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DEVELOPMENT OF A HACCP PLAN FOR *Listeria monocytogenes* HAZARD ASSOCIATED WITH RAW MILK CHEESE ARTISANAL PRODUCTION PROCESS

Mara GEORGESCU[1], Constantin SAVU[1], Ileana PĂUNESCU[1], Dragoş GEORGESCU[2]

[1]University of Agronomic Sciences and Veterinary Medicine, Veterinary Medicine College, Splaiul Independentei 105, District 5, 050097, Bucharest, Romania, Phone: 021.318.04.69
[2]University of Medicine and Pharmacy Carol Davila, 37 Dionisie Lupu Str., District 1, Bucharest, Romania

Corresponding author email: dr_georgescu_mara@yahoo.com

Abstract

Listeria monocytogenes is a challenging problem still associated with traditional raw milk cheese manufacturing, as it can contaminate milk at reception but it can also occur at later production stages. A model HACCP plan was developed for L. monocytogenes hazard, associated with raw milk cheese production in a local artisanal type manufacturing facility. Ripening was identified as CCP. Critical limits were set considering previous scientific findings related to L. monocytogenes development and fluctuations in such cheeses. The prerequisite programs and the management of the established control system for L. monocytogenes were designed in such manner as to allow the artisanal production by traditional means and to ensure the safety of the final product.

Keywords*: Listeria monocytogenes, raw milk cheese, HACCP plan.*

INTRODUCTION

According to Codex Alimentarius Food Hygiene Basic Texts, Hazard Analysis and Critical Control Points (HACCP) is a system that identifies, evaluates and controls hazards that are significant for food safety. The same document states that a HACCP plan is the document prepared in accordance with the principles of HACCP to ensure control of significant food safety hazards in the segment of the food chain under consideration (Codex Alimentarius, 2009).

Listeria monocytogenes is a well-known significant food safety hazard for which there is extensive literature data regarding surveillance, outbreak and control in food industry (FDA/USDA, 2003). Occurrence of *L. monocytogenes* in raw milk and milk products is common and widely documented (Eck et al., 2000; Amezquita et al., 2004; Mohammad Mehdi Mahmoodi, 2010). Therefore, scientifically supported control measures within the HACCP plan have grown to be considered the main instrument of *Listeria* spp. hazard management, especially for raw milk commodities (Augustin et al., 2005; Beaufort, 2011). Nevertheless, finding the most effective control measure for the management of *Listeria* spp. hazard is still considered challenging for artisanal and traditional raw milk cheese manufacturing, as appropriate control generally interferes with the design, arrangement and facilities of pastoral complexes and the handcrafted manufacturing technology of such products (Nicolaescu, 2008; Nicolaescu et al., 2007).

This study aimed to develop a model HACCP plan for *L. monocytogenes* for raw milk artisanal cheese manufacturing process that would be suitable for an efficient hazard control considering the specific production methods used in small, traditional facilities.

MATERIALS AND METHODS

In order to develop a HACCP plan for the control of *L. monocytogenes* hazard in a small, artisanal raw milk cheese manufacturing facility, the Codex Alimentarius (FAO, 1997) basic recommended steps were followed as means for implementing a food safety management tailored plan based on HACCP system principles:

→ Conduct a hazard analysis (HA),
→ Identify the critical control points (CCP) using the decision tree diagram tool (Figure 1),
→ Establish the critical limits (CL),
→ Monitor all CCP's,
→ Establish the corrective actions,
→ Establish verification procedures and
→ Establish a record keeping system.

The plan was developed for a private milk processing station. The process considered for the development of a tailored food safety management system was the manufacturing of a traditional kneaded cheese, made of a mixture of cow's and ewe's milk, called burduf.

The process follows the traditional manufacturing methodology and is tailored for the facility and the available equipment.

Microbiological, chemical and physical parameters used to describe the product were analyzed according to previously described methods (Nicolaescu, 2008).

Appropriate control measures were developed for the identified CCP in order to keep the involved parameters inside the critical range of limits. Also, verification and record systems were established in order to assess the effectiveness and prove the *Listeria* spp. hazard-free condition of all finished products.

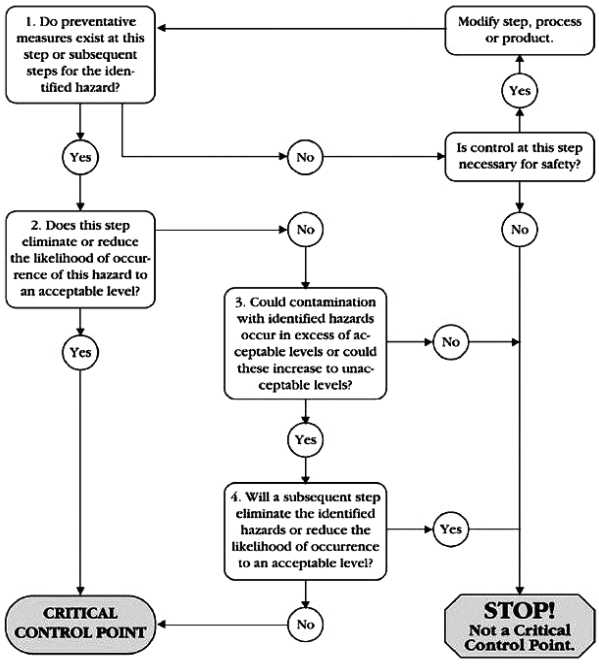

Figure 1. Decision tree diagram for CCPs (FAO, 1997)

RESULTS AND DISCUSSIONS

Before proceeding with the development of a HACCP plan, the preliminary tasks were followed in a logical sequence pending the food safety system implementation for *Listeria monocytogenes* control:

1. A multidisciplinary team was established using internal and external resources.
2. The product was described using data from former analysis reports and verification analysis bulletins for 20 samples of burduf cheese, individually packaged using fir bark, pork bladder or artificial membrane. Product description and the intended use specification tasks were fulfilled by the HACCP team and the producer. The product characteristics regarding chemical composition and physical properties relevant for the growth of most bacteria are detailed in Table 1.
3. The flow diagram developed by the producer was verified on site and necessary details were added for relevance and transparency (Figure 2). The finished product is a soft, creamy, powerfully flavoured cheese, packaged in either natural/artificial membrane or in fir bark.

Table 1. Product composition (average values at membrane filling)

Parameter	Value
pH	5.3
TA (titratable acidity)	187
% H_2O	47.5
a_w (water activity)	0.959*
% NaCl	2.03
% protein	24.3
Fat/dry content, %	52.6

*estimated according to Raoult's Law (Grummer et al., 2011)

The prerequisite programs (PRPs). The producer follows basic and standard good manufacturing and hygiene practices (GMPs and GHPs) and a number of standard operational procedures (SOPs) were available for employee's to use in daily operations.

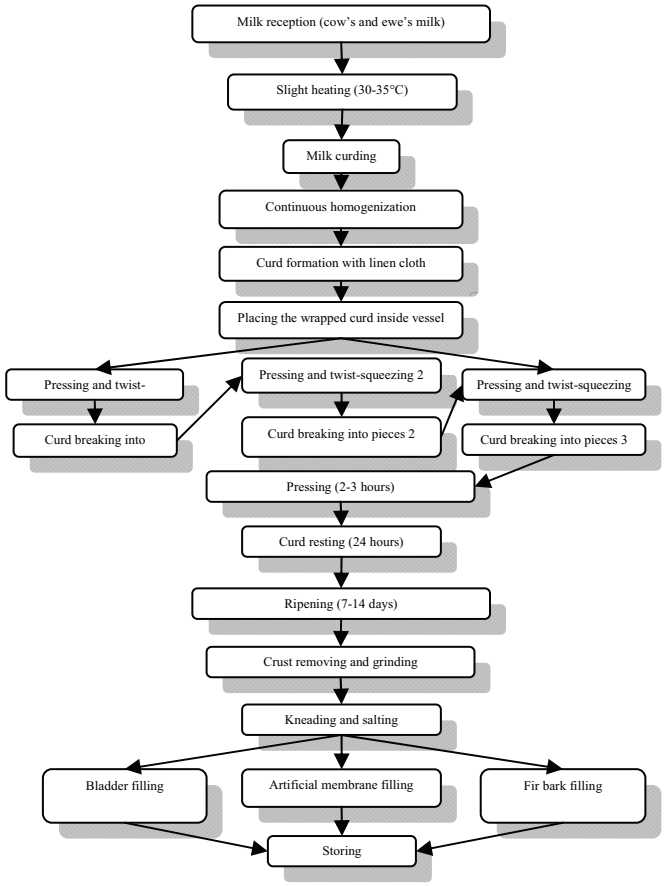

Figure 2. Diagram flow for "burduf cheese"

Nevertheless, numerous deviations were noticed when comparing the PRPs in place with the requirements of ISO/TS 22002-1:2009:

- 4.3. No controlled access inside facility areas.
- 5.2. No cross-contamination-free interior design for employee and product traffic.
- 6.1. Water supply from potable source but not monitored inside facility.
- 8.2. The hygienic design of equipment not available for all items: extensive use of fir wood for surfaces in storage, ripening and processing areas.
- 9.2. No available monitoring system for suppliers.
- 10.2d) no logical traffic of employees and equipment for cross/contamination prevention and 10.2e) no controlled air pressure between contaminated and non contaminated areas.
- 15. No recall procedures
- 18. No food defense procedures.

The working surfaces, equipment and shelves in storage areas were tested for *Listeria* spp. contamination but no positive results were obtained.

Hazard analysis and hazard control measures are detailed below and listed in Table 2.

1. **Reception.** 32% of the raw milk samples taken in a 30 days period of time tested positive for *Listeria* spp. At reception, milk was not tested from individual producers, due to the fact that the facility received collection milk, as well as milk from individual producers. Therefore milk reception was considered a point in the process flow that requires control for *Listeria* spp. hazard and was considered for determination of critical control point (CCP) status.
2. **All stages from reception to storage of final product** are under risk of contaminating the product if not properly sanitized. Appropriate PRPs may cover this aspect, but the mentioned deviations remain a problem to be addressed.
3. No sufficient heating is used in the process; therefore **no listericidal processing step is available for the control of *Listeria* spp. microbiological hazard.** Scientific literature indicates that pasteurization at 71-72^{0}C for 15 seconds would be enough to eliminate *Listeria monocytogenes*. Yet, traditional cheese manufacturing assumes using raw milk as prime material, therefore other control measures must be considered to eliminate this hazard or to reduce the risk to the safety level.
4. **Ripening** is an important stage in raw milk cheese manufacturing, as many sensory features are connected to the ripening conditions and time. In addition, ripening was proven to be an important tool for *Listeria* spp. elimination from cheeses (Eck et al., 2000).
5. **Storing, as well as most processing and handling steps,** may be considered as a potential contamination step considering the risk of *Listeria* spp. presence in the environment. Yet, the

risk is low due to the packaging solutions for the cheese considered (natural or artificial membrane, or fir bark cylinders). Moreover, hazard control for this step is covered by PRPs.

The control measures indicated by scientifically based data include (CAC/GL 61-2007; Codex Alimentarius, 2009):

→ Listericidal processes applied in cheese manufacturing, such as milk pasteurization or sterilization prior to curding, should be validated to ensure that the treatments are effective and can be applied consistently. Yet, for the cheese considered in this study, no heating is accepted above the limit of 35^0C, because of the traditional feature of the recipe.

→ Single or combination parameters, such as a pH less than 4.4, a water activity less than 0.92 or freezing, may be relied upon to prevent *L. monocytogenes* growth. However, average values of pH and a_w (Table 1) place this product outside the limit range of possible prevention.

→ The storage step requires time/temperature combination control. According to Codex Alimentarius guidelines, the product temperature should not exceed 6^0C (preferably 2-4^0C), while monitoring and controlling refrigerated storage temperatures are key control measures.

→ Literature indicates additional control measures that limit *Listeria* spp. growth, such as freezing the product, shortening the shelf-life, reformulation of the product.

→ When necessary and applicable, alternative post-packaging listericidal treatment options are available for controlling this hazard: heating, high-pressure treatment, irradiation. Unfortunately, the nature of the packaging materials of burduf cheese prevents us from applying these controls.

Critical control points (CCPs) and Critical limits. Elevated temperatures or sodium chloride levels are not applicable for *Listeria* spp. hazard control. According to Eck et al. (2000), *Listeria* spp. can grow at 10% NaCl. Due to specific product chemical features (Table 1), pH and water activity cannot be controlled down to the required levels for safety. Therefore, raw milk quality monitoring and appropriate GMPs and GHPs for adequate sanitation during processing remain on top of the list of control measures that may be selected for the considered process. Nevertheless, as previously stated, monitoring raw milk for *Listeria* spp. is hard to accomplish because it assumes intensive financial resource investment, milk is collected from multiple sources, samples from private producers are intensively contaminated and an adequate local producers' selection is difficult.

Regarding time-temperature control for food safety at storage, this is a valid control measure for *Listeria* spp. growth prevention, but according to recent studies, it may be more that just that. Recent studies proved that despite the rise of pH in cheeses during ripening, which would be favorable for the growth of *Listeria* spp., traditional raw milk cheeses reveal a significant descending shift in the level of listeric contamination after the ripening period. When the ripening period is long enough, contaminated cheeses become *Listeria* spp.-free. Our previous studies associated the natural loss of *Listeria monocytogenes* with the bacterial competition between *Listeria* spp. and *Lactobacillus* spp. (Nicolaescu et al., 2007). Other authors also indicate ripening as a potential control measure for *Listeria* spp. in Camembert cheese (Eck et al., 2000) or raw milk hard cheeses (Rodriguez et al., 2001), some suggesting that the inhibitory effect occurs due to the competition with bacteria belonging to a balanced natural ecosystem (Brasca et al., 2004) or to bacteria of wood shelves biofilm (Mariani et al., 2008).

The results of our previous research, regarding a 90 days ripening period necessary for Listeria elimination from raw milk cheeses (Nicolaescu et al., 2007) are consistent with other reports such as the study of Pirisi et al. (2008), which indicates the elimination of *Listeria monocytogenes* in PDO Pecorino Romano cheese, after 90 days of ripening.

Critical control points (CCPs) were searched for every step in the diagram flow using the decision tree diagram (Figure 1).

Reception of milk, ripening and storing were considered for evaluation to decide if they may be critical control points for *Listeria monocytogenes*.

Raw milk quality may be controlled through adequate PRPs concerning supplier selection and monitoring. No control was considered necessary at this step for food safety, because there is no achievable method of elimination, due to the specific conditions necessary for processing (no pasteurization allowed). Moreover, there is a further step in the diagram that allows hazard control.

Ripening was considered to be a critical control point (CCP1) because it does prevent the growth of *Listeria monocytogenes* or reduces to a safety level the risk of listeric proliferation in cheese.

Storage was also considered to be a critical control point (CCP2), because contamination could increase to unacceptable levels or remain to unacceptable levels if time-temperature levels are not followed. A new, simplified diagram flow, including information on the CCPs was developed (Figure 3).

The HACCP team considered these correlations and set up as critical limits:

→ CCP1 - Ripening: temperature of maximum 6^0C for the period of 7-14 days required prior to the following steps of grind removing, salting, grinding and membrane filling. Maintaining this temperature limit would prevent *Listeria monocytogenes* growth in the environment considered otherwise favorable: pH = 5.3 (greater that the minimum limit of 4.4), a_w = 0.95 (greater than 0.92).

→ CCP2 – Storing: temperature of maximum 6^0C, for minimum 90 days prior to delivery, to assure the second ripening (according to the process diagram flow).

It is important to maintain the minimum ripening time and to assure that the maximum temperature is consistently observed through ripening and storage. The HACCP master plan is presented as described, in Table 3.

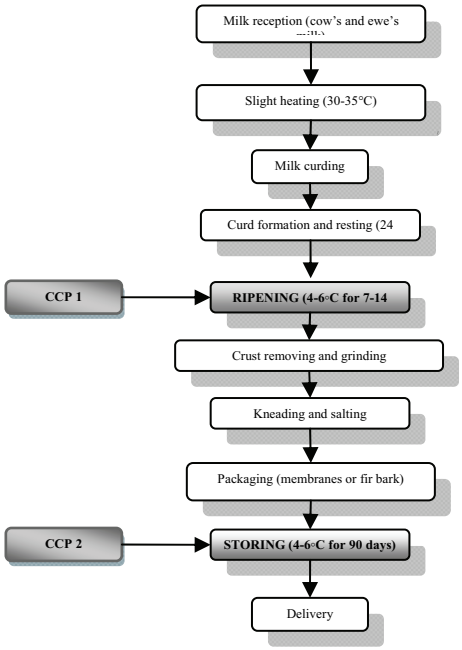

Figure 3. Modified flow diagram "burduf cheese", with critical control points

Verification. Visual inspections are to be performed on a regular basis by the employees, to assure the SOPs covering the basic GHPs are followed consistently throughout the process.

An initial verification was performed for all batches of product before delivery, for 3 months. All analyzed samples were free from *Listeria monocytogenes.*

On a regular basis (depending on the production), and whenever considered necessary, samples of the final product are to be taken for *Listeria* spp. analysis. The food safety outcome is to be considered no *Listeria monocytogenes* contamination in final product.

Validation. Initial validation of this HACCP plan was performed to determine its effectiveness to keep the *Listeria monocytogenes* hazard to the established level.

In addition, systematic revalidations are to be performed, following these steps:

→ review the HACCP plan;

→ verify records of CCPs' monitoring, critical limits observing, deviations registered, corrective actions performed;

→ verify the final product by random sampling and analysis for *Listeria monocytogenes.*

CONCLUSIONS

The HACCP plan developed for *L. monocytogenes,* based on a new, simplified diagram flow for burduf cheese manufacturing, is focused on two CCPs. The effectiveness of the plan relies upon the correction of eight identified deviation of the PRPs, according to ISO/TS 22002-1:2009. The management full commitment, complete awareness and training of personnel and continuous verification of the new plan for food safety management are crucial for guaranteeing final product safety.

Table 2. *Listeria monocytogenes* hazard analysis, control measures and hazard control at control points and critical control points

Process	Activities	Risk of occurence	Control measures	PRP*/CCP
Reception	Reception of mixture of cow's and ewe's milk from private producers	High	Controlled by PRPs and subsequent steps	PRP-chapter 9
Heating	Heating to maximum 35^0C	Low		PRP-chapters 8, 10.2, 11, 13
Curding	Addition of rennet	Low		PRP-chapters 8, 10.2, 11, 13
Curd formation	Curd colleting and cheese formation	Low		PRP-chapters 8, 10.2, 11, 13
Ripening	Resting and ripening for 7-14 days at max. 6^0C	High	Not exceeding the temperature of 6^0C	CCP1
Grinding	Crust removing and grinding	High	Controlled by PRPs and subsequent steps	PRP-chapters 8, 10.2, 11, 13
Kneading and salting	Kneading by hand and salting the curd	High		PRP-chapters 8, 10.2, 11, 13
Packaging	Filling natural /artificial membranes of fir bark cylinders	High		PRP-chapters 8, 10.2, 11, 13
Storing	Ripening for at least 90 days at max. 66^0C	High	No deliveries allowed before 90 days ripening. No exceeding 6^0C.	CCP2

*According to ISO/TS 22002-1:2009 requirements.

Table 3. HACCP Master plan for *Listeria monocytogenes* hazard in raw milk cheese burduf

CCPs	Critical limits	Monitoring	Corective actions	Verification	HACCP records
CCP1	Maximum 6^0C	Temperature control	Maintenance of refrigeration system in case of malfunction	Temperature check	Maintenance and performance records;
CCP2	Maximum 6^0C Minimum 90 days	Temperature control Number of ripening days	Maintenance of refrigeration system in case of malfunction Zero tolerance for cheeses younger than 90 days	Temperature check Verification of labeling, stock rotation and ripening age at delivery Random sampling of final product for *Listeria monocytogenes*	Maintenance record Batch record Storage record Delivery record Analysis bulletin for *Listeria monocytogenes*

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