Hygienic and sensory quality factors affecting the shelf-life of *Fruhe* (*Casu axedu*) traditional Sardinian fresh cheese

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Abstract

A study was conducted to evaluate the durability of the traditional fresh soft cheese *Fruhe* manufactured in Sardinia either from goats' or sheep's milk. Four farmstead cheese-making plants were visited three times during the *Fruhe* cheese-making season. During each visit environmental samples were collected from food contact and non-food contact surfaces in order to evaluate the presence of *Enterobacteriaceae*, *Escherichia coli*, *Pseudomonas* spp. and *Listeria* spp. In a total of 60 environmental samples, *Escherichia coli* and *Listeria* spp. were never detected, while contamination with *Enterobacteriaceae* and *Pseudomonas* spp. was observed respectively in 48% and 43% of samples. The microbiological profile of 48 *Fruhe* cheese samples was assessed at different time points during the product shelf-life. Aerobic mesophilic bacteria, *Enterobacteriaceae*, *E. coli*, *Pseudomonas* spp., *Bacillus cereus* and *Listeria monocytogenes* were investigated at 0, 7, 14 and 21 days after production. *E. coli*, *L. monocytogenes* and *B. cereus* were never detected in the product. *Enterobacteriaceae* contamination was observed, showing decreasing levels over time. *Pseudomonas* spp. was recovered in only two *Fruhe* samples (3.3%) at day 0. Sensory analysis was also conducted using a triangle test to determine whether a difference between *Fruhe* samples at 14 and 21 days of shelf-life exists. Based on the evolution of the microbiological profile and the sensory attributes observed in the present study, it is reasonable to assume that the product shelf-life can be feasibly extended up to 21 days.

Introduction

The terms *Fruhe* and *Casu axedu* are used to refer to a traditional soft fresh cheese manufactured in Sardinia (Italy) from pure cows', sheep's, goats' milk or from a mixtures of these. It is also known with many other synonyms according to the different areas of production in the regional territory. The name *Merca* indicates the cheese aged in brine. The production of *Fruhe* is typically conducted at artisanal level or in farmstead cheese-making facilities. Although *Fruhe* manufacturing process may vary from producer to producer, it is usually obtained from whole raw or heat-treated milk. Lactic acid bacteria (LAB) are added with natural or commercial starter culture and the milk is coagulated with liquid calf or kid rennet or lamb paste rennet. Clotting occurs into plastic containers within 15-30 min, while hardening of the curd takes up to 4-5 h (LAORE Sardegna, 2013). In order to aid the syneresis, the curd is then cut into slices and the excess of whey discarded. After 24 h ripening, the containers are sealed with a plastic film and the *Fruhe* is stored at refrigeration temperature. The final product is a fresh compact coagulum with sour taste and with the typical flavour of the milk of the species of origin. It is presented in rectangular blocks of variable size and weight (about 500 g), immersed in the residual whey. The shelf-life is defined under the responsibility of the food business operator and varies from 10 days up to 2 weeks under refrigerated storing conditions.

Regulation (EC) No 852/2004 (European Commission, 2004) on the hygiene of foodstuffs states that the primary responsibility for food safety rests with the food business operators (FBOs), which are legally responsible for the determination of the date of minimum durability of the foodstuffs they place on the market. FBOs are also responsible for the compliance of the foodstuffs with microbiological criteria defined by Regulation (EC) No 2073/2005 (European Commission, 2005). Contamination of the environment where food is prepared is recognised as an important transmission route of microorganism in ready-to-eat foods (Health Protection Agency, 2009). Testing the food environment to monitor the presence of spoilage and pathogen microorganisms is an essential strategy to verify whether good hygienic practices (GHP) are correctly applied (Tompkin *et al*., 1999). Indicator microorganisms, such as *Enterobacteriaceae* and *Escherichia coli,* are frequently used as a measure of the effectiveness of sanitation in a food processing environment and they can also be used to assess post-process contamination in ready-to-eat foods (Jay, 1992; Kornacki, 2001; Tompkin, 2004). Indicator microorganisms are sensitive to the action of sanitiser, so they can be adequately removed from the environment. Therefore, their presence is mainly due to a reintroduction in the processing environment and they can be referred to as transient microflora (Tompkin, 2004). Unlike transient microflora, other microrgansims such as *Listeria monocytogenes* and *Pseudomonas* spp. can persist over time established in niches in the food processing environment behaving as resident microflora and serving as a potential source for post-process contamination (ICMS, 2002; Tompkin, 2004). Microbiological characteristics of the product, with particular regard to pathogenic and spoilage bacteria, are essential in order to determine the shelf-life of foods based upon valid scientific evidence. The terms *best before date* or *expiration date* are used to define product shelf-life taking into account the deterioration of organoleptic properties of the food. Therefore, when defining the shelf-life of their products, FBOs should conduct, in addition to microbiological testing, also sensory analysis evaluations.

Little research has been conducted to define the hygienic and organoleptic quality of *Fruhe* cheese produced in Sardinia (Murgia *et al*., 2009). The aim of the present study was to obtain valuable information to be used in the determination of the shelf-life of farmstead *Fruhe* cheese. Therefore, the assessment of environmental hygienic and manufacturing condition of four Sardinian farmstead cheese-making plants was conducted. Determination of microbiological profile and sensory charac-

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teristics of *Fruhe* cheese during shelf-life was also conducted under the foreseeable distribution, storage and use conditions.

Materials and Methods

The survey was conducted during the cheese-making season 2012 enrolling four (A-D) farmstead artisanal cheese-making facilities manufacturing *Fruhe* cheese. The farms were selected among those fulfilling the following criteria: willingness to participate the survey, artisanal production, similar manufacturing process, same packaging and product size, distribution of products covering the whole regional territory. Although in Sardinia small ruminants milk production goes from November/December to June/July, *Fruhe* production goes from February to July only. In order to take into account variation during the cheese-making season, the facilities were visited three times at 2-month-intervals (March, May and July). *Fruhe* was manufactured in one plant from goat's milk (A), one plant from sheep's milk (B) and two plants from a mixture of ovine and caprine milk (C and D).

Environmental sampling

During each visit, environmental samples were collected from processing areas and equipment. In order to reflect working condition hygiene, samples collection was performed during production activities. Environmental sampling included food contact surfaces (jars used to pour milk into plastic containers) and non-food contact surfaces (floors, floor drains and shelves) from the cheese-making and warm rooms. Environmental sampling was conducted using sterile sponges pre-moistened with Buffered Peptone Water (3M; St. Paul, MI, USA). Samples were stored at refrigeration temperatures until analysis, which were performed within 24 h after collection. As a general indication of food-processing hygiene condition, the detection of indicator microorganisms such as *Enterobacteriaceae* (ISO 21528-1:2004; ISO, 2004a) and *E. coli* (ISO 16649-1:2001; ISO, 2001) was conducted. The presence or absence of potential resident microflora such as *Pseudomonas* spp. (ISO/TS 11059:2009; ISO, 2009) and *Listeria* spp. (UNI EN ISO 11290-1:2005; ISO, 2005) was also determined.

Presumptive *Pseudomonas* spp. positive samples were confirmed by molecular identification. After DNA extraction (Cattoir *et al*., 2010), two different polymerase chain reaction (PCR) protocols were used to identify *Pseudomonas fluorescens* (Scarpellini *et al*., 2004), *Pseudomonas* spp. and *Pseudomonas aeruginosa* (De Vos *et al*., 1997).

Durability study

Fruhe cheese samples manufactured during each visit were collected the day after they were sealed. The production batches were identified as batch 1, 2 and 3, indicating the cheese produced during the first, the second and the third visit conducted at each farm, respectively. *Fruhe* cheese samples were transported under refrigeration conditions to the laboratory. Samples were analysed at the following sampling times: T0 (the day of sampling), T7, T14 and T21 (respectively 7, 14 and 21 days after production). According to the foreseen storage condition of storage during shelf-life, the samples were kept refrigerated (4±2°C) until analysis. For each sample, microbiological and intrinsic properties analysis such as pH and water activity (a_W) were conducted. Microbiological analysis were conducted according to international standard methods and included the following parameters: aerobic mesophilic bacteria (ISO 4833; ISO, 2003), *Enterobacteriaceae* (ISO 21528-1; ISO, 2004a), *E. coli* (ISO 16649-2:2001; ISO, 2001), *Listeria monocytogenes* (ISO 11290-1/2; ISO 1996, 1998), *Pseudomonas* spp. (ISO/TS 11059:2009; ISO, 2009), and *Bacillus cereus* (ISO 7932; ISO, 2004b). Cheese pH and a_W were measured using pH meter GLP22 (Crison Instruments, Barcelona, Spain) and water activity meter Aqualab 4TE (Decagon, Pullman, WA, USA), respectively.

Sensory evaluation

During each visit additional *Fruhe* samples were collected for sensory analysis, which was performed at the LAORE laboratory. A panel of 30 assessors familiar with basic sensory evaluation techniques was recruited. Sensory properties of *Fruhe* cheese of the same batch were compared at 14 and 21 days of shelf-life by triangle test (UNI U590A2520:2001; UNI, 2001). Sensory analysis was conducted in 12 separate sessions, one for each of the 4 farms and of the 3 sampling times. Prior to the evaluation, *Fruhe* samples were cut into pieces of about 70 g, identified by a three digit code, allowed to reach room temperature (20°C) and randomly distributed to panelists. The forced-choice procedure was used, in which panelists were asked to identify the odd sample (which one of the three samples was perceived to be different as compared to the other two), choosing =0.05, =0.10 and Pd=40% the number of corrected responses to determine a significant difference between samples was 15.

Results

Environmental contamination

From the environment of the 4 farmstead cheese making plants 48 samples from non-food contact surfaces and 12 from food contact surfaces were collected. *Listeria monocytogenes* and *Escherichia coli* were never detected. *Enterobacteriaceae* were recovered in 9 out of 12 (75.0%) floor samples collected from the cheesemaking room and in 6 out of 12 (50.0%) floor samples in the warm room. Floor drains showed *Enterobacteriaceae* contamination in 7 out of 12 samples (58.3%). *Enterobacteriaceae* were constantly recovered from jars in two farms (D and A), while they were never detected in the other two (B and C). A sporadic contamination of the shelves was detected.

Pseudomonas spp. were recovered in 5 out of 12 (41.7%) floor samples collected from the cheesemaking room and in 7 out of 12 samples (58.3%) from the warm room. Floor drains showed *Pseudomonas* spp. contamination in 5 out of 12 samples (41.7%). *Pseudomonas* spp. contamination was observed in 4 out of 12 shelf samples (30.0%) and in 5 out of 12 (41.7%) samples food contact surfaces. Molecular identification showed that 40 strains isolated from environmental surfaces were confirmed to belong to the genus *Pseudomonas*, 17 of which (42.5%) were identified as *Pseudomonas fluorescens*. Detailed results of environmental contamination for each target microorganism by farm and sampling site over time are reported in Table 1.

Microbiological profile and intrinsic properties

A total of 48 *Fruhe* cheese samples were analysed to assess the presence of background and pathogen microorganisms and for the determination of pH and a_W. In all analysed samples *Listeria monocytogenes*, *Escherichia coli* and *Bacillus cereus* were always below the detection limit of the methods. Mean aerobic mesophilic bacteria counts [$\log_{10}$ colony forming units (CFU)/g±standard deviation (SD)] ranged between 9.44±0.85 at T0 and 8.43±0.90 at T21, showing significant differences ($P<0.05$) only in farms B and C (Table 2). *Enterobacteriaceae* were constantly recovered from *Fruhe* samples collected from farm A and showed a significant decrease ($P<0.05$) from T0 to T21 (Table 2). They were never detected in *Fruhe* samples collected from farm C, while contamination occurred in 8 out of 12 samples (66.7%) and in 4 out of 12 samples (33.3%) in farm D and B, respectively. *Pseudomonas* spp.

was recovered from two *Fruhe* samples (one from farm A and 1 from farm D) both samples at T0. Table 3 reports the evolution of pH and a_W of *Fruhe* samples during shelf-life.

Sensory analysis

Overall, 12 sensory analysis sessions with 30 panelists each were conducted. No significant difference was observed by panelist between *Fruhe* at 14 and 21 days of shelf-life produced during visit one (in March), while *Fruhe* samples produced during visit two (in May) showed significant differences in all farms. Triangle test conducted on samples collected during visit three (July) showed significant differences only for farm B, where 17 out of 30 panelists recognised the odd sample. Triangle test results for each session and by farm are reported in Table 4.

Discussion

Indicator microorganisms are generally recognised as a good measure of hygienic conditions in food processing environment. Testing for *Enterobacteriaceae* family, instead of coliforms, provides more accurate information on the correct application of GHP in food processing plants. *Enterobacteriaceae* are sen-

Table 1. Detection of microflora from environmental samples collected from 4 farmstead cheese-making plants.

Parameters	Farmstead cheese-making plant	Cheesmaking room						Warm room						Equipment		
		Floor			Floor drain			Floor			Shelves			Jars		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Enterobacteriaceae	A	+	+	+	+	+	-	+	+	-	-	-	+	+	+	+
	B	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-
	C	+	+	+	+	+	-	+	+	-	-	-	-	-	-	-
	D	+	+	-	+	+	-	-	+	-	-	-	-	+	+	+
Pseudomonas spp.	A	+	-	-	+*	-	-	-	-	+	+	+	-	-	+	-
	B	+	-	-	+*	-	-	+*	-	+	-	-	-	+*	+	-
	C	-	-	+*	-	+	+	+*	+	-	+*	-	-	-	+	-
	D	+*	-	+	+	-	-	+	+*	-	+	-	-	+*	-	-

1, Samples collected at visit 1; 2, samples collected at visit 2; 3, samples collected at visit 3; +, presence of the target microorganism was observed; -, presence of the target microorganism was not observed. **Pseudomonas fluorescens.*

Table 2. Microbiological profile (log_{10} cfu g^{-1};mean±standard deviation) of *Fruhe* cheese manufactured in 4 farmstead cheese-making plants during shelf-life.

Parameters	Farmstead cheese-making plant	T0	T7	T14	T21
Aerobic mesophilic bacteria	A	9.78±1.40^{a} (n=3/3)	9.13±0.22^{a} (n=3/3)	8.96±0.45^{a} (n=3/3)	8.17±0.94^{a} (n=3/3)
	B	9.25±0.22^{a} (n=3/3)	9.29±0.229^{a} (n=3/3)	8.93±0.63^{ab} (n=3/3)	8.14±0.89^{b} (n=3/3)
	C	9.00±0.27^{a} (n=3/3)	8.81±0.45^{a} (n=3/3)	7.86±0.33^{b} (n=3/3)	7.89±0.43^{b} (n=3/3)
	D	9.70±1.13^{a} (n=3/3)	9.55±0.36^{a} (n=3/3)	9.58±0.23^{a} (n=3/3)	9.52±0.31^{a} (n=3/3)
Enterobacteriaceae	A	5.13±0.86^{a} (n=3/3)	4.26±0.33^{ab} (n=3/3)	3.94±0.29^{b} (n=3/3)	2.66±0.25^{c} (n=2/3)
	B	3.52±0.00 (n=1/3)	3.43±0.00 (n=1/3)	3.01±0.00 (n=1/3)	2.99±0.00 (n=1/3)
	C	0.0±0.00 (n=0/3)	0.0±0.00 (n=0/3)	0.0±0.00 (n=0/3)	0.0±0.00 (n=0/3)
	D	4.73±0.09^{a} (n=2/3)	4.16±0.06^{ab} (n=2/3)	3.33±0.56^{b} (n=2/3)	3.34±0.00^{b} (n=1/3)
Pseudomonas spp.	A	4.36±0.00 (n=1/3)	0.0±0.00 (n=0/3)	0.0±0.00 (n=0/3)	0.0±0.00 (n=0/3)
	B	0.0±0.00 (n=0/3)	0.0±0.00 (n=0/3)	0.0±0.00 (n=0/3)	0.0±0.00 (n=0/3)
	C	0.0±0.00 (n=0/3)	0.0±0.00 (n=0/3)	0.0±0.00 (n=0/3)	0.0±0.00 (n=0/3)
	D	4.51±0.00 (n=1/3)	0.0±0.00 (n=0/3)	0.0±0.00 (n=0/3)	0.0±0.00 (n=0/3)

The sampling times T0, T7, T14 and T21 refer to the days (0, 7, 14, and 21, respectively) elapsed during the shelf-life. Means in the same row with different superscript letters are significantly different ($P<0.05$).

Table 3. Evolution of pH and a_W (mean±standard deviation) of *Fruhe* cheese manufactured in 4 farmstead cheese-making plants during shelf-life.

Farmstead cheese-making plant	pH				a_W			
	T0	T7	T14	T21	T0	T7	T14	T21
A	4.45±0.06^{a}	4.42±0.10^{a}	4.39±0.10^{a}	4.38±0.06^{a}	0.986±0.01^{a}	0.993±0.01^{a}	0.996±0.00^{a}	0.994±0.00^{a}
B	4.44±0.08^{a}	4.50±0.18^{a}	4.47±0.16^{a}	4.41±0.04^{a}	0.987±0.02^{a}	0.993±0.00^{a}	0.993±0.00^{a}	0.994±0.00^{a}
C	4.40±0.08^{a}	4.44±0.15^{a}	4.44±0.14^{a}	4.45±0.14^{a}	0.988±0.00^{a}	0.994±0.00^{a}	0.993±0.00^{a}	0.988±0.00^{a}
D	4.52±0.05^{a}	4.56±0.22^{a}	4.48±0.16^{a}	4.45±0.10^{a}	0.983±0.01^{a}	0.995±0.00^{b}	0.994±0.00^{b}	0.987±0.01^{ab}

a_w, water activity. The sampling times T0, T7, T14 and T21 refer to the days (0, 7, 14, and 21, respectively) elapsed during the shelf-life. Means in the same row with different superscript letters are significantly different ($P<0.05$).

Table 4. Triangle test of *Fruhe* cheese at 14 and 21 days of shelf-life in three production batches by 30 panelists.

Farmstead cheese-making plant	Batch*	Samples correctly detected		
		Correct answer	%	Significance
A	1	10/30	33.3	NS
B	1	9/30	30.0	NS
C	1	10/30	33.3	NS
D	1	12/30	40.0	NS
A	2	17/30	56.7	P<0.05
B	2	15/30	50.0	P<0.05
C	2	16/30	53.3	P<0.05
D	2	18/30	60.0	P<0.05
A	3	9/30	30.0	NS
B	3	13/30	43.3	NS
C	3	17/30	56.7	P<0.05
D	3	12/30	40.0	NS

NS, not significant. *Batch 1, 2 and 3 correspond to *Fruhe* production carried out during visit 1 (March), visit 2 (May) and visit 3 (July), respectively.

sitive to sanitiser and heat treatment, therefore they can be effectively used either as indicators of hygiene or of post-heat processing contamination. In the present study, post-operational surfaces showed *Enterobacteriaceae* contamination to some extent, which is to be expected. For cheese made from milk that has undergone a heat treatment, *Enterobacteriaceae* are not included in the process hygiene criteria laid down by Regulation (EC) No. 2073/2005 (Euroepan Commission, 2005). However, their frequent recovery in the final product (3 to 5 $\log_{10}$ cfu/g) could indicate a failure in the hygienic preparation and handling of *Fruhe* or that underprocessing occurred, *e.g.* inadequate pasteurization (FSNZ, 2001). In the two farms where *Enterobacteriaceae* were constantly recovered from *Fruhe* cheese samples, a significant decrease of the contamination level was observed over time. It may be explained with the low pH (4.45±0.11) of the product which can contribute to the inactivation of these microorganisms.

The Regulation (EC) 2073/2005 (Euroepan Commission, 2005) on microbiological criteria for foodstuffs includes another indicator microorganism, *E. coli*, as process hygiene criteria for cheese. *E coli* was never detected in *Fruhe* samples, indicating compliance of the product with this specific hygiene criteria either at the beginning or at the end of the shelf-life. Another important aspect of environmental monitoring in food processing plants is the detection of pathogen microorganisms such as *L. monocytogenes*. It is well established the ability of *Listeria monocytogenes* to persist in food processing environment (Carpentier and Cerf, 2011) representing a potential source of food post-process contamination (Tompkin, 1999). In the frame of the EC Regulation 2073/2005 (Euroepan Commission, 2005), sampling programmes to detect the presence of *L. monocytogenes* should be conducted on processing areas and equipment.

In the present study *L. monocytogenes* nor listeria-like organisms were detected on food contact and non-food contact surfaces. This indicates that *Fruhe* cheese farmstead production seems to be associated with a low risk of *Listeria* contamination. In the determination of a food shelf-life it is essential to consider whether the product supports or not the growth of *L. monocytogenes*. Ready-to-eat products with pH≤4.4 and a_W≤ 0.92 or pH≤5.0 and a_W≤0.94, and products with shelf-life of less than five days are considered unable to support the growth of *L. monocytogenes*. *Fruhe* cheese is characterised by pH ranging from 4.3 to 4.8 and a_W ranging from 0.983 to 0.996 and shelf-life of about two weeks. Therefore, FBOs should demonstrate the compliance with absence in 25 g criteria before the food has left its immediate control and with the limit of 100 CFU/g during the entire shelf-life. *Listeria monocytogenes* was never detected at each of the sampling time showing compliance with the most restrictive criteria during *Fruhe* shelf-life. However, more information should be provided on the behaviour of *L. monocytogenes* artificially inoculated in *Fruhe* cheese samples. Sensory evaluation showed differences between *Fruhe* cheese samples at 14 and 21 days of shelf-life mostly limited to the production carried out in May and with a number of correct responses observed really close to the expected number (15) of corrected responses required to determine a significant difference between the samples. Overall, the sensory evaluation indicates that none or limited differences exist between the product at 14 and 21 days of shelf-life.

Conclusions

Farmstead *Fruhe* cheese production is characterised by a wide operational and post-operational environmental contamination with *Enterobacteriaceae* indicating the need for good hygiene practice improvement. On the other hand, the absence of pathogenic bacteria such as *Listeria monocytogenes*, in combination with the sensory analysis, supports a possible extension of *Fruhe* shelf-life up to three weeks.

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