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THE EFFECT OF ANTIMICROBIAL SUBSTANCES TO INHIBIT THE GROWTH OF *Listeria monocytogenes* INTO THE READY-TO-EAT PRODUCTS

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Abstract

Listeria monocytogenes (LMO) is one of the most pathogenic agents that can contaminate the ready-to-eat (RTE) products, of animal and non-animal origin and, due to its special resistance, it justifies specific measures on the RTE-processing flow. It is a continuing concern for the prevention of LMO infections in humans through the food consumption. In the reduction of LMO growth, the bioprotectors use is increasingly being considered. In this paper we are presenting the antimicrobial effect of substances added to RTE products to suppress or limit the growth of LMO in Chorizo sausages. One of these is the bioprotection culture that contain strains of Pediococcus acidilactici. P. acidilactici produces pediocin which is a bacteriocin with strong antagonistic properties against LMO. The results support the recommendations of P. acidilactici use as an additional culture in the production of dry or semi-dry fermented sausages below 26ºC. Under these conditions, the addition of the culture to the normal recipe will ensure an efficient reduction of LMO growth, and does not significantly affect the acidification profile.

Key words*: RTE, LMO, bioprotection substances, Pediococus acidilactici.*

INTRODUCTION

Listeria monocytogenes (LMO) is one of the most important pathogenic agents that can contaminate the ready-to-eat (RTE) products, of animal and non-animal origin. Human listeriosis may affect some risk category that include immunocompromised patients, pregnant women, neonates, and the elderly. It causes pathologies such as meningitis, septicemia, encephalitis and abortions (Vázquez-Boland et al., 2001; Di Pinto et al., 2010; Lopez-Valladares et al., 2018).

The establishment of listeriosis as a major foodborne infection began in 1983 when human outbreaks were reported in North America and Europe (Vázquez-Boland et al., 2001). Various food products have been reported as sources of infection for humans (e.g., soft cheeses, dairy products, sausages, smoked fish, and salads) but they were usually refrigerated ready-to-eat products (McLauchlin et al., 1990; Farber et al., 1991; Rocourt, 1996). *Listeria monocytogenes* is widespread in the environment and is a potential risk because there is a wide variety of foodstuffs, including the dry fermented sausages, that may be contaminated with LMO (Meloni, 2015). These categories of products, asRTE products, could be contaminated with LMO if the raw meat is contaminated. Also, during the fermentation, ripening and drying steps of manufacturing, LMO, which is ubiquitous, psychrotrophic and relatively resistant bacteria to the action of curing agents, could survive and multiply (Johnson et al., 1988; Junttila et al., 1989).

The pathophysiology of LMO in humans and animals infection it is not fully known (Vázquez-Boland et al., 2001). The primary site of LMO entry into the host is thought to be the gastrointestinal tract with contaminated food as the major source of infection (Farber & Peterkin, 1991). The minimum infectious dose in humans is unknown, levels of food contamination as low as 10^2 to 10^4CFU/g of LMO have been associated with human listeriosis (Farber et al., 1991; McLauchlin, 1991). In the presentation of clinical disease, the number and pathogenic properties of bacteria ingested with food and susceptibility, health and immunological status of the patient play major roles (Vázquez-Boland et al., 2001).

Bioprotective action of some bacterial cultures against LMO has been described in several

scientific papers (Tomé et al., 2008; Garriga et al., 2015; Winkelströter et al., 2015; Saraoui et al., 2018; Aymerich et al., 2019).

The addition of the bioprotection culture that contains strains of *Pediococcus acidilactici* proved to be efficient to suppress or limit the growth of LMO in dry or semi-dry fermented sausages below 26ºC, throughout the shelf life of the product, since this microorganism produces pediocin which is a bacteriocin with strong antagonistic properties against LMO (Silberiagel et al., 2004; Johnson & Mills, 2013). The positive effects were observed with use of bacteriocin and organic acid supplement-ation and the best antilisterial effects were achieved by combined use of bacteriocin and organic acid (Ustudang & Ozdogan, 2017).

In this paper we are presenting the antimicrobial effect of substances added to RTE products to suppress or limit the growth of LMO in Chorizo sausages.

MATERIALS AND METHODS

In order to evaluate the antimicrobial effect of *Pediococcus acidilactici* culture (B-LC-20 SafePro^{TM}, Chr. Hansen) added to dry fermented sausages below 26ºC, two Chorizo sausages batches with LMO contaminated meat (100 CFU/g) were prepared in accordance with the manufacturing process: (1) chopping and mixing of ingredients, (2) stuffing the meat mixture, (3) fermentation and smoking (alternative stages of fermentation and smoking), (4) ripening and storage steps. The LMO inoculum was added directly to the meat recipe, into the bowl chopper, during the products manufacturing.

Batch I

Chorizo sausages with the bioprotection culture *Pediococcus acidilactici* culture 6.1×10^7 CFU/g (SafePro® B-LC-20, CHR. Hansen Holding, Hoersholm, Denmark) and the starter culture *Pediococcus pentosaceus* plus *Staphylococcus carnosus* 5×10^6 CFU/g) (BFL-F02, Chr. Hansen Holding, Hoersholm, Denmark). The starter culture is intended for the dry meat products to ensure the characteristics of the meat mixture during fermentation step (the acidification process).

The evaluation of *Pediococcus acidilactici* in the reduction of LMO growth was performed with VIDAS® *Listeria monocytogenes* II (LMO2) (bioMérieux, Marcy-l'Etoile, France) on 25 g of samples taken on days 0, 2, 5 and 9. Each sample was tested in quintuplicate.

Batch II

Chorizo sausages with the starter culture *Pediococcus pentosaceus* plus *Staphylococcus carnosus* 5×10^6 CFU/g) (BFL-F02, Chr. Hansen Holding, Hoersholm, Denmark). The evaluation of LMO growth was perfomed with VIDAS® *Listeria monocytogenes* II (LMO2) (bioMérieux, Marcy-l'Etoile, France) on 25 g of samples taken on days 0, 2, 5 and 9. Each sample was tested in quintuplicate.

LMO detection by using VIDAS® *Listeria monocytogenes* II (LMO2) (bioMérieux, Marcy-l'Etoile, France)

VIDAS® *Listeria monocytogenes* II (LMO2) (bioMérieux, Marcy-l'Etoile, France) is an automated enzyme-linked fluorescent immunoassay (ELFA) for the specific detection of *Listeria monocytogenes* antigens in foods with a detection limit $P < 0.05$ (Silberiagel et al., 2004; Johnson & Mills, 2013).

The protocol

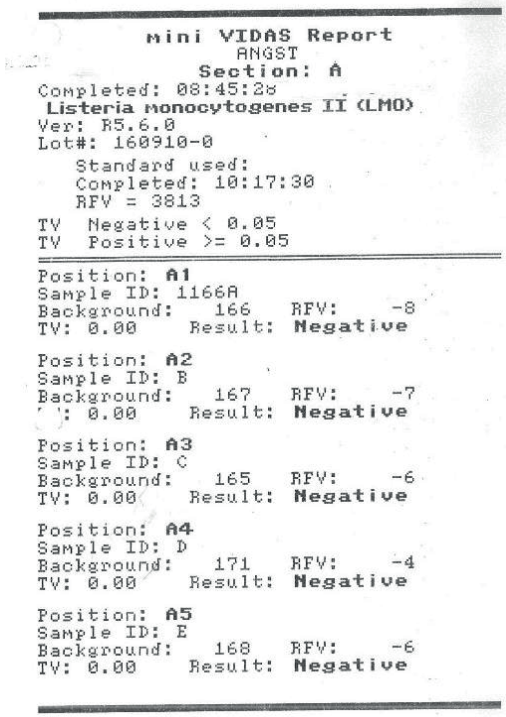
- In a blender bag, aseptically add: 25 g of sample and 225 ml of Half-FRASER broth;
- The mixing using a paddle blender;
- Incubation for 24-26 hours at 30°C ± 1°C;
- After incubation, mixing the contents of the blender bag manually and transfer 1 mL of the suspension into 10 ml of FRASER broth (enrichment broth);
- Incubating for 24-26 hours at 30°C ± 1°C;
- Performing the VIDAS® assay (Figures 1 and 2).

Figure 1. VIDAS STR for *Listeria monocytogenes*

Figure 2. Performing LMO detection by *using* VIDAS® *Listeria monocytogenes* II (LMO2) (bioMérieux, Marcy-l'Etoile, France)

Results are analyzed automatically by the computer (Figures 3 and 4).

Figure 3. Mini VIDAS *Reporting* for VIDAS® *Listeria monocytogenes* II (LMO2) (bioMérieux, Marcy-l'Etoile, France)



```
mini VIDAS Report
ANGST
Section: A
Completed: 08:45:28
Listeria monocytogenes II (LMO)
Ver: R5.6.0
Lot#: 160910-0
   Standard used:
   Completed: 10:17:30
   RFV = 3813
TV  Negative < 0.05
TV  Positive >= 0.05

Position: A1
Sample ID: 1166A
Background:   166   RFV:    -8
TV: 0.00    Result: Negative

Position: A2
Sample ID: B
Background:   167   RFV:    -7
TV: 0.00    Result: Negative

Position: A3
Sample ID: C
Background:   165   RFV:    -6
TV: 0.00    Result: Negative

Position: A4
Sample ID: D
Background:   171   RFV:    -4
TV: 0.00    Result: Negative

Position: A5
Sample ID: E
Background:   168   RFV:    -6
TV: 0.00    Result: Negative
```

Figure 4. Mini VIDAS Report *for* LMO perfomed with VIDAS® *Listeria monocytogenes* II (LMO2) (bioMérieux, Marcy-l'Etoile, France)

RESULTS AND DISCUSSIONS

In the first two days, all results of batches I and II were positive for LMO (Tables 1 and 2). On testing on the fifth day, three of the five samples analyzed in Batch I were negative. Testing on day nine provided only negative results, suggesting that, in the Chorizo sausages with the bioprotection culture *Pediococcus acidilactici* culture 6.1×10^7CFU/g (SafePro® B-LC-20, Chr. Hansen Holding, Hoersholm, Denmark) and the starter culture *Pediococcus pentosaceus* plus *Staphylococcus carnosus* 5×10^6CFU/g) (BFL-F02, Chr. Hansen Holding, Hoersholm, Denmark) LMO growth was efficiently reduced.

These results are in agreement with the observations of Heller-Stahnke (2005) who found that "*The use of an adjunct culture such as B-LC-20 provides a unique anti-listerial reduction for fermented sausages since it was found that Pediococcus acidilactici is a strong producer of pediocin (which destroys Listeria monocytogenes) at European fermentation temperatures (< 26°C) while not being a strong acidifier at 10 this temperature*" (Heller-Stahnke, 2005).

Table 1. Results obtained in Chorizo sausages with the bioprotection culture* at VIDAS® *Listeria monocytogenes* II (LMO2) testing

Sample code	Sampling day	Result of VIDAS® *Listeria monocytogenes* II (LMO2) testing	
		Pozitiv (≥ 0.05)/25 g	Negativ (< 0.05)/25 g
T0 A	0	x	-
T0 B		x	-
T0 C		x	-
T0 D		x	-
T0 E		x	-
T1A	2	x	-
T1 B		x	-
T1 C		x	-
T1 D		x	-
T1 E		x	-
T2 A	5	x	-
T2 B		-	Absent/25 mg
T2 C		-	Absent/25 mg
T2 D		x	-
T2 E		x	Absent/25 mg
T3A	9	-	Absent/25 mg
T3B		-	Absent/25 mg
T3C		-	Absent/25 mg
T3D		-	Absent/25 mg
T3E		-	Absent/25 mg

*Chorizo sausages with the bioprotection culture *Pediococcus acidilactici* culture 6.1×10^{7}CFU/g (SafePro® B-LC-20, Chr. Hansen Holding, Hoersholm, Denmark) and the starter culture *Pediococcus pentosaceus* plus *Staphylococcus carnosus* 5×10^{6}CFU/g) (BFL-F02, Chr. Hansen Holding, Hoersholm, Denmark)

Al samples of the in Chorizo sausages without the bioprotection culture at VIDAS® *Listeria monocytogenes* II (LMO2) provided positive results in days 0, 2, 5 and 9 of study.

Foegeding et al. (1992) were interested in finding out whether pediocin is produced by *Pediococcus acidilactici* and has effective antilisterial activity during sausage fermentation. Their research indicated that the dry sausage fermentation process can reduce *L. monocytogenes* populations when the pH at the end of the fermentation process was less than 4.9 and during drying portions of manufacturing (Foegeding et al., 1992). The action of *P. acidilactici* at this pH is supported by de Bhunia et al. (1988) study that proves the sensitivity of pediocin to proteolytic enzymes resistant to heat and organic solvents, and activity of pediocin at a wide range of pH. In the same study, the pediocin produced by *P. acidilactici* inhibited many food spoilage bacteria and foodborne pathogens (e.g., *Staphylococcus aureus*, *Clostridium perfringens* and *Listeria monocytogenes*).

Table 2. Results obtained in Chorizo sausages without the bioprotection culture* at VIDAS® *Listeria monocytogenes* II (LMO2) testing

Sample code	Sampling day	Result of VIDAS® *Listeria monocytogenes* II (LMO2) testing	
		Pozitiv (≥ 0.05)/25 g	Negativ (< 0.05)/25 g
C0 A	0	x	-
C0 B		x	-
C0 C		x	-
C0 D		x	-
C0 E		x	-
C1A	2	x	-
C1 B		x	-
C1 C		x	-
C1 D		x	-
C1 E		x	-
C2 A	5	x	-
C2 B		x	-
C2 C		x	-
C2 D		x	-
C2 E		x	-
C3A	9	x	-
C3B		x	-
C3C		x	-
C3D		x	-
C3E		x	-

*Chorizo sausages with the starter culture *Pediococcus pentosaceus* plus *Staphylococcus carnosus* 5×10^{6}CFU/g) (BFL-F02, Chr. Hansen Holding, Hoersholm, Denmark)

The contamination of fermented sausages with LMO can be from various sources (e.g., slaughterhouse environments, raw meat, production processes, and post-processing conditions) and the use of starter cultures and the correct drying can minimize the potential for LMO growth in this type of sausages (Meloni, 2015). In our study the use of bioprotection cultures proved that the *Pediococcus acidilactici* culture (B-LC-20

SafeProTM, Chr. Hansen) improve microbial safety in the production of food products.

In a review made by Mehta et al. (2013), it is shown that pediocins are of great interest to the food industry for their strong activity against food spoilage and pathogenic bacteria. Moreover, pediocin produced by *Pediococcus acidilactici*, has been recognized as safe (Mehta et al., 2013).

In the future, more scientifically studies should be performed to identify other isolates with properties similar to those demonstrated by the cultures used in this study. These studies should also be encouraged by recent study of Bungenstock et al. (2020) who identified among the 169 collected isolates, two new bacteriocin-producing isolates which have the potential to contribute to product and consumer safety: *Pediococcus pentosaceus* LMQS 331.3 and *Pediococcus acidilactici* LMQS 154.1 (Bungenstock et al., 2020).

CONCLUSIONS

This study showed the LMO reduction after the fermentation stage and till the end of ripening and drying stage, compared to a control starter culture alone.

The bioprotection culture is able to inhibit the growth of LMO in Chorizo sausages, proving that is suitable to use for this purpose, very important for the food safety aim and the human health.

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