THE SMART NATURAL SOLUTION

BIENCA

Lactoperoxidase system: New alternative to chemical preservatives

Alma Flores

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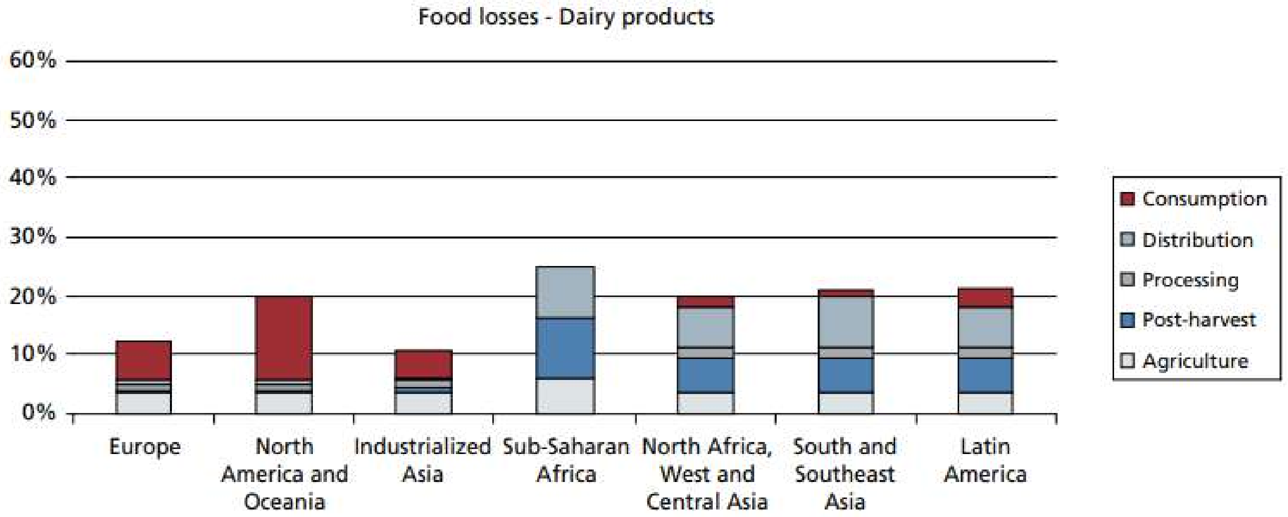
Our mission

Bienca, a Belgian company that develops and provides innovative and by nature inspired solutions for the microbial safety of food products.

The challenge

Figure 9. Part of the initial milk and diary production lost or wasted for each region at different stages in the FSC



Source: FAO. 2011. Global food losses and food waste – Extent, causes and prevention. Rome.

- Dairy products are among the top three food groups being lost and wasted, with fluid milk being responsible for 2/3 of the volume attributed to dairy products.
  (International Dairy Federation. (2024). IDF Dairy Sustainability Outlook – Dairy Processing (Issue N° 8). https://doi.org/10.56169/KZTD1887)

- 55% of the waste of fresh dairy products comes from consumer side. Therefore, reducing waste of dairy products not only requires initiatives on the production side, but also innovative solutions to help consumers decrease waste.
  (IDF communications. 2019. How the Dairy Sector is Tackling Food Waste)

Undesired microbial growth has an impact on dairy products

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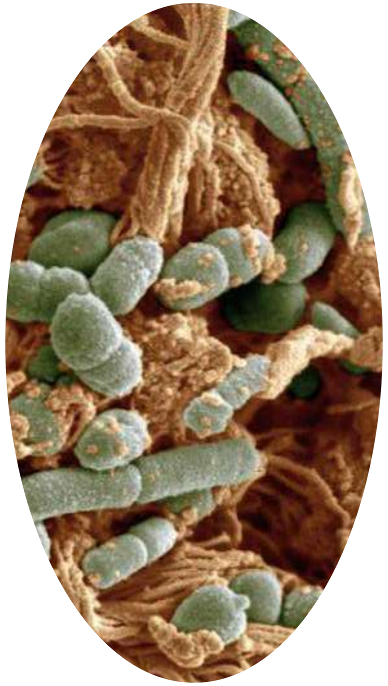


Pathogens

Spoilage

- Psychrophilic and psychrotolerant bacteria (e.g. *Pseudomonas spp*)
- Sporeforming bacteria (e.g. *Paenibacillus* and *Clostridium*)

Effects of undesired microbial growth

- **Acidification :** typical cause of bad smell and taste.
- **Gas development :** typical cause of inflated packages.
- **Production of proteases** : enzymes that affect the texture, typical cause of release of liquid and bitter taste of milk.
- **Production of lipases :** enzymes that act on fat, typical cause of rancid taste.
- **Production of toxins**

How to avoid undesired microbes?

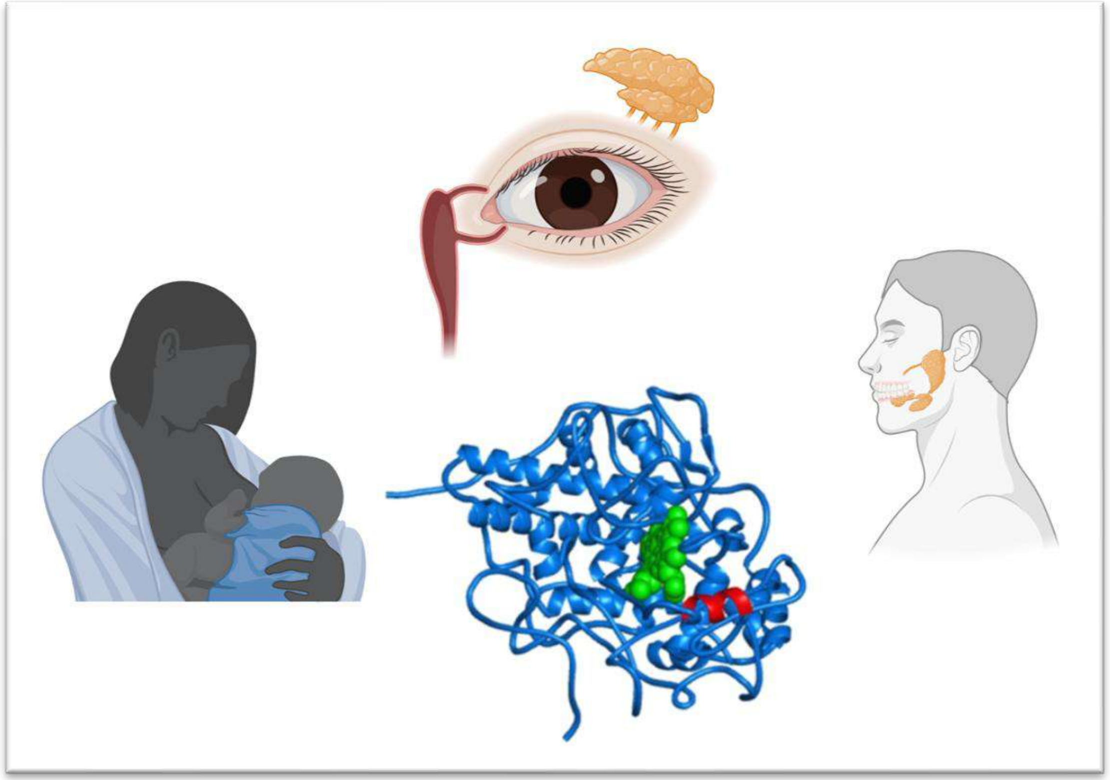
- Physical treatment (e.g. heat or pressure) not always possible due to impact on product characteristics.

- Chemical additives (e.g. sorbates and benzoates) are being related to negative side-effects and are less and less accepted by the consumer

- Protection based on natural systems

The lactoperoxidase system

- The lactoperoxidase system (LPS), a natural antimicrobial system.
- Lactoperoxidase is found in the mammary, salivary, and lachrymal glands of mammals and in their respective secretions, e.g., milk, saliva, and tears[1].
- LP is one abundant enzyme in bovine milk. Its concentration is about 30 mg L–1, corresponding to about 1% of whey protein[2].
- LP is one of the most heat-stable enzymes. It inactivates above 70°C[3].
- It is resistant to acidity up to a pH equal to 3, and also to the proteolytic action of gastric juice[3].



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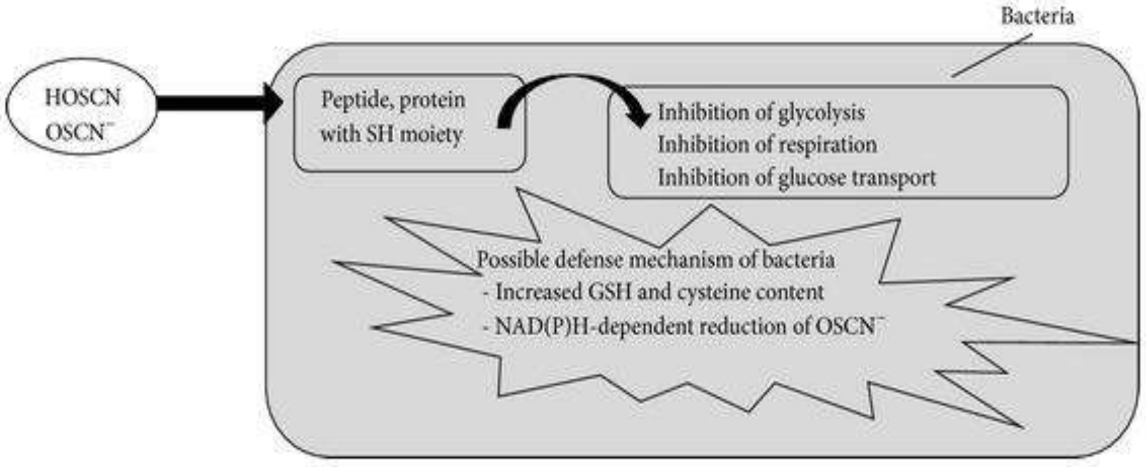
Sources:

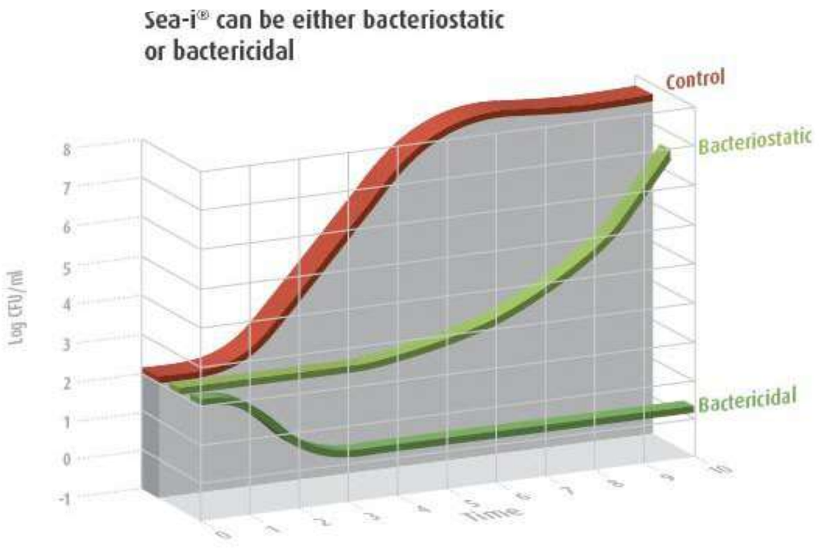
1. Wolfson et al. (1993). Antibacterial Activity of the Lactoperoxidase System: A Review. Wolfson et al. Journal of Food Protection, Vol. 56, No. 10, Pages 887-892 (October 1993)
2. Silva E. et al. (2020): Lactoperoxidase system in the dairy industry: Challenges and opportunities. Czech J. Food. Sci., 38: 337–346.
3. Kussendrager *et al.*(2000) Lactoperoxidase: physico-chemical properties, occurrence, mechanism of action and applications. British Journal of Nutrition, 84, Suppl. 1, S19-S25

How does it work

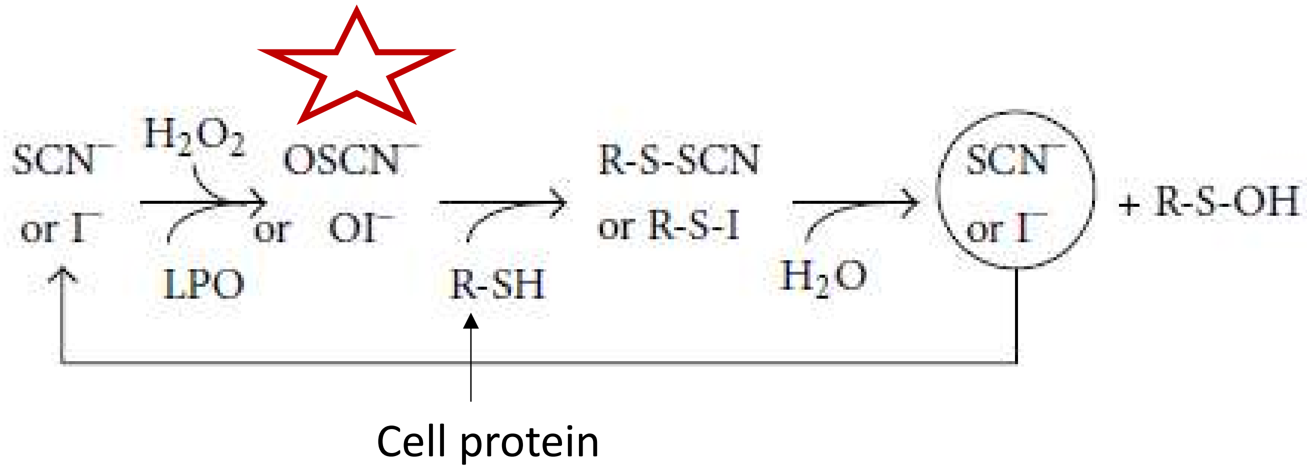
- It generates an unstable substance, with a strong and selective antimicrobial activity, reacting with thiol groups in membrane proteins of micro-organisms, leading to reduced growth (bacteriostatic) and even killing (bactericidal)



Source: *Bafort et al.* (2014) Enzyme Research



Reaction mechanism



Source: Bafort et al. (2014). Mode of action of lactoperoxidase as related to its antimicrobial activity: A review. Enzyme research, ol 2014, Article ID 517164.

- The sulfhydryl moiety is essential for the activity of numerous proteins. I.e. the disruption of the respiration and glucose transport.
- Not al sulfhydryl's are equally sensitive. β-lactoglobuline is poorly oxidized.
- Reversible inhibition is observed when cells recover after $OSCN^-$ is depleted.
- Irreversible inhibition is observed with long-term incubation or high levels of $OSCN^-$.

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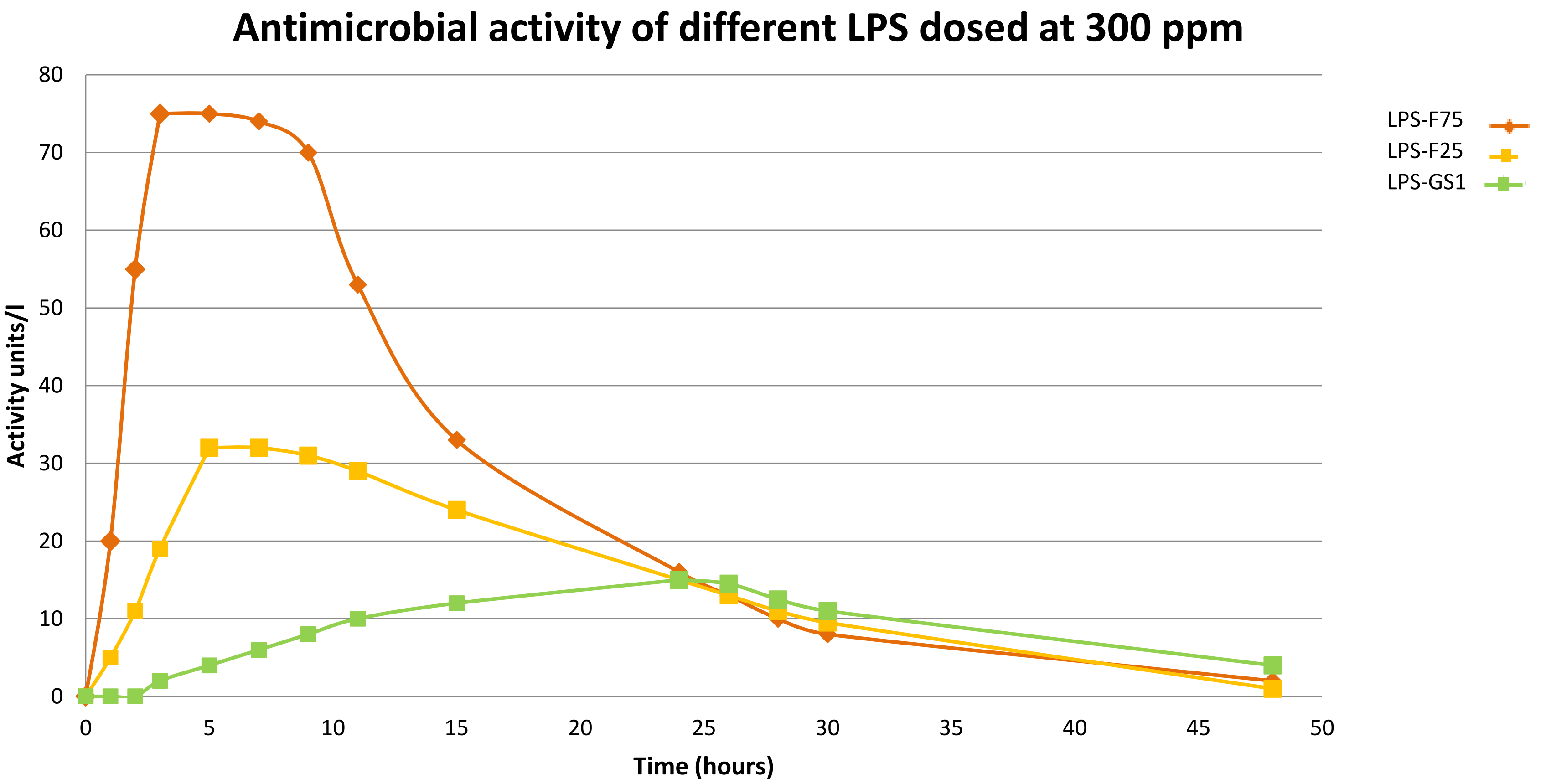
Table 2. Antimicrobial spectrum of the lactoperoxidase system

Microorganism	Donor	Effect	References
Gram positive bacteria			
Streptococcus cremoris	SCN^-	Oxygen uptake	Modi, Deodhar, Behere, and Mitra (1991)
Streptococcus lactis	SCN^-	Growth inhibition	Marshall and Reiter (1980)
Streptococcus agalactiae	SCN^-	Sugar transport	Mickelson (1977)
Streptococcus mutans	SCN^-	Glucose uptake	Loimaranta, Tenovuo, and Korhonen (1998)
Streptococcus mutans	SCN^-	Enzyme inhibition	Korpela et al. (2002)
Streptococcus sanguis	I^-	Bactericidal	Courtois, Vanden Abbeele, Amrani, and Pourtois (1995)
Gram negative bacteria			
Actinobacillus actinomycetemcomitans	SCN^-/I^-	Bactericidal	Ihalin, Loimaranta, Lenander—Lumikari, and Tenovuo (1998)
Actinobacillus actinomycetemcomitans	I^-	Growth inhibition	Ihalin, Pienihäkkinen, Lenander-Lumikari, Tenovuo, and Jousimies-Somer (2003)
Fusobacterium nucleatum	SCN^-/I^-	Bactericidal	Ihalin, Loimaranta, Lenander-Lumikari, and Tenovuo (2001)
Helicobacter pylori	SCN^-	Bactericidal	Shin, Yamauchi, Teraguchi, Hayasawa, and Imoto (2002)
Porphyromonas gingivalis	SCN^-/I^-	Bactericidal	Ihalin et al. (2001)
Porphyromonas gingivalis	SCN^-	Bactericidal	Fadel and Courtois (1999, 2001)
Prevotella loescheii	SCN^-	Bactericidal	Fadel and Courtois (2001)
Prevotella intermedia	SCN^-	Bactericidal	Fadel and Courtois (2001)
Prevotella melaninogenica	SCN^-	Bactericidal	Fadel and Courtois (2001)
Yersinia enterocolitica	SCN^-	Growth inhibition	Farrag, El-Gazzar, and Marth (1992)
Fungi			
Candida albicans	SCN^-	Loss of viability	Lenander-Lumikari (1992)
Virus			
HIV-1	SCN^-	Enzyme inhibition	Wang, Ye, and Ng (2000)

SCN^-, thiocyanate ion; I^-, iodide ion; HIV, human immunodeficiency virus.

Source: E. Seifu et al. (2005) Trends in Food Science & Technology 16. 137–154

Antimicrobial activity of products based on the Lactoperoxidase System (LPS)



Application areas

SEA-i®

Literature review - Silva E., Oliveira J., Silva Y., Urbano S., Sales D., Moraes E., Rangel A., Anaya K. (2020): Lactoperoxidase system in the dairy industry: Challenges and opportunities. Czech J. Food. Sci., 38: 337–346.

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Table 4. Effect of the lactoperoxidase system (LPS) on the production processes of dairy products for different species

Products	Species	Effect of the LPS
Milk curd	buffalo	Decreasing diacetyl and acetoin content and proteolytic activity
Fresh cheese	cow	Slow acidification, low moisture retention with satisfactory texture
Gouda cheese	goat	Improving microbiological quality and taste
Manchego cheese	sheep	Preventing excessive proteolysis and softening
Cottage cheese	cow	In sensory tests, taste and different taste from control, increase in cheese yield
Acidophilic milk	cow	Lower content of diacetyl and acetoin and lower proteolytic activity
Mozzarella cheese	buffalo	Lower retention of moisture, slow acidification. Longer time (2 h) to reach the curd stretching stage
Yoghurt	cow buffalo	No difference in chemical composition and sensory qualities No effect on body and texture
Canned cheese	cow and buffalo	Lower processing time and economic use of whey, greater serum expulsion Higher cheese yield and coagulation time, reduced curd tension, increased moisture content and decreased acidity, satisfactory quality and high score

Source: Adapted from Seifu et al. (2005)

Literature review: Malireddy S Reddy. The Effect of all-Natural Biological Milk Silo Culture (MSC) to Protect Human Health by Improving the Safety, Quality and Quantity of the Dairy Food Products through Activation of Milk's Natural Lactoperoxidase (LP) System. LOJ Phar & Cli Res 3(2)-2023.

- Lara *et. al.* reported increased yields for cheese made with LPS activated milk (>2 Kg/100 Kg of milk).

- Bjorck *et. al.* reported that the activated LPS prevented the growth of psychrotropic bacteria for up to 5 days in raw milk.

- Malireddy makes a summary of the following benefits of using the LPS:
    - *Salmonella, S. aureus, Listeria* – bacteriostatic and bactericidal action
    - *Campylobacter jejuni* – antibacterial effect
    - *Bacillus cereus* – Inhibition in raw milk
    - *Brucella melitensis* – bactericidal effect
    - Aflatoxins – mould produced aflatoxins are degraded or destroyed by the LPS.

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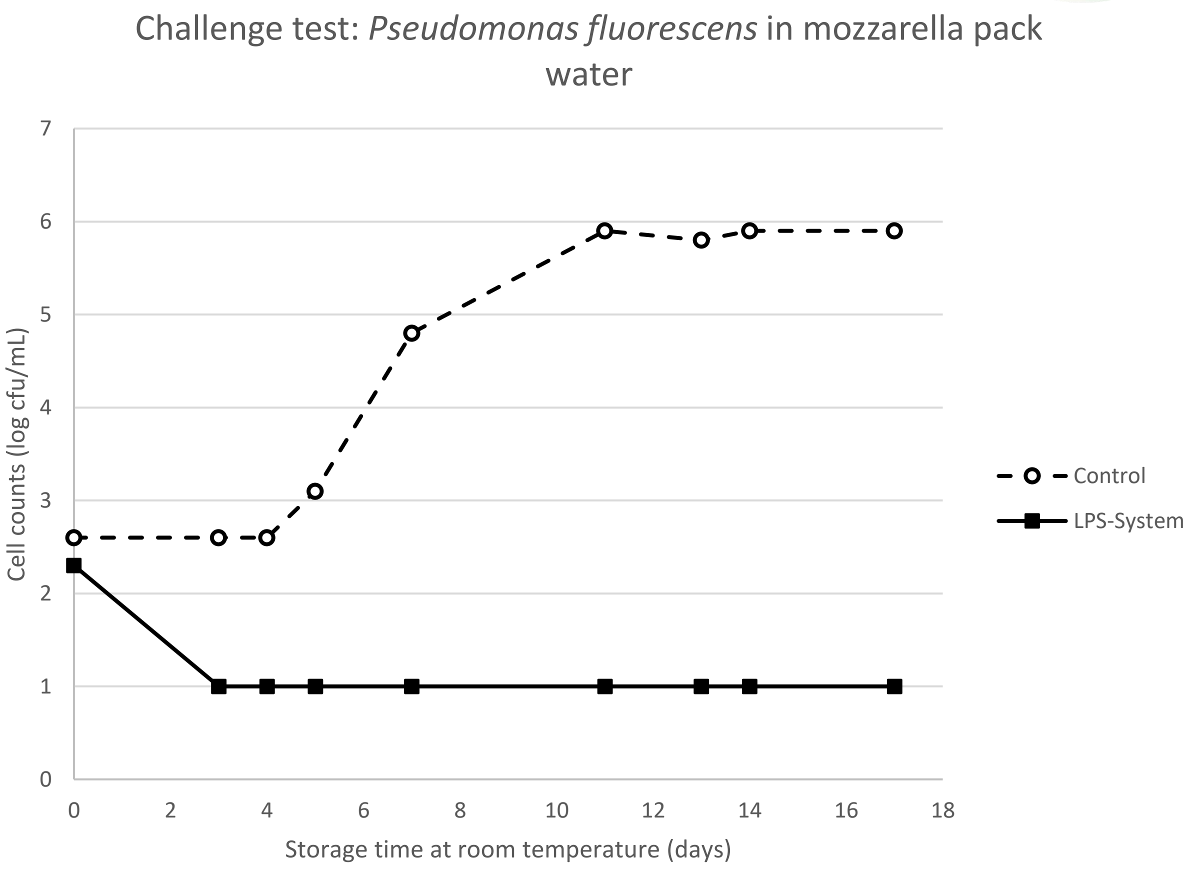
Commercial applications

RAW MILK	Growth control of psychrotrophic bacteria
MILK	Inactivation of *Listeria monocytogenes*
	Inhibition of *Escherichia coli*
	Growth control of psychrotrophic bacteria
CREAM	Growth control of the spoiling microorganisms
FRESH CHEESE	Prevention of development of residual culture
	Growth control of the spoiling microorganisms
COTTAGE CHEESE	Prevention of development of residual culture
	Stabilization of the acidity levels
CHEESE IN BRINE	Growth control of the spoiling microorganisms
MOZZARELLA	Inactivation of *Listeria monocytogenes*
	Inhibition of *Pseudomonas fluorescens*
	Texture improvement throughout shelf life
	Growth control of the spoiling microorganisms
ICE-CREAM MIX	Growth control of the spoiling microorganisms

FRESH MOZZARELLA : CHALLENGE TEST PSEUDOMONAS

- Pack water was inoculated with 1-2 log cfu/mL *Pseudomonas fluorescens,* treated with 300 ppm LPS_F75 and stored at 7°C.

- No development of *Pseudomonas fluorescens* in mozzarella pack water treated with the LPS_F75

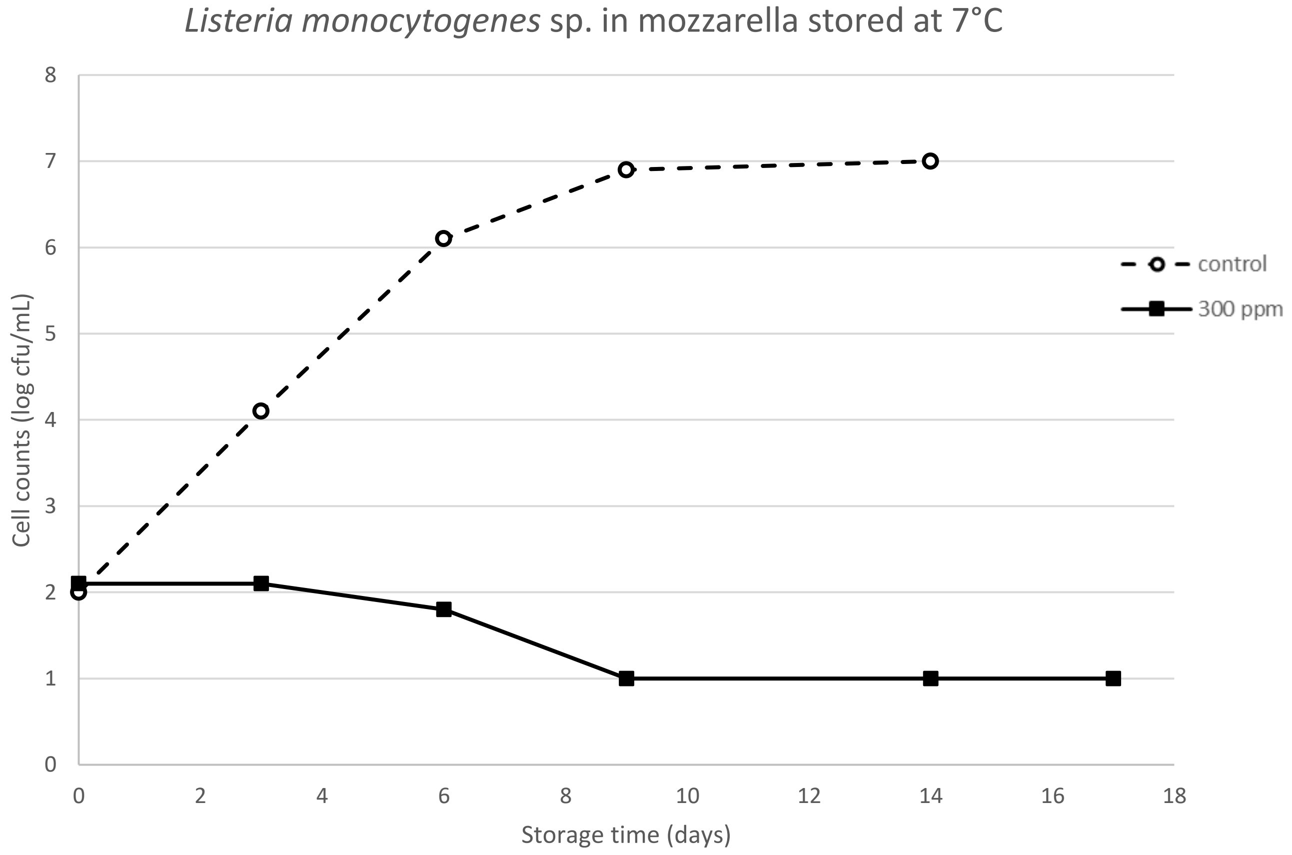


FRESH MOZZARELLA : CHALLENGE TEST LISTERIA

- Minced mozzarella was inoculated with a cocktail of *Listeria monocytogenes* at a level of 60 cfu/g, followed by addition of 300 ppm of LPS_F75 to the pack water and storage at 7°C.

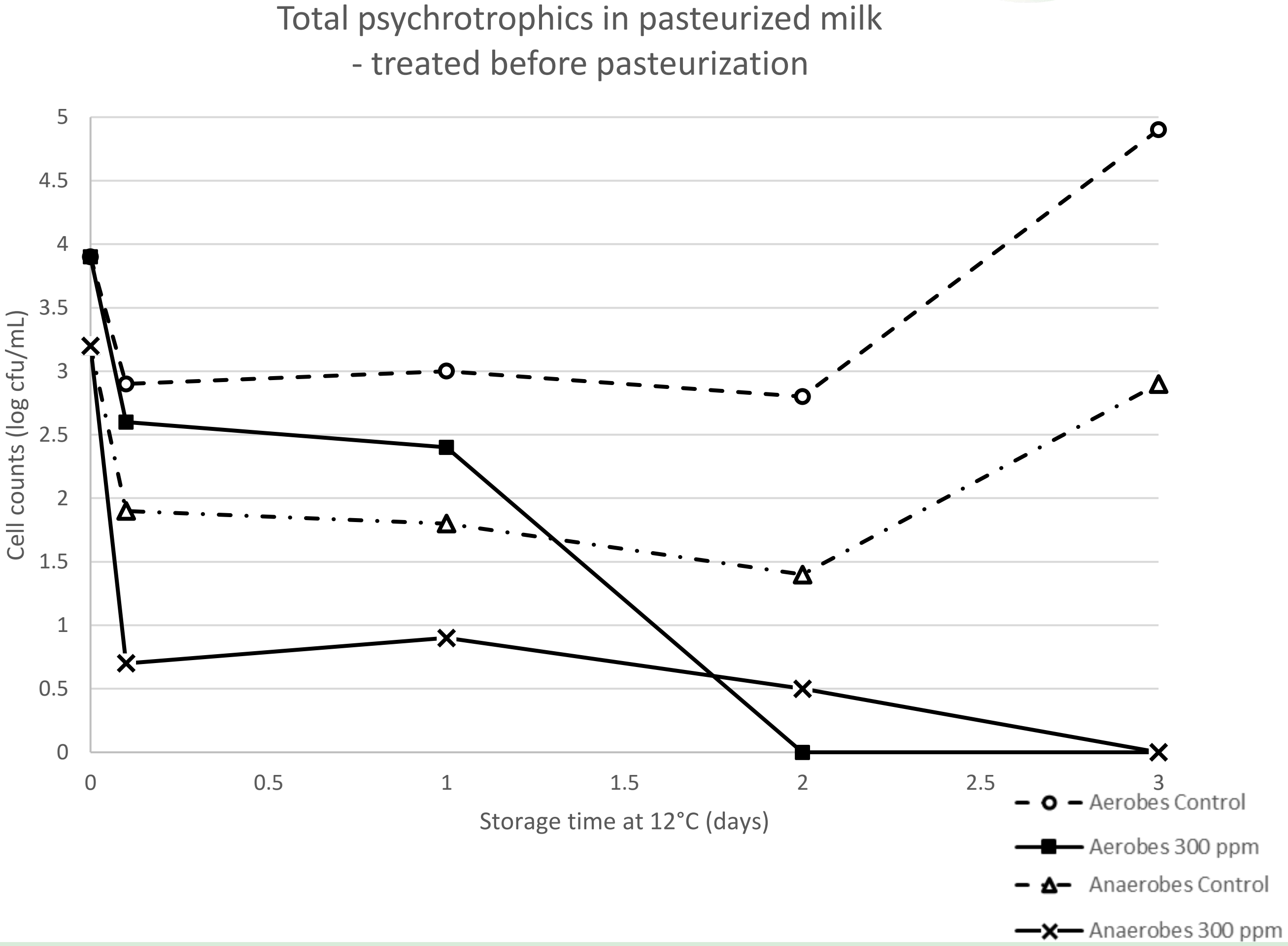
- Treatment with LPS_F75 effectively inhibits *Listeria* growth.

- LPS_F75 is an extra hurdle in the food safety management.



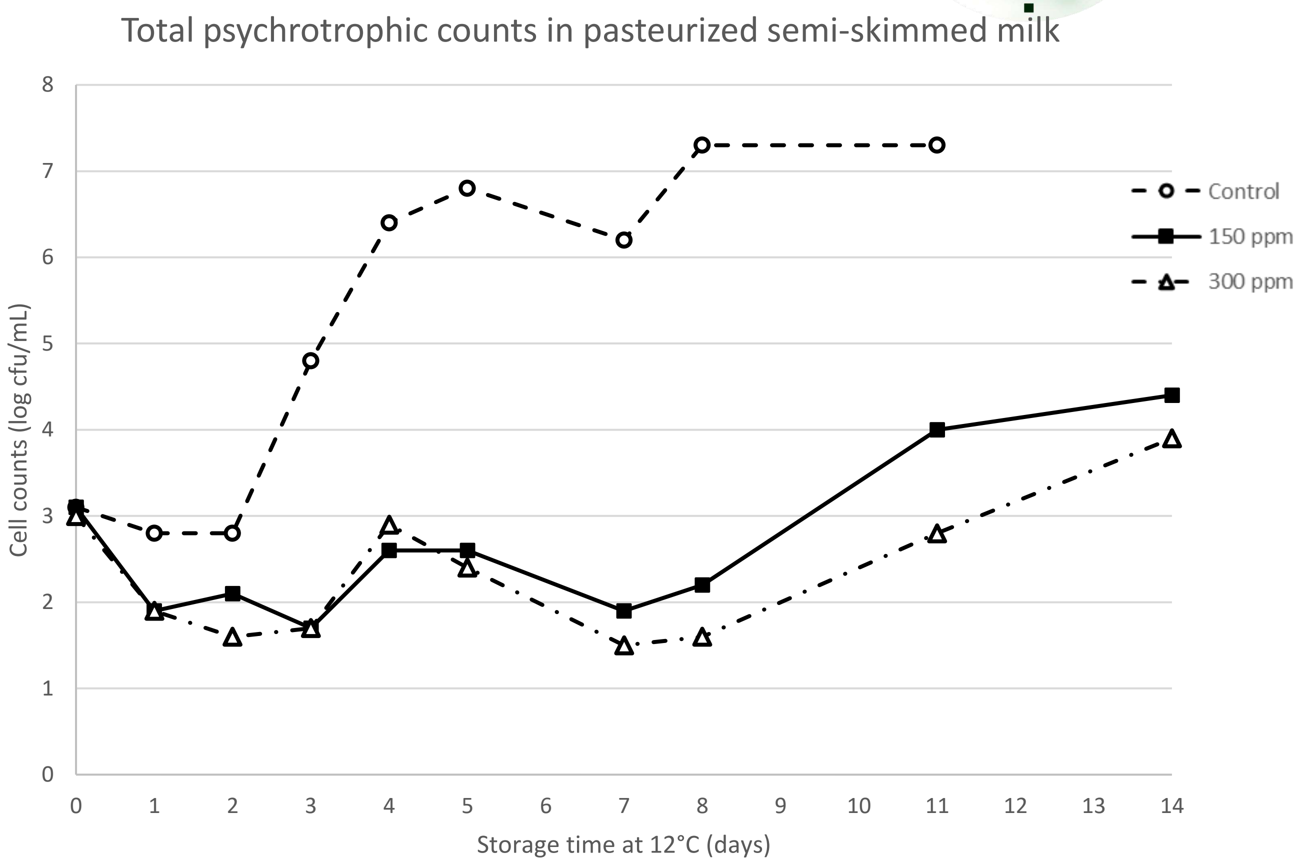
PASTEURIZED MILK (treatment before heating)

- LPS_F75 was added in raw fresh milk at a concentration of 300 ppm.
- After 8 hours at 7°C the raw milk was pasteurized for 15 sec. at 72°C and cooled down in ice water.
- The samples were stored at 12°C.
- The efficiency of the heat treatment was improved with the use of LPS_F75.



PASTEURIZED MILK (treatment after heating)

- LPS_F75 was added to pasteurized semi-skimmed milk in different concentrations: 0 ppm, 150 ppm, 300 ppm.
- The semi-skimmed milk was incubated at 12°C.
- The LPS system reduced the growth of the psychrotrophic bacteria.

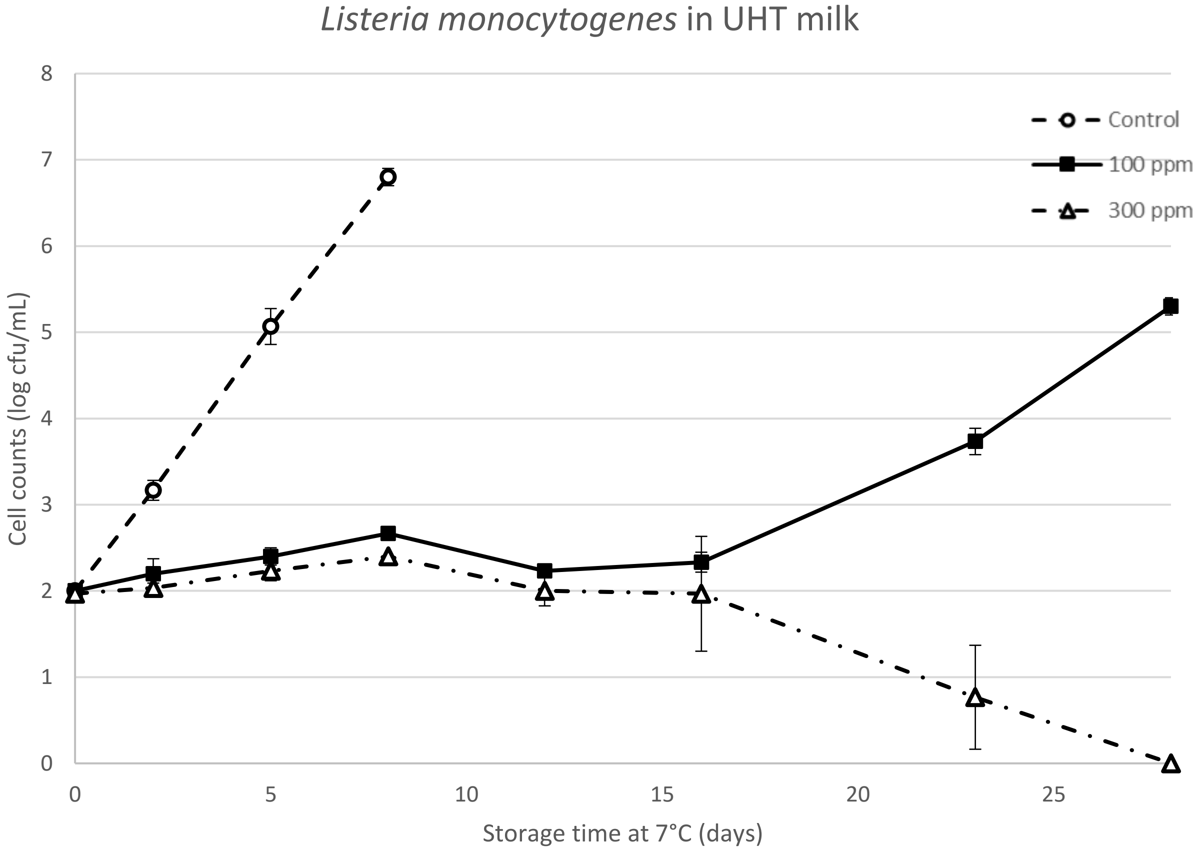


MILK : CHALLENGE TEST LISTERIA

- UHT milk which was aseptically divided into smaller portions and inoculated with a cocktail of *L. monocytogenes* strains at a level of 50 CFU/mL was treated with LPS_F75 in different concentrations: 0, 100 and 300 ppm and stored at 7°C.

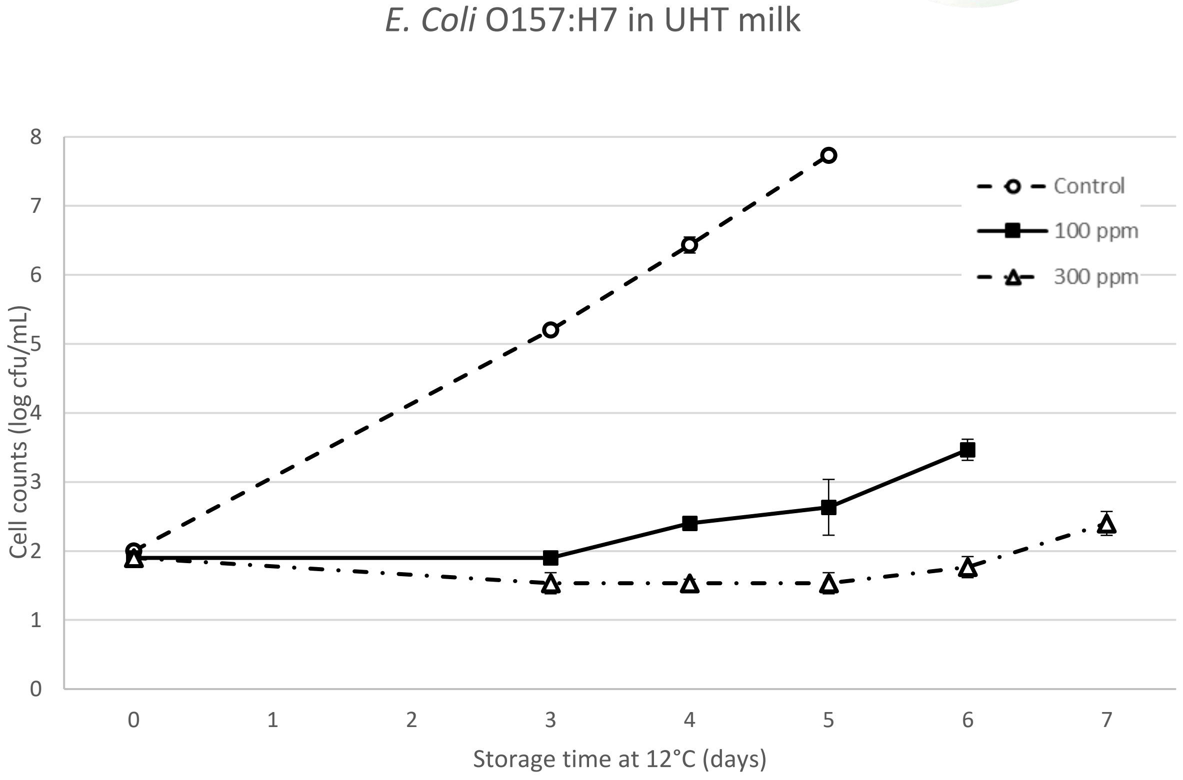
- The performed challenge tests prove the anti-listeria effect of LPS_ F75 in UHT milk stored at 7°C.

- The intermediate concentration gave a growth delay (longer lag phase and slower growth rate) while the highest concentration induced an inactivation of the target micro-organism over time.



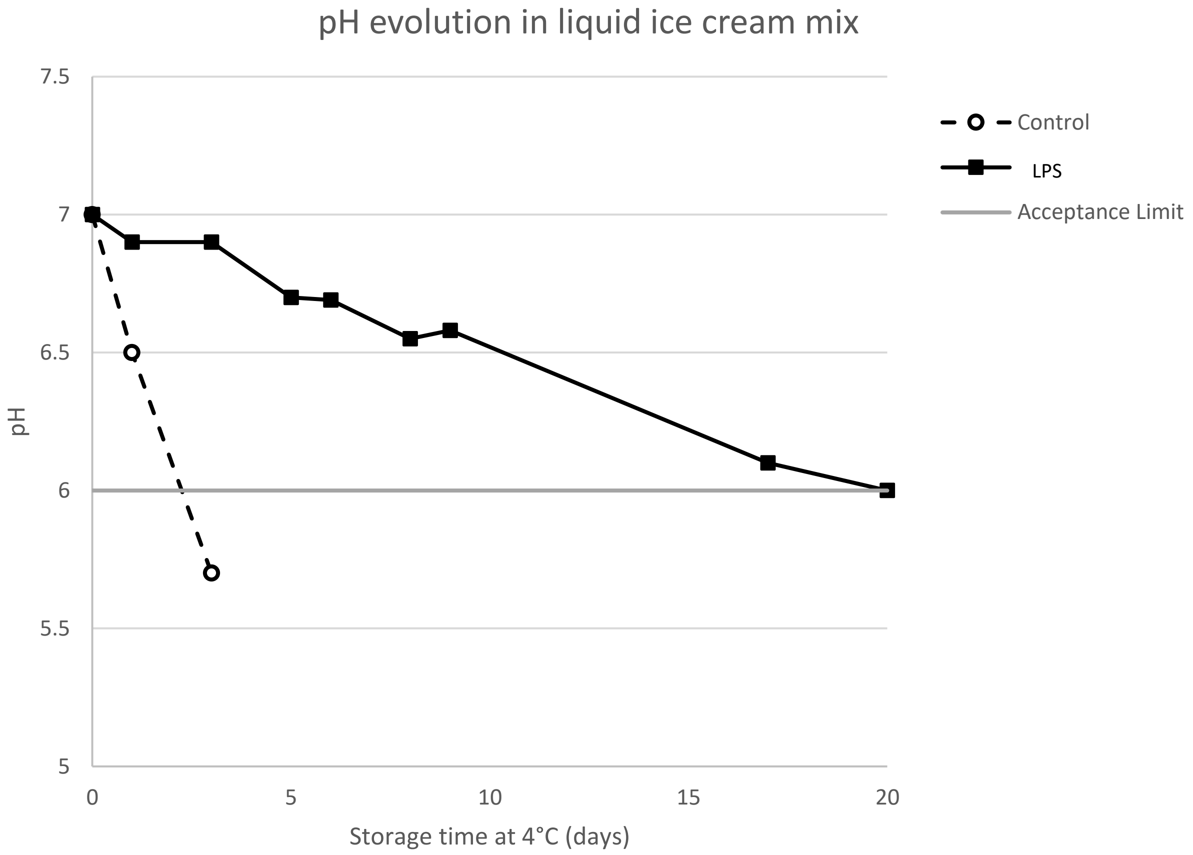
MILK : CHALLENGE TEST *Escherichia coli* O157:H7

- Semi-skimmed UHT milk was inoculated with a cocktail of *Escherichia coli* O157:H7 strains at a level of 50 cfu/ml.
- LPS_F75 was added in different concentrations: 0ppm, 100ppm, 300ppm. The milk was divided in portions and stored at 12°C.
- LPS_F75 clearly suppressed the growth of a mixture of *Escherichia coli* O157:H7 strains inoculated.
- In milk at 300 ppm, no growth of the pathogen was observed after 7 days.



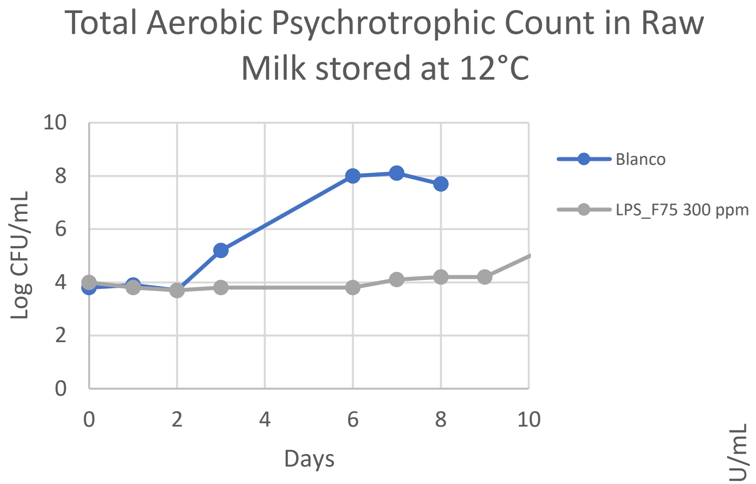
ICE CREAM MIX : PH EVOLUTION

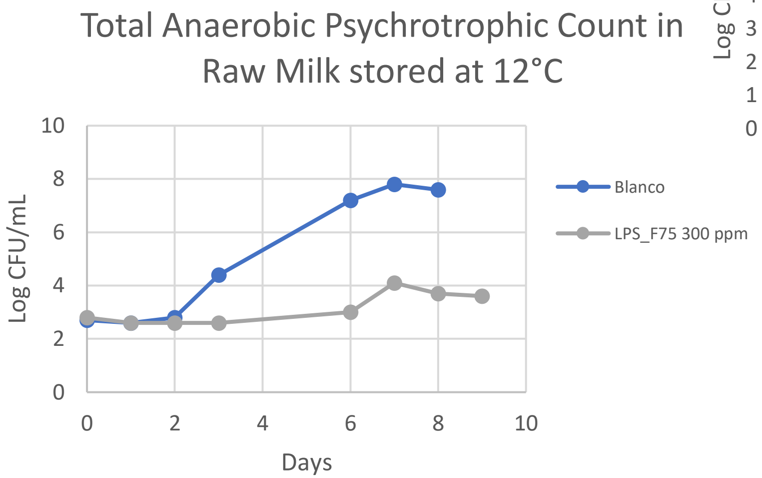
- LPS was added before pasteurization (150 ppm) and after pasteurization (150-250 ppm).
- With LPS pH was acceptable for 20 days.
- Without LPS, pH was below acceptance threshold after 3 days.

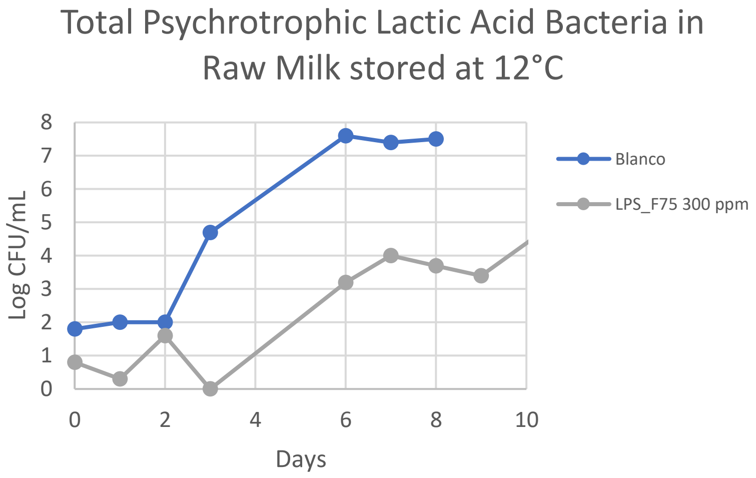


EFFECT OF PASTEURIZATION

- LPS_F75 was added in raw milk.
- After 4 hours at 4°C the raw milk was pasteurized for 15 sec. at 72°C.
- The samples were stored at 12°C.
- When using LPS in the raw milk, we observe a growth delay (longer lag phase and slower growth rate).

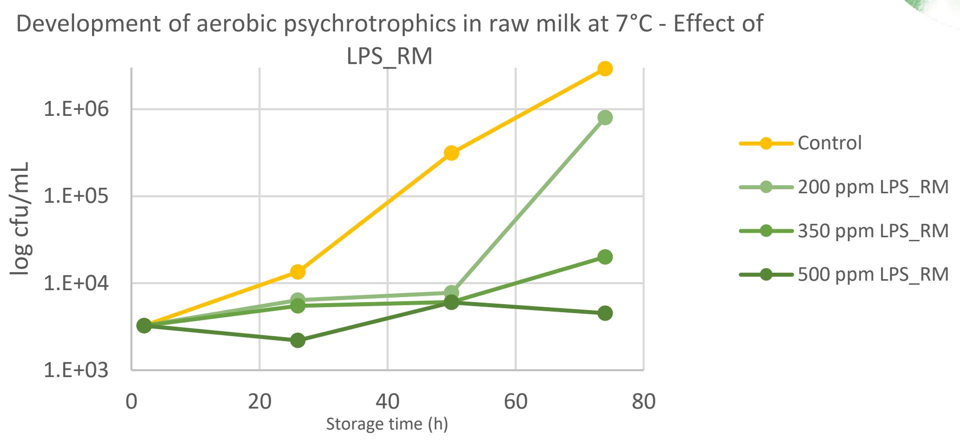


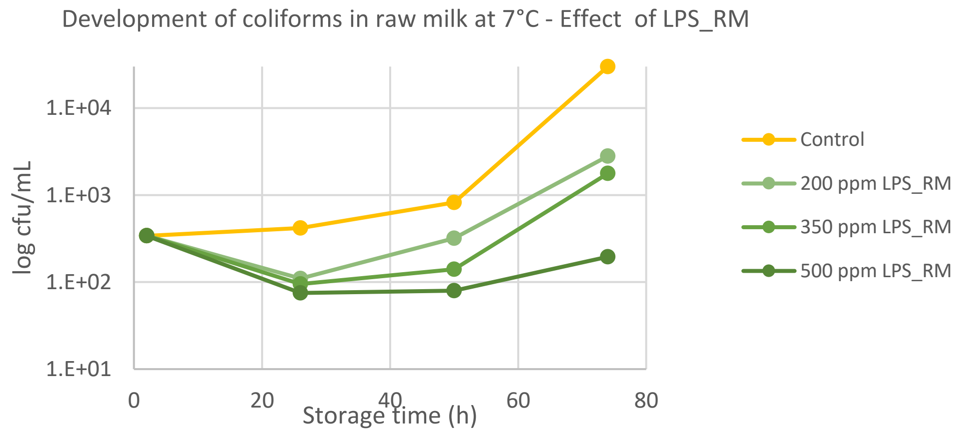




INDUSTRIAL TEST M

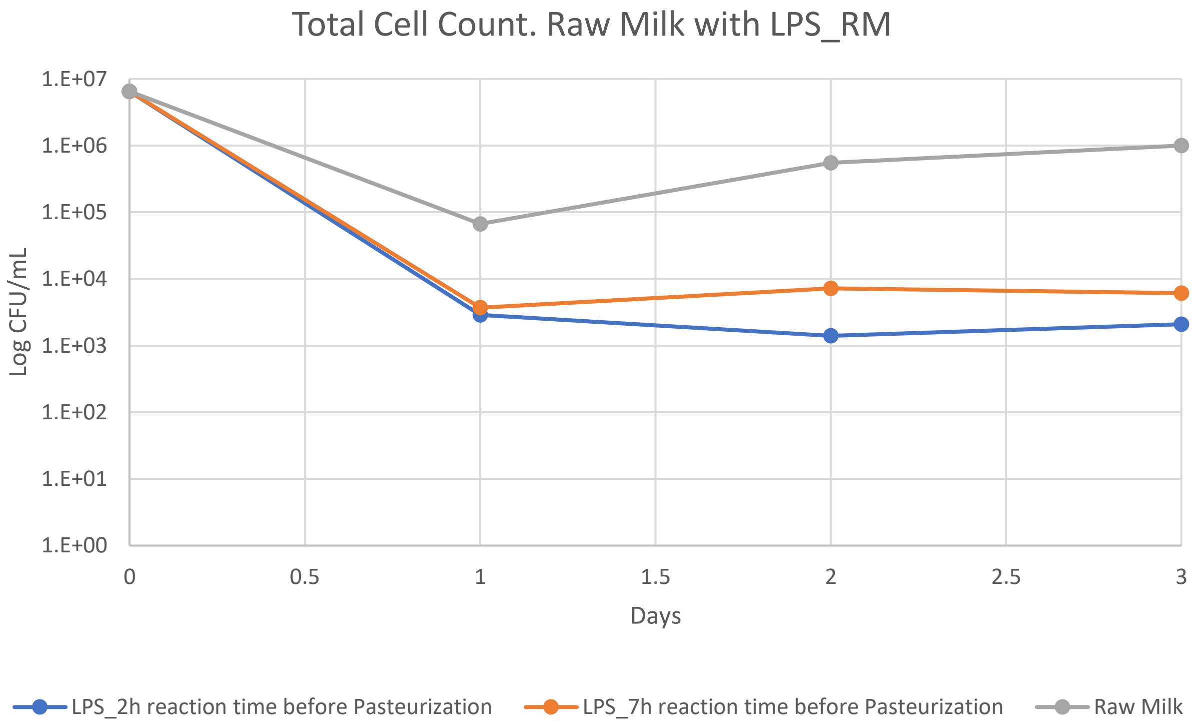
- LPS_F75 was added in raw milk.
- The samples were stored at 7°C for 3 days.
- When using LPS in the raw milk, we observe a growth delay (longer lag phase and slower growth rate).





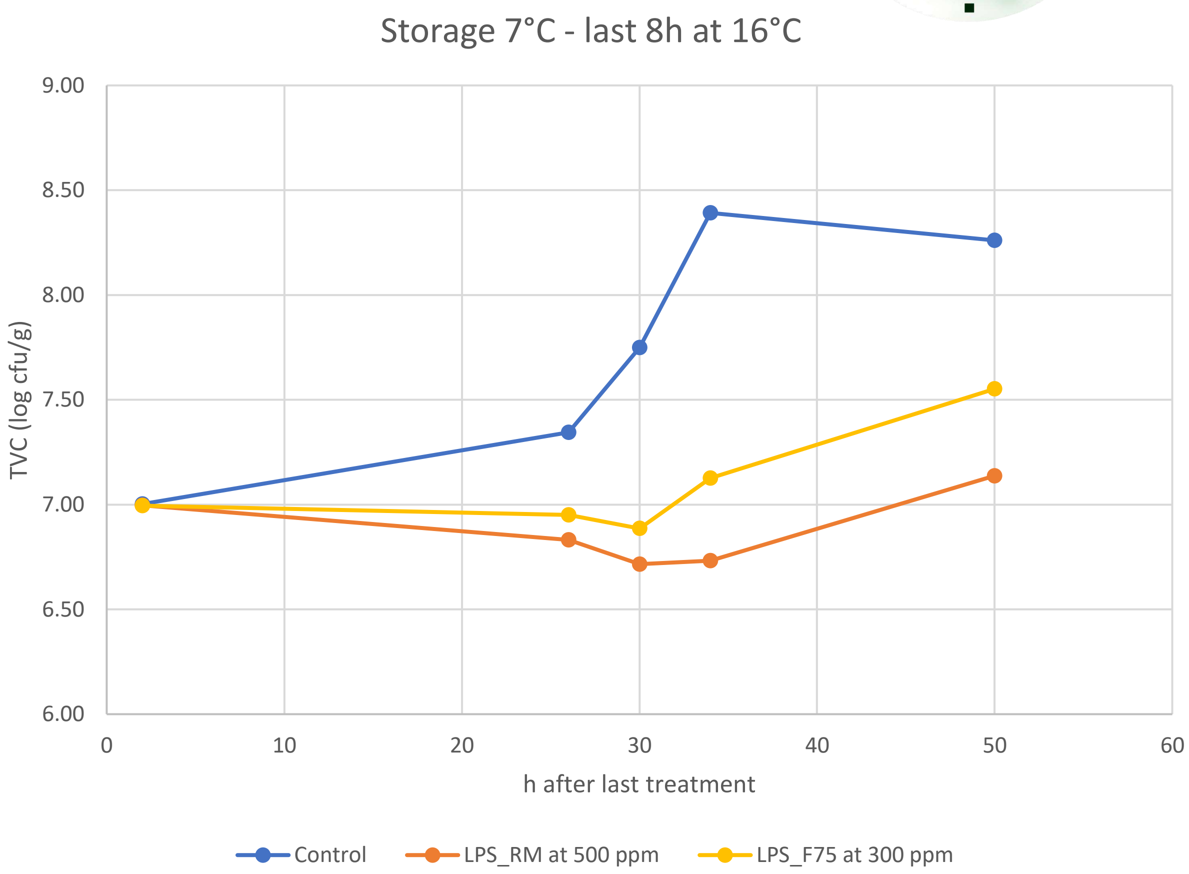
INDUSTRIAL TEST Z – Effects after pasteurization

- LPS_F75 was added in raw milk.
- The milk was then pasteurized at 72°C/15 seconds
- The samples were stored at 7°C for 3 days.
- The use of LPS increases the efficiency of the heat treatment by decreasing cell counts and delaying microbial growth (longer lag phase and slower growth rate).



INDUSTRIAL TEST B

- LPS_F75 was added in raw milk.
- The samples were stored at 7°C for 2 days.
- The last 8 hours the milk was at 16°C due to transport conditions.
- When using LPS in the raw milk, we observe a growth delay (longer lag phase and slower growth rate).



INDUSTRIAL TEST Farm A_ZA

- 3 repetitions
- LPS_F75 was added in raw milk.
- Samples were analysed the day after (>20 h)
- When using LPS in the raw milk, we observe a growth delay.

Summary

- LPS exerts bacteriostatic activity.
- It is naturally present in milk.
- It is already applied to dairy matrixes to improve the sensorial and microbiological quality and extend shelf life.
- It is a tool to prevent food waste.

Thank you for your attention

QUESTIONS?