized Grade A milk standards in Europe (Barbano et al. 2006; Turkish Food Codex 2000). After the pasteurization total aerobic bacteria count was 3.18 log cfu/ml and the other microorganism counts were below the limit is due to the heat application process at 72°C for 15 s.

Average total mesophilic aerobic bacteria, lactic acid bacteria, yeasts and moulds counts in the cheese vat and vat equipments were determined as 4.86, 1.11 and 2.36 log cfu/cm<sup>2</sup>, respectively, pathogens were not detected in the vat. These conditions stated that cleaning and disinfection of the cheese vats were efficient. Especially stainless-steel vats in dairy plants together with using sodium hypochlorite solution and the steam sterilization can be effective to maintaining hygienic conditions. In contrast Temelli et al. (2006) found very high microbial populations in Turkish white cheese vats and equipments in their study.

*Enterobacter* is a typical opportunistic pathogen that cause disease. Since the 1980s *Enterobacter* has been documented as being an important source of nosocomial infections, e. g., urinary tract infections, endocarditis, sepsis and wound infections (Yazici et al. 2004). In this study *Enterobacteriaceae* counts were determined as 2.3 and 1.23 log kob/ml for the brine solution and Dil cheese. Thermophilic culture of cheese, workers hands and room floors were harbor of the *Enterobacteriaceae* in the dairy plant. Ortigosa et al. (2008) found 5 and 3.2 log kob/ml for brine solution and one day old cheese from the farm cheese making plants in Spain, respectively. In the

**Table 1** Media and incubation conditions used in the microbial analyses

Microorganism	Media	Incubation Temperature °C	Incubation Time (hours or days)	Method
Total aerobic mesophilic bacteria	Plate count agar	31	72 h	Gonzales-Fandos et al. (2000)
<i>Enterobacteriaceae</i> count	Violet red bile dextrose (VRBD) agar	37	24 h	Govaris et al. (2007)
Lactic acid bacteria count	Man Rogosa Sharpe agar	30	72 h	Whitley et al. (2000)
Yeasts and moulds counts	Yeast extract glucose chloramphenicol agar	25	5–7 days	Gonzales-Fandos et al. (2000)
<i>S. aureus</i>	Baird-Parker agar	37	48 h	The Oxoid Manual (1998)
<i>E. coli</i>	Eosin methylene blue agar	37	24 h	The Oxoid Manual (1998)
<i>Salmonella</i> spp.	Xylose lysine deoxycholate agar	35	24 h	BAM Manual (1999)
<i>Pseudomonas</i> spp.	<i>Pseudomonas</i> CFC selective agar	25	44 h	ISO/WD 13720 (2000)

**Table 2** Results of the microbiological analyses of the samples ( $n = 2 \times 3$ ) collected from "Dil" cheese production stages

Samples	Total mesophilic bacteria	<i>Enterobacteriaceae</i>	<i>Pseudomonas</i> spp.	Lactic acid bacteria	<i>E. coli</i>	<i>S. aureus</i>	<i>Salmonella</i> spp.	Yeasts and moulds
Raw milk	7.8 ± 1.4 <sup>a</sup>	4.83 ± 0.9	4.86 ± 0.3	5.83 ± 2.06	3 ± 3.2	3.2 ± 1.8	2.5 ± 1.7	6.53 ± 0.49
Milk after the pasteurization	3.18 ± 2.0	0.59 ± 0.4	0	0	0	0.23 ± 0.6	0	0
Cheese curd (41°C)	3.35 ± 1.1	1.49 ± 0.5	0	3.61 ± 1.7	0	1.3 ± 0.7	0	2.54 ± 1.23
Cheese after the pressing	4.87 ± 1.2	1.89 ± 0.7	1.6 ± 2.1	4.2 ± 1.8	2.7 ± 1.2	2.19 ± 1.2	0.5 ± 2.1	4.26 ± 1.0
Kneaded cheese	3.55 ± 1.0	1.39 ± 0.8	2 ± 3.4	2.72 ± 0.9	0.69 ± 2.7	1 ± 0.9	0.3 ± 1.1	3.39 ± 0.8
Brine solution for kneading	5.94 ± 0.9	0	0	0	3.5 ± 2.1	1.84 ± 1.2	0	0
Brine solution	6.06 ± 0.6	2.3 ± 1.3	0	5.04 ± 1.2	3.1 ± 0.8	3.23 ± 2.6	1.4 ± 1.3	4.82 ± 1.9
Thermophilic culture	7.27 ± 0.2	3.4 ± 1.2	2.6 ± 1.8	5.88 ± 2.7	1.7 ± 0.5	2.73 ± 1.5	0.8 ± 1.7	4.73 ± 0.6
Rennet	4.2 ± 2.9	1.9 ± 2.3	0	2.56 ± 1.3	0	2.43 ± 2.4	0.2 ± 1.9	0
CaCl <sub>2</sub>	3.53 ± 0.5	1.14 ± 2.8	1.2 ± 1.7	<1	<1	<1	<1	2.34 ± 0.7
Packaging material	1.32 ± 1.1	<1	<1	<1	<1	<1	<1	1.11 ± 0.8
Worker hand 1	6.3 ± 1.0	2.8 ± 1.7	1.2 ± 1.9	4.25 ± 1.9	0	3.4 ± 0.7	0	4.73 ± 0.3
Worker hand 2	5.75 ± 1.2	1.63 ± 1.3	1.53 ± 0.8	2.34 ± 1.4	0	3.81 ± 2.3	0	3.55 ± 1.5
Room floor	4.92 ± 0.4	2.34 ± 2.1	3.25 ± 0.3	2.38 ± 2.4	2.84 ± 0.6	1.2 ± 1.8	0	4.59 ± 1.6
Room wall	2.71 ± 0.8	0	0	0	0	2.47 ± 2.1	0	3.2 ± 1.8
Room air	2.25 ± 1.7	0	0	0	0	0	0	5.11 ± 0.5
Cheese vat and equipments	4.86 ± 1.1	0	0	1.11 ± 0.7	0	0	0	2.36 ± 1.1
Vacuum packaged Dil cheese	5.98 ± 1.7	1.23 ± 2.5	0.56 ± 1.9	2.86 ± 0.4	2.64 ± 0.8	2.28 ± 0.7	0	4.47 ± 0.3

<sup>a</sup> Mean of log. Microbial count ± SD

study of Salo et al. (2006), from the three Estonian dairies shown that food contact surfaces were mostly clean from *Enterobacteria* but non-contact surfaces in food processing were in most cases contaminated with *Enterobacteria*.

*Pseudomonas* spp. indicates post-manufacturing contaminations in the plants. They were easily destroyed by pasteurization and cooking temperatures but generally contaminations can be occurred after the productions (Farkye and Vedamuthu 2002). In the Dil cheese plant room floor and thermophilic culture were the mostly contaminated steps in the Dil cheese process.

*E. coli* in cheese is frequently used as indicators of hygienic quality and shows lack of microbiological safety. *E. coli* can be embedded in the organic matrix of the biofilm and cause hygiene problems, if the biofilm formed is not removed in the cleaning procedure. *E. coli* can also survive for extended periods in several acidic foods, e. g. cheese and yogurt. Acid-adapted *E. coli* O157:H7 has shown enhanced survival and prevalence in biofilms on stainless-steel

surfaces (Salo et al. 2006). Mucchetti et al. (2008) determined  $3.3 \times 10^3$  cfu/g of *E. coli* in Vasteda cheese due to the post-contaminations in the dairy plants. Aygun et al. (2005) found  $4.27 \times 10^3$  cfu/g of Carra Turkish cheese and for improving the microbiological quality of Carra cheese, the processing, ripening and storage should be carried out under good hygienic conditions in the production. Tekinsen and Ozdemir (2006) considered 2.4, 3.4, and 3.82 log cfu/g *E. coli* in Turkish White, Savak, Tulum cheeses respectively and they also determined 3.68 log cfu/g *E. coli* in Van Otlu cheese samples.

*S. aureus* is recognized worldwide as the most important pathogen causing intramammary infections in dairy cows. *S. aureus* is an important cause of morbidity and mortality in humans. Recently, the increasing prevalence of methicillin-resistant *S. aureus* (MRSA) strains has become an additional infection control problem in human and veterinary medicine. Cheese and other industrialized dairy products can be sources of food poisoning *S. aureus* from infected udders may contaminate bulk milk and subsequently,

milk products. It was stated that some authors found high percentages of *S. aureus* in white cheese samples around the world. *S. aureus* were responsible for 59% of the 177 outbreaks implicating milk and milk products in France over a 10-year period. In another study in the Bologna area, *S. aureus* was present in 16.3% of the cheese samples manufactured from pasteurized milk, with a density ranging from  $10^1$  to  $3.1 \times 10^5$  cfu/g. Minas Frescal cheese is widely consumed by the Brazilian and the study about this cheese shows that *S. aureus* isolates from the cheese were the same as isolated from the raw milk (Andre et al. 2008; Charlier et al. 2009). Carvalho et al. (2007) have not detected any coagulase positive *S. aureus* in Minas Frescal cheese process and they concluded that due to its closed system process with no handling the product after pasteurization. In this study Dil cheese was also contaminated with *S. aureus* and it isn't comparable to Turkish cheese standards. Post-contaminations are seen in this plant and brine solution, workers hands and thermophilic cheese cultures were contaminated with this pathogen seriously.

Generally cultures for Mozzarella type cheese are combinations of *S. thermophilus* and either *Lb. delbrueckii* subsp. *bulgaricus* or *Lb. helveticus*. The starter culture contributes to functional properties related to this use such as stretchability and heat-induced browning. Frozen concentrated starter cultures should be contain  $10^{10}$ – $10^{11}$  cfu/g a sufficient activity for bulk culture preparation (Hassan and Frank 2001). Starter culture of Dil cheese have contained 5.88 log cfu/ml lactic acid bacteria. After the pressing the cheese has contained 4.2 log cfu/g lactic acid bacteria and then their amount has decreased to 2.72 cfu/g in hot brine kneaded Dil cheese. Nevertheless, all the other bacterial and pathogen counts were very high in the starter culture. This case is probably due to the lack of a hygienic starter culture preparation room and preparations of cultures by non-expert personnel in the plant. Temelli et al. (2006) determined high microbial counts of total aerobic mesophilic bacteria, coliforms, *Enterobacteriaceae*, *Staphylococci*, psychrophilic bacteria and yeasts and moulds in Turkish white cheese starter culture also. These microbial contaminations in the culture can cause inactivity of the lactic acid bacteria and stop the inhibitory conditions for the pathogens in the cheese. Purity and activity of the starter cultures are highly important and application of hazard analysis for the critical control points (HACCP) is normally adopted during the production of these cultures (Tamime 2002).

*Salmonella* was not detected in the Dil cheese samples. But mainly the brine solution was the carrier of the *Salmonella* and from the brine solution post-contaminations to cheese is possible. After the packaging of cheese samples *Salmonella* has been eliminated and there was not any risk about it. Tekinsen and Ozdemir (2006) found *Salmonella* in 6% (3/50) of Van otlu cheese samples. In the study of Colak et al. (2007) *Salmonella* spp. was detected in 4.8% of 250 samples of Tulum cheeses sold in various markets of Istanbul. Carvalho et al. (2007), Aygun et al. (2005), Tamagnini et al. (2006), Mucchetti et al. (2008) could not detect any *Salmonella* in the Minas Frescal Brazilian soft and fresh cheese (Turkish traditional Carra cheese, Argentinean soft goat cheese, Vastedda Sicilian pasta filata sheep cheese samples). The Turkish microbiological standards for the cheese at retail point permit a maximum count of *S. aureus*  $10^2$  cfu/g, *E. coli* 2 MPN/g and not the presence of *Salmonella* in 25 g of sample (Anonymous 2001).

Yeast and moulds can grow easily on Dil cheese because of the moisture content ( $\sim 50\%$  w/w) and the low salt content ( $\sim 1.8\%$  w/w) of Dil cheese (Kamber 2005). Post-contaminations of yeast and moulds cause big problems in the production process. In this research thermophilic culture and room air heavily contaminated with yeast and moulds after the raw milk and Dil cheese contains high counts of yeast and moulds with 4.47 log cfu/g. Aygun et al. (2005) found  $4.8 \times 10^7$  cfu/g yeasts and moulds in Carra Turkish cheese, Temelli et al. (2006) determined 4.15 log cfu/g in Turkish white cheese, Tamagnini et al. (2006) observed higher yeast counts with 5.27 log cfu/g (in summer) and 6.34 log cfu/g (in winter) of Crottin goat's cheese. Mucchetti et al. (2008) determined lower counts ( $3.5 \times 10^4$ ) of yeasts and moulds in pasta filata sheep cheese "Vastedda" from the Dil cheese samples. Kure et al. (2008) stated that mould contamination is periodically a problem in production of semi-hard cheese and to avoid problems with mould, it is important to monitor the hygienic quality of the air regularly and follow up with corrective actions to prevent contamination.

#### 4 Conclusion

It was concluded that to obtained application of improved hygienic conditions in collecting raw milk, pasteurization of brine solution after collecting up the cheeses, preparation of starter culture with properly complying hygienic conditions, training personnel and routine cleaning and disinfection of

worker's hands, equipments, production room and also a filtration system for production room's air would be helpful to eliminate contaminations for this or similar cheese processes in industry.

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