

THE SMART NATURAL SOLUTION



BIENÇA

SEA-i[®], bringing a by nature inspired antimicrobial system to the food industry

SASDT WEBINAR

19/07/2023

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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.



Bienca's partners world-wide



Alimentum Natura d.o.o.



Effects of undesired microbial growth

Undesired microbial growth is responsible for deterioration of the quality of a food product. This can result in :

- **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) not always possible due to impact on product characteristics
- Chemical additives (e.g. sorbates and benzoates, buffered organic acids) are being related to negative side-effects and are less and less accepted by the consumer
- Protection based on natural systems



Source : Internation Journal of Scientific and Research Publications, 2015

What is SEA-i[®]?

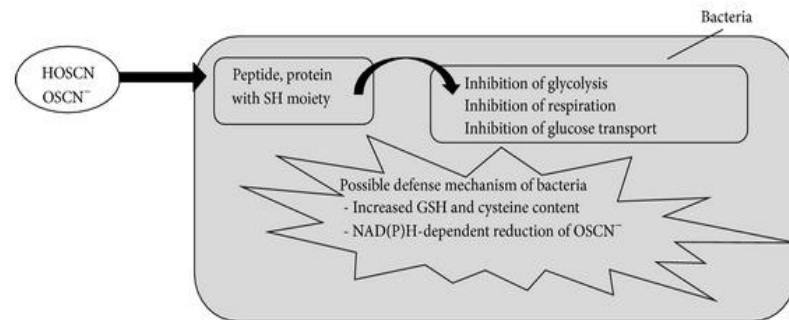
- SEA-i[®] is based on the lactoperoxidase system (LPS), a natural antimicrobial system



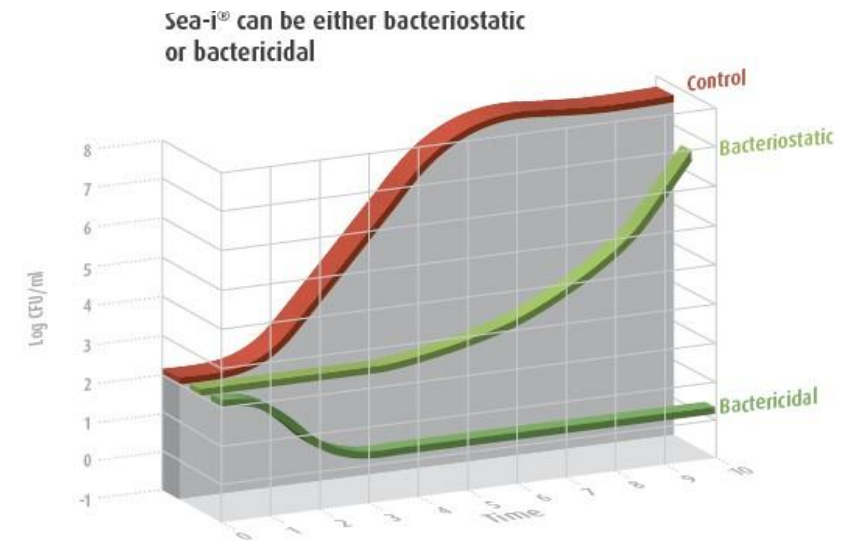
- SEA-i[®] can be used as a label-friendly processing aid and is an agent against microbial spoilage of food products as alternative to chemical preservatives

Antimicrobial activity of the Lactoperoxidase System

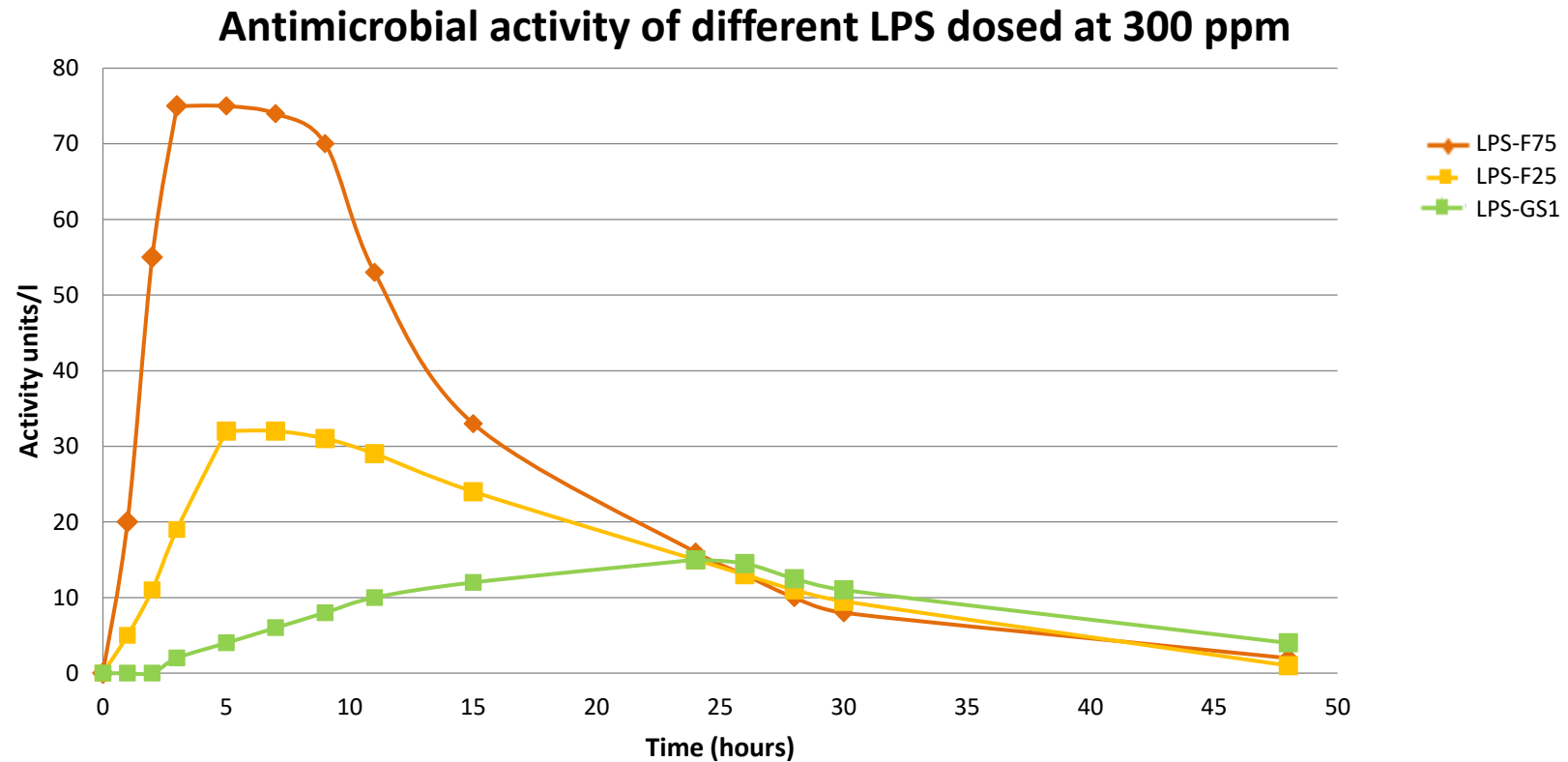
- The system is widely present in nature (e.g. mother's milk, tears, saliva) and involved in host-defence mechanisms
- It generates an unstable substance, with a strong and selective antimicrobial activity, reacting with thiol groups in membrane proteins of micro-organisms, leading to reduced growth (bacteriostatic) and even killing (bacteriocidal)



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

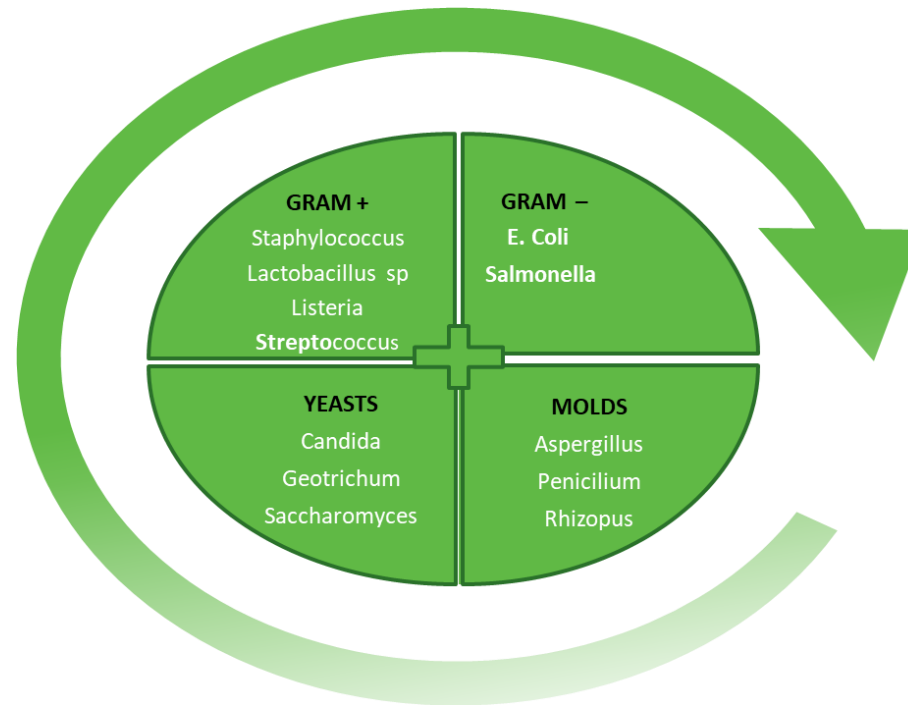


Antimicrobial activity of products based on the Lactoperoxidase System (LPS)



Antimicrobial activity of the Lactoperoxidase System

Through its antimicrobial action, the Lactoperoxidase System offers a broad protection and is an additional hurdle in food safety system





Application areas

Dairy applications



RAW MILK	Growth control of psychrotrophic bacteria
MILK	Inactivation of <i>Listeria monocytogenes</i> Inhibition of <i>Escherichia coli</i> 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 <i>Listeria monocytogenes</i> Inhibition of <i>Pseudomonas fluorescens</i> Texture improvement throughout shelf life Growth control of the spoiling microorganisms
ICE-CREAM MIX	Growth control of the spoiling microorganisms



Fresh Cheeses

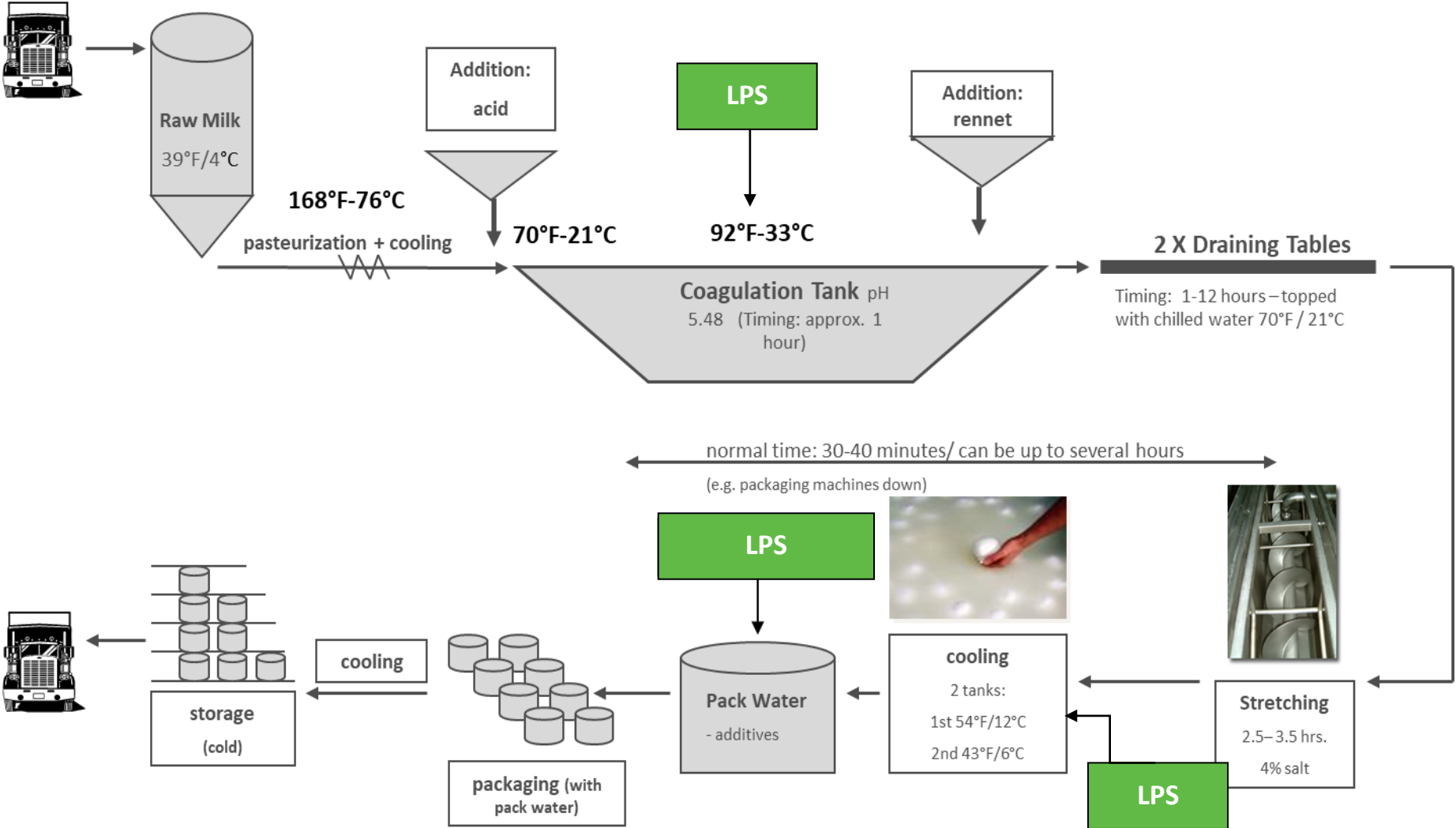




Fresh Mozzarella



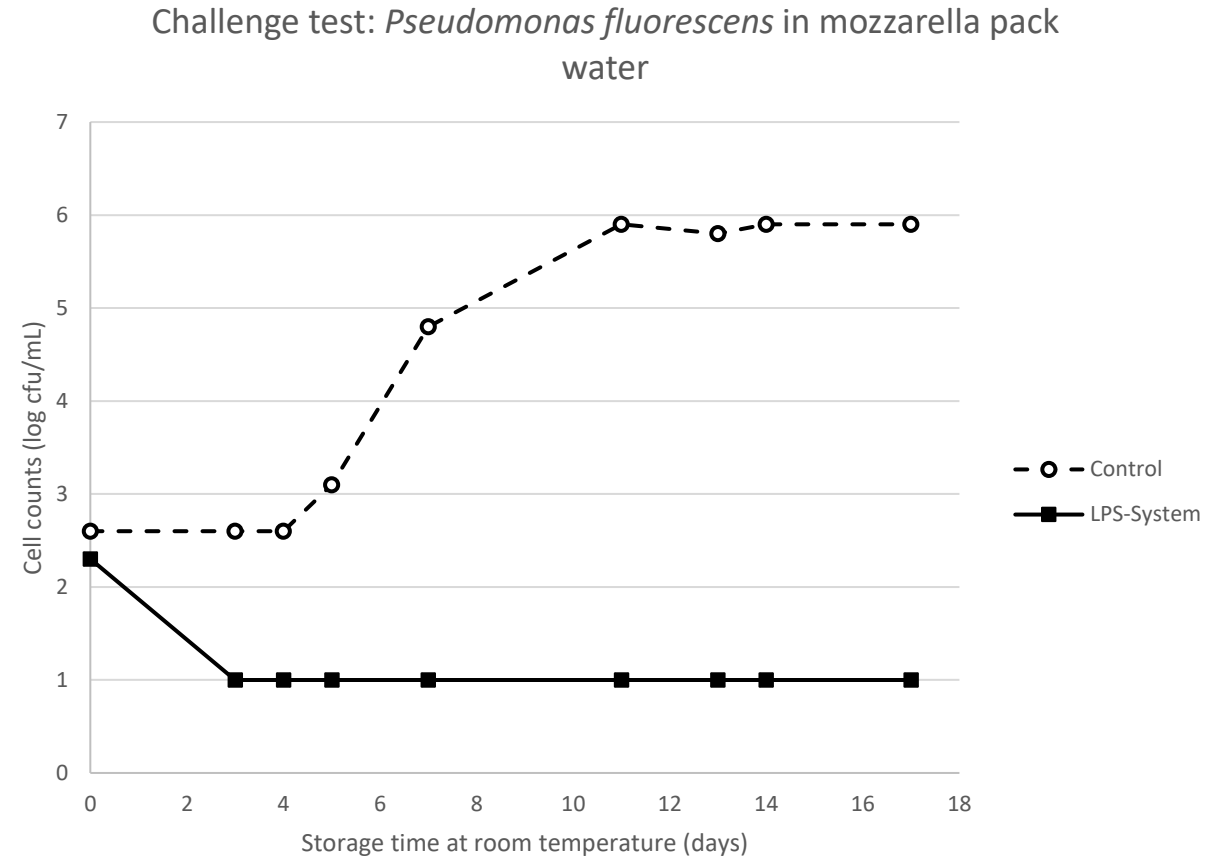
Production process fresh mozzarella



FRESH MOZZARELLA : CHALLENGE TEST PSEUDOMONAS



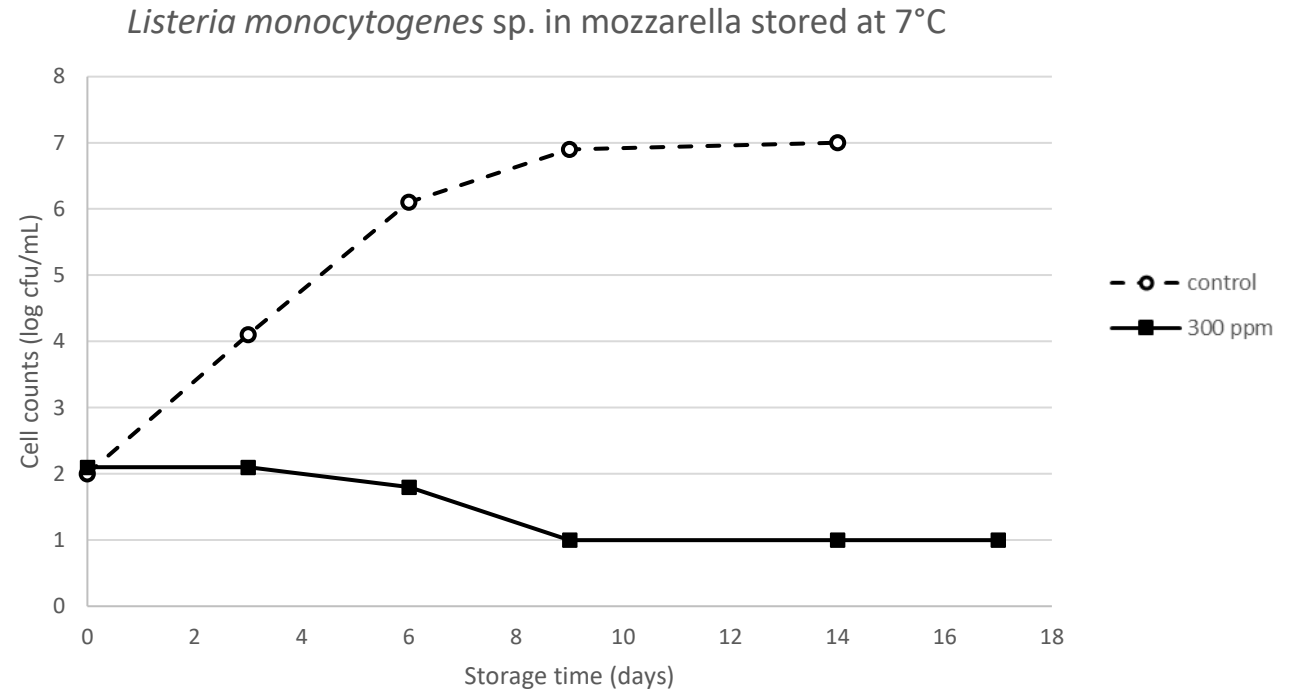
- Pack water was inoculated with 1-2 log cfu/mL *Pseudomonas fluorescens*, treated with 500 ppm LPS_F75 and stored at 7°C.
- No development of *Pseudomonas fluorescens* in mozzarella pack water treated with the LPS_F75



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



FRESH MOZZARELLA : IN PACK WATER



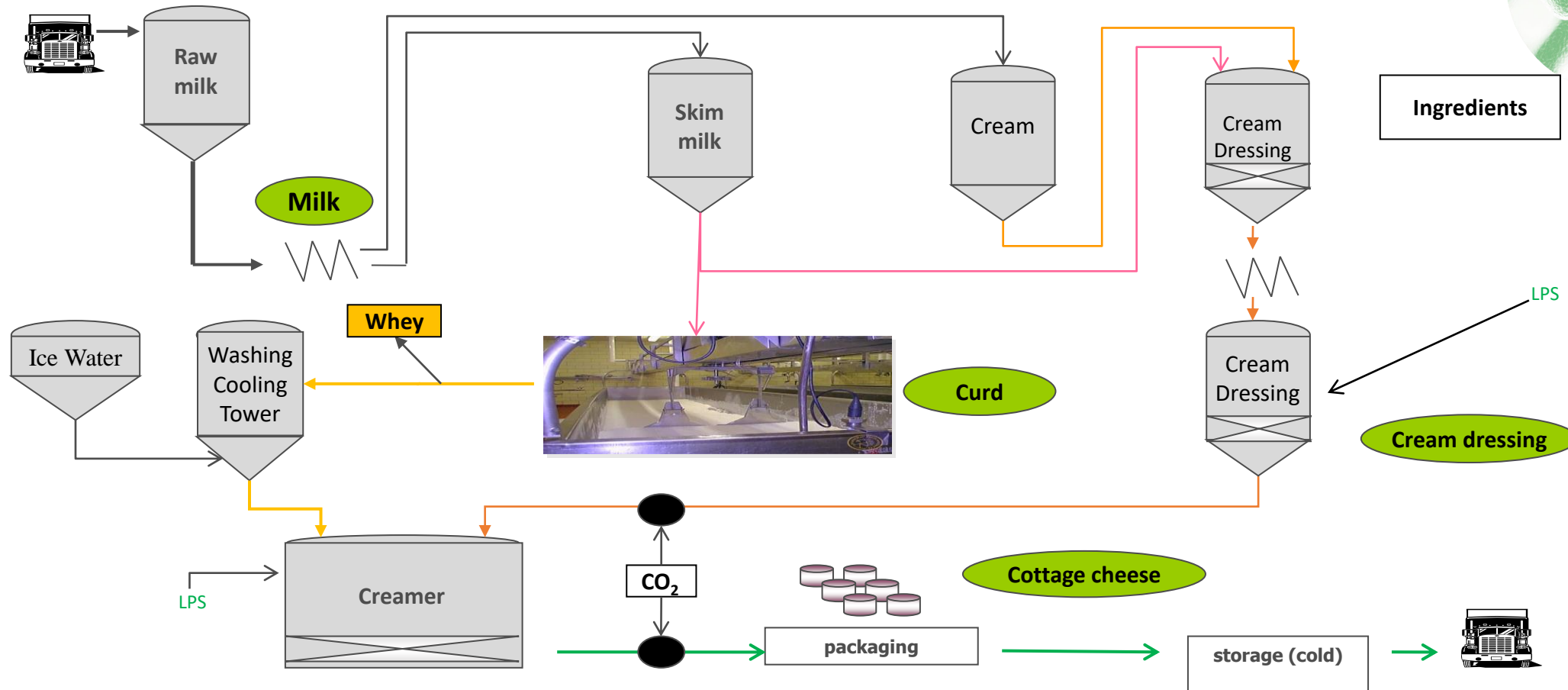
- LPS maintains the quality of the product for 1 month.
- Without SEA-i[®] the pH was below acceptance threshold after 3 days (data not shown)

Consistency of Fresh Mozzarella		
Days	Control	0,04% SEA-I in Pack Water
0	OK	OK
5	Slightly soft	OK
10	Slightly soft	OK
15	Soft	OK
20	Softer	OK
25	Too soft	OK
30	Too soft	OK

Cottage Cheese



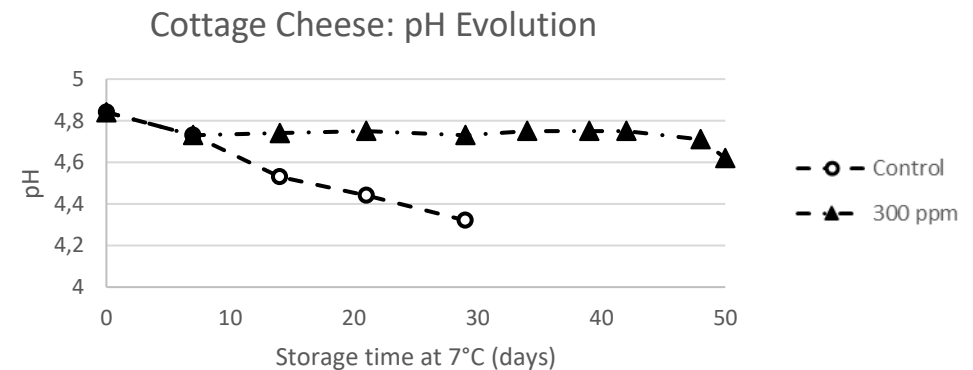
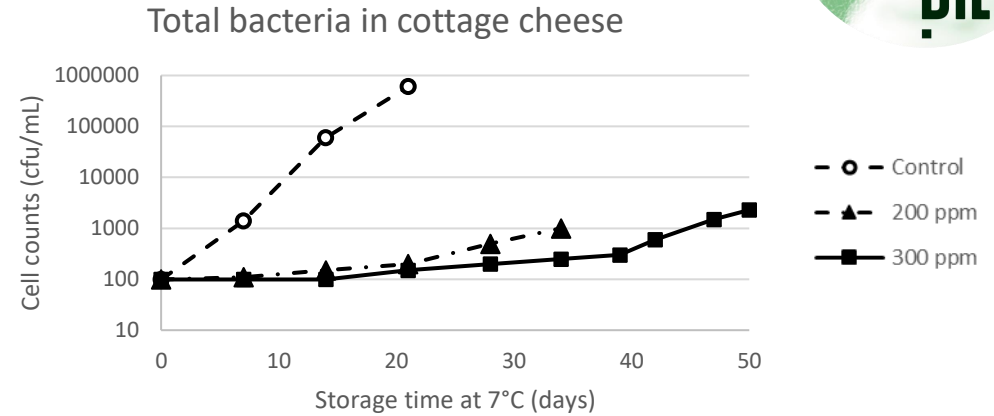
Cottage Cheese



LPS is sprinkled on the curd just prior to dressing (open vat: in vat; with creamer: in creamer)

COTTAGE CHEESE

- LPS was inoculated in the dressing before being mixed with the curd grains.
- The treated Cottage Cheese was stored at a temperature of 7 °C.
- Treatment with LPS of the cottage cheese is effectively controlling the growth of the microbiota and the redevelopment of the residual culture.
- Treatment with LPS of the cottage cheese is stabilizing the pH during shelf life .



Queso Fresco

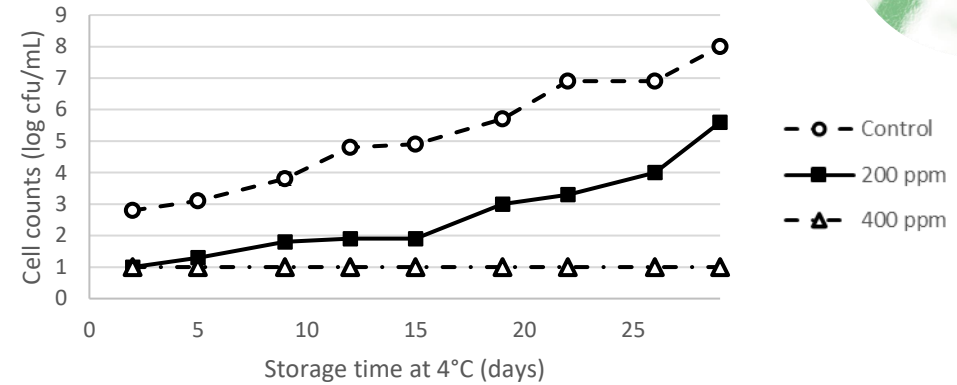


QUESO FRESCO

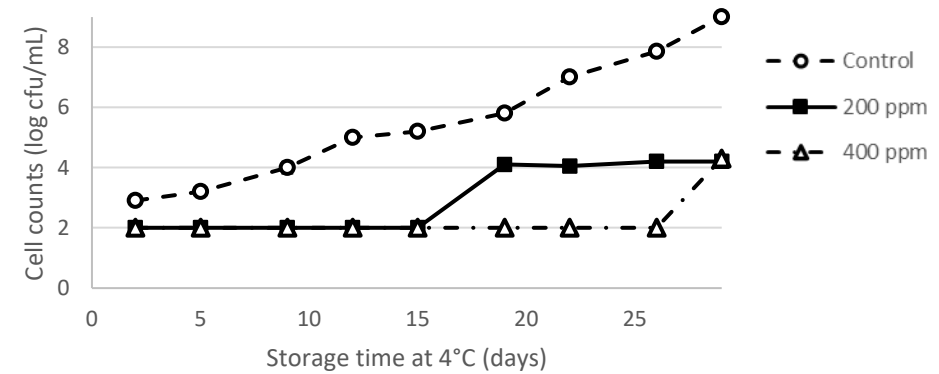
- Fresh Burgos cheese was produced with addition of the LPS in the milk after pasteurization and standardization. The fresh cheese was stored at 4°C



Enterobacteria in queso fresco



Yeasts and moulds in queso fresco





Dairy Beverages



THE CHALLENGES OF THE DAIRY BEVERAGES INDUSTRY



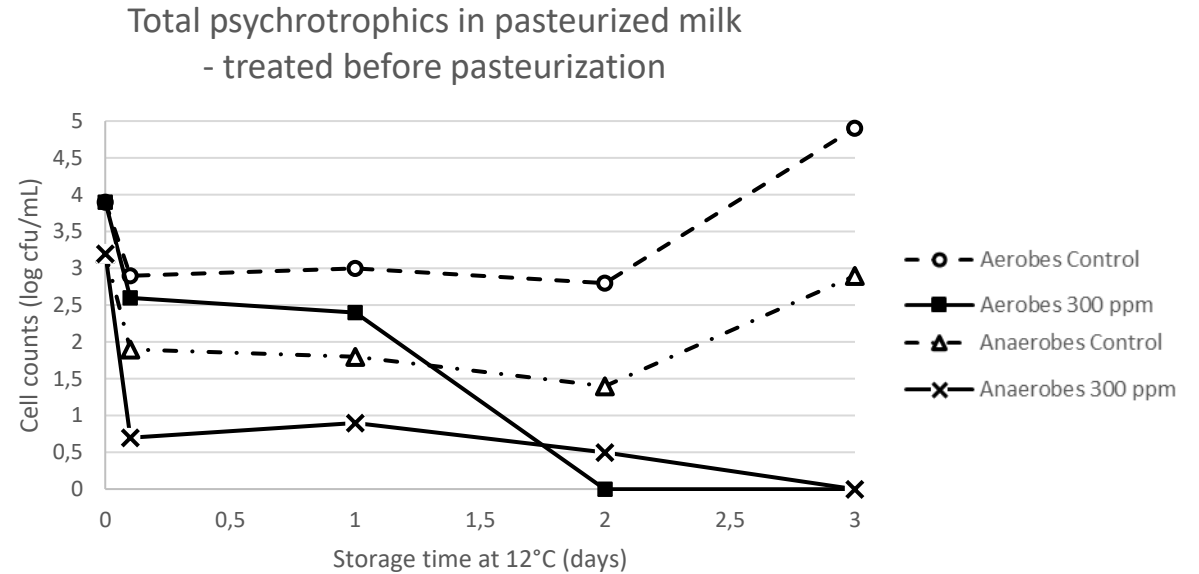
- **One of the biggest challenges of this industry is to find the right balance between shelf-life and original taste.**

- Selecting the right heating process T° is always a challenging choice for:
 - More shelf-life
 - or
 - More original taste and behavior

PASTEURIZED MILK (treatment before heating)



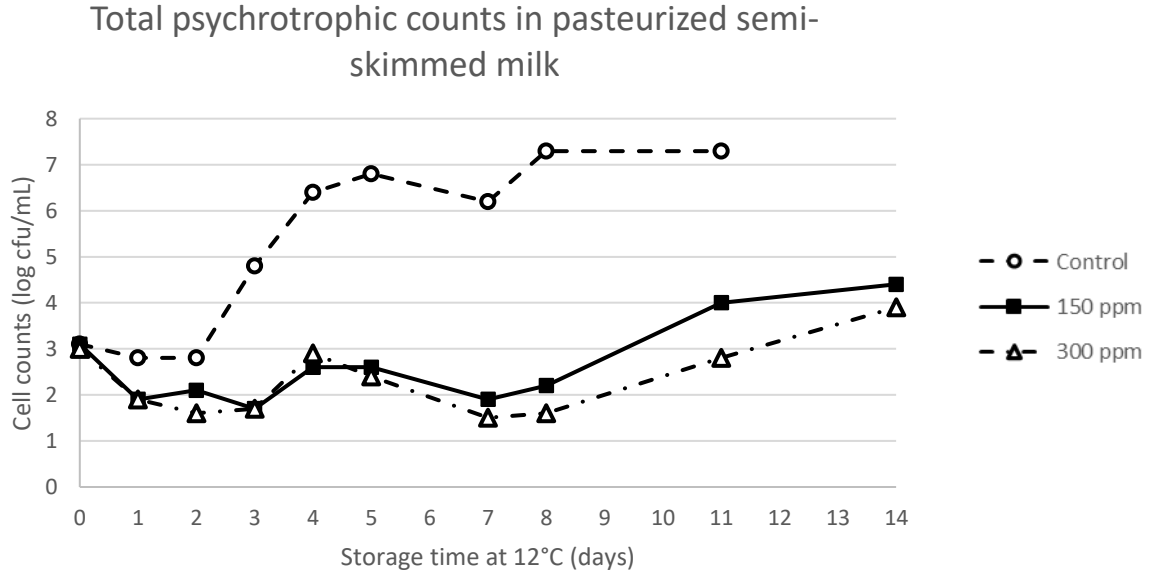
- LPS_F75 was added in raw fresh milk at a concentration of 300 ppm.
- After 8 hours at 7°C the raw milk was pasteurized for 15 sec. at 72°C and cooled down in ice water.
- The samples were stored at 12°C.
- The efficiency of the heat treatment was improved with the use of LPS_F75



PASTEURIZED MILK (treatment after heating)



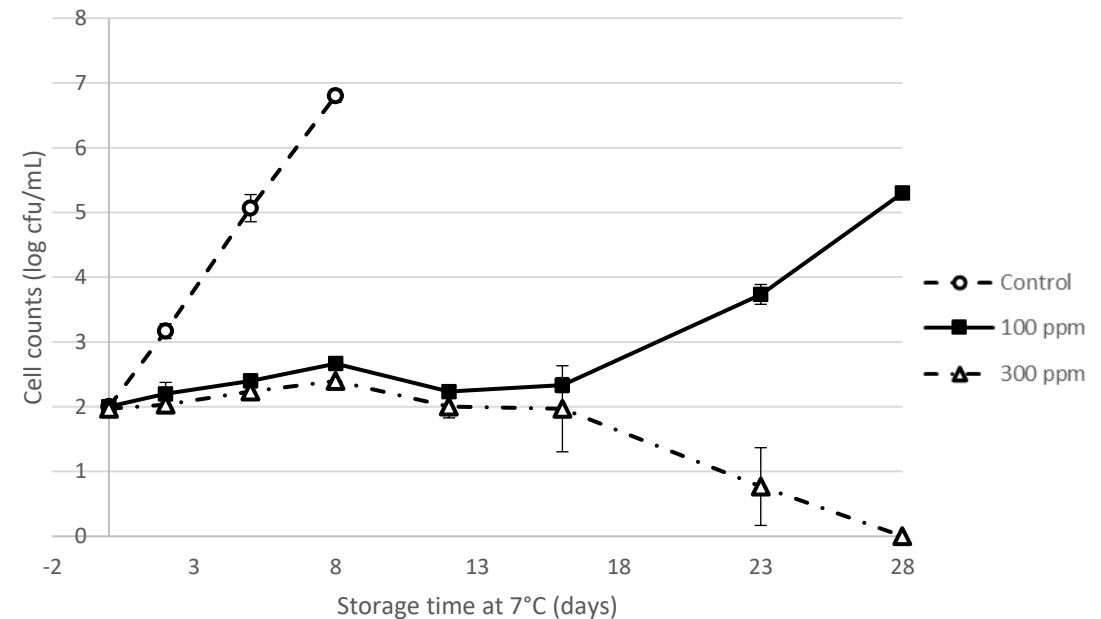
- LPS_F75 was added to pasteurized semi-skimmed milk in different concentrations (0 ppm, 150 ppm, 300 ppm).
- The semi-skimmed milk was incubated at 12°C.
- The LPS system reduced the growth of the psychrotrophic bacteria.



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.

Listeria monocytogenes in UHT milk

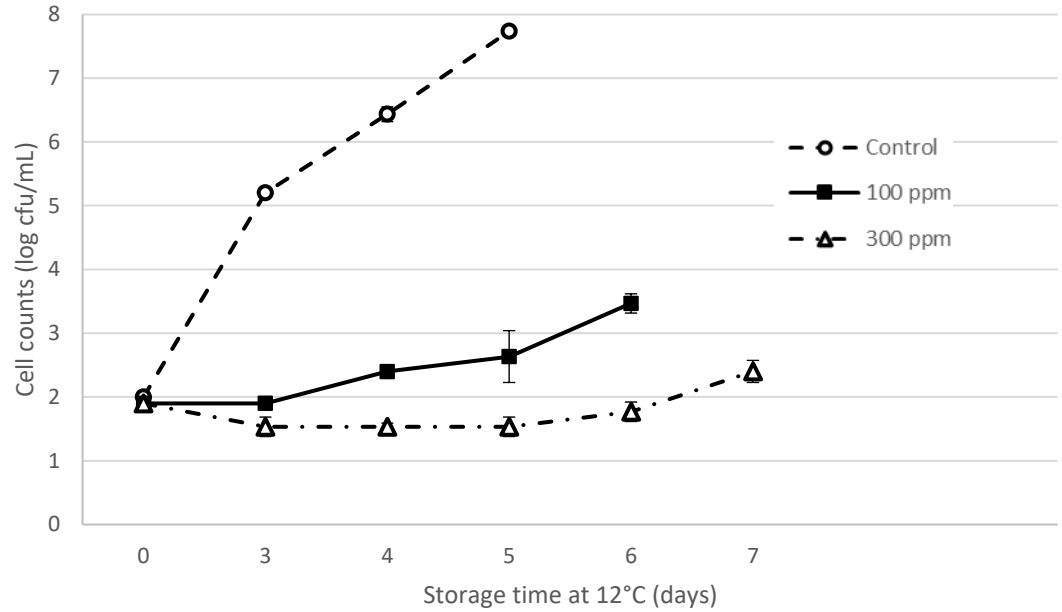


MILK : CHALLENGE TEST *Escherichia coli* O157:H7



- 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

E.Coli O157:H7 in UHT milk





Liquid Ice Cream Mix



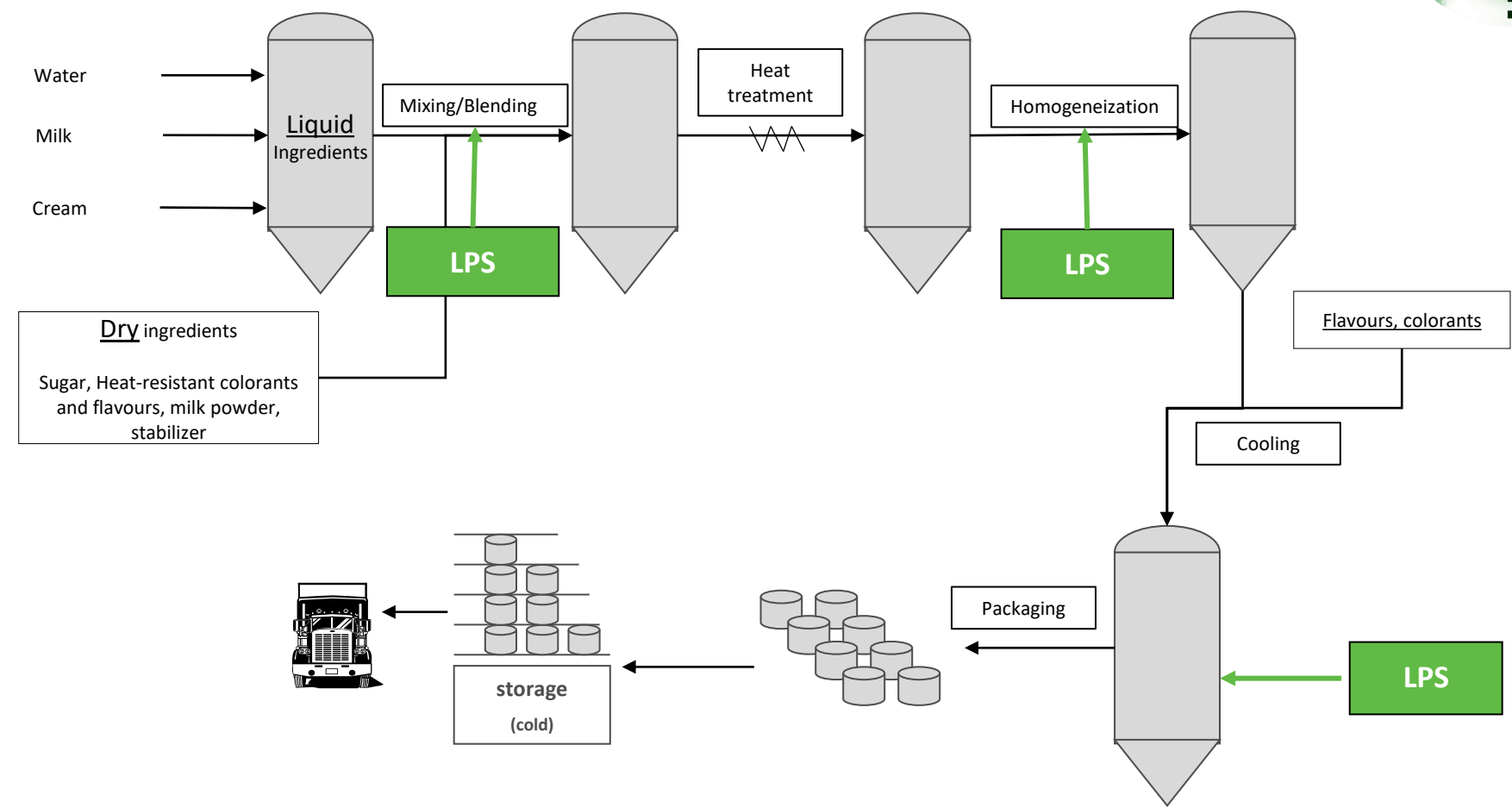
CHALLENGES FOR LIQUID ICE CREAM INDUSTRY

- **Mix has to be produced on order due to limited shelf life of the product.**
- **Unpredictable weather conditions have a big Influence on the sales of Ice cream.**
- **A lot of different and often small batches due to the assortment of flavors.**
- **Production planning**



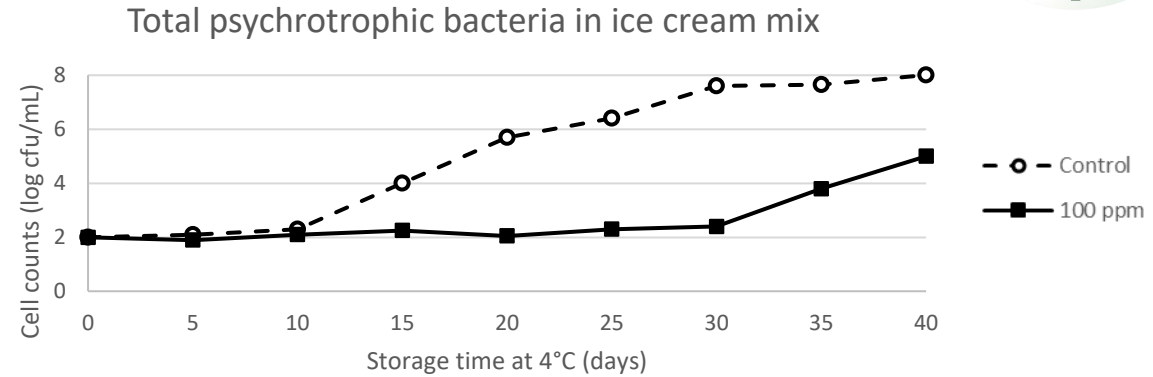


Production process Liquid Ice Cream mix



ICE CREAM MIX

- Ice cream mix was treated with 100 ppm LPS and shelf life was monitored by microbiological and organoleptic parameters.
- Treatment with 100 ppm LPS results in a shelf life extension of at least 20 days
- The longer shelf life allows to optimize production :
 - Process of larger batch sizes
 - Reduction in the number of productions during off season
 - Reduction in the production cost /start ups / shutdowns

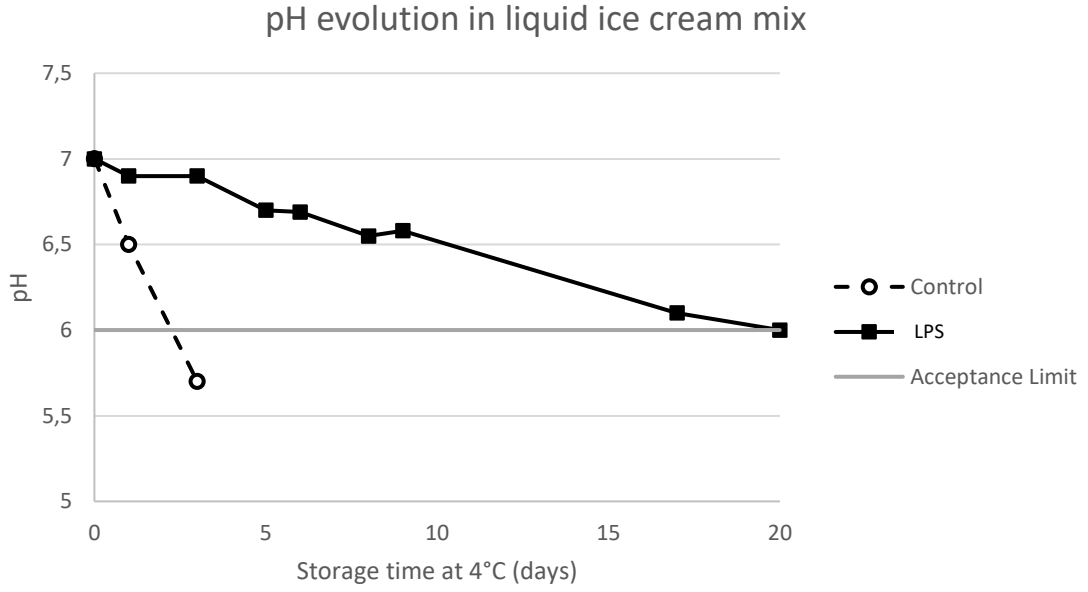


Storage at 4°C (days)	Control	0,01% LPS
1	OK	OK
6	OK	OK
11	OK	OK
15	OK	OK
20	Slight off flavor	OK
25	Sour	OK
29	Sour	OK
34	Sour	OK
39	Sour	OK
44	Sour	OK

ICE CREAM MIX : PH EVOLUTION



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



Raw Milk

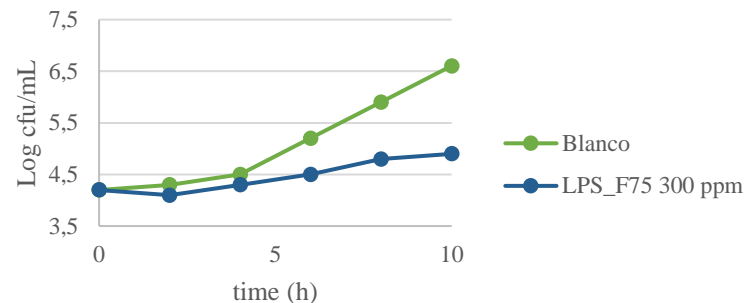




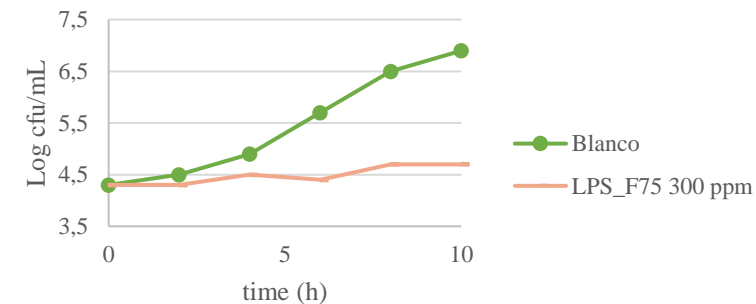
Lab tests at 25°C

- LPS_F75 was added in raw fresh milk.
- The samples were stored at 25°C.
- When using LPS in the raw milk, we observe a growth delay (longer lag phase and slower growth rate).

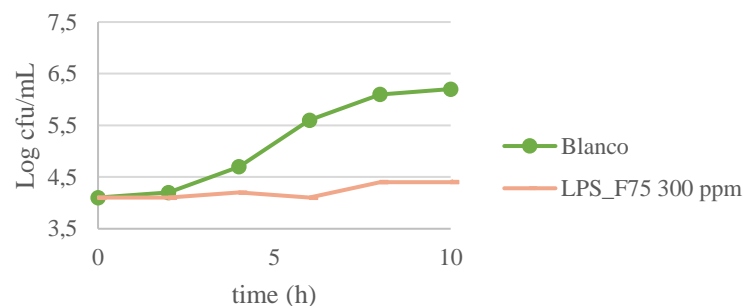
TVC in raw milk stored at 25°



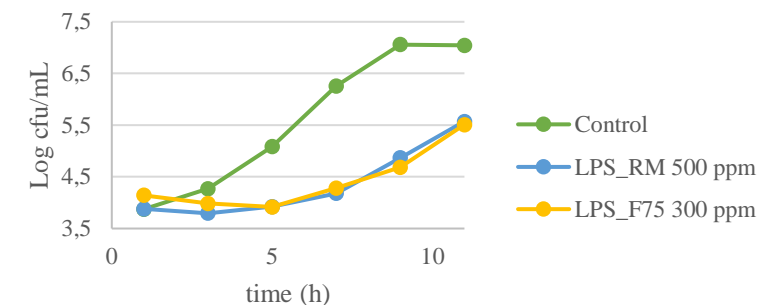
TVC in raw milk at 25°C – repetition 1



TVC in raw milk at 25°C – repetition 2



TVC in raw milk at 25°C – repetition 3

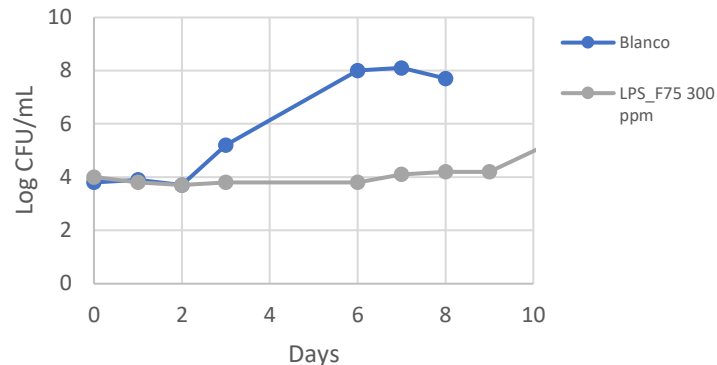




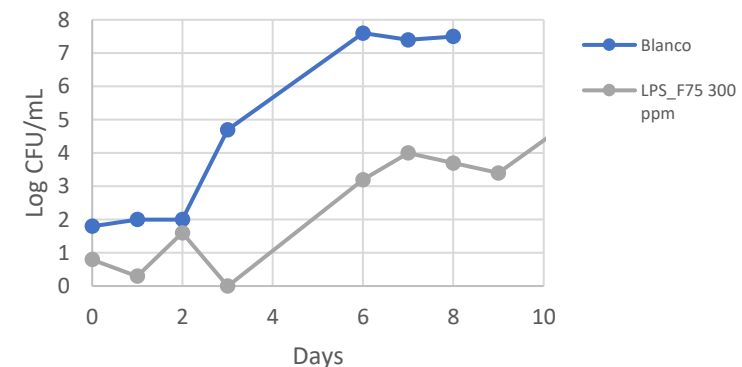
Effect of pasteurization

- 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).

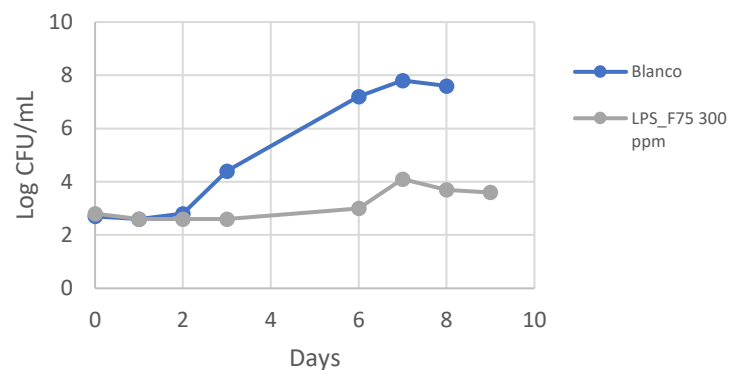
Total Aerobic Psychrotrophic Count in Raw Milk stored at 12°C



Total Psychrotrophic Lactic Acid Bacteria in Raw Milk stored at 12°C



Total Anaerobic Psychrotrophic Count in Raw Milk stored at 12°C

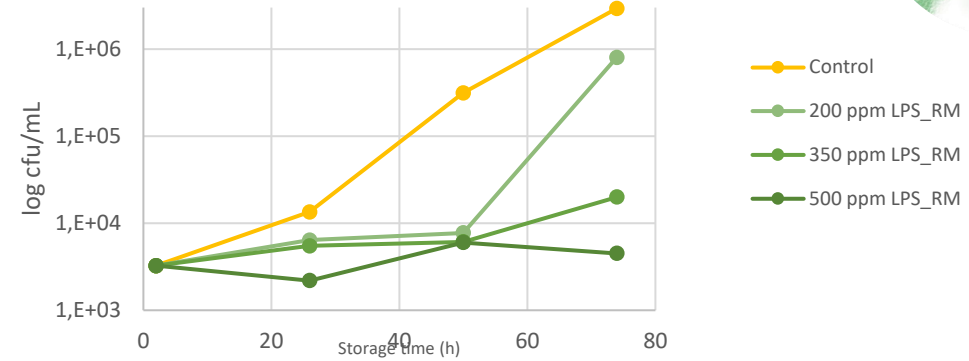




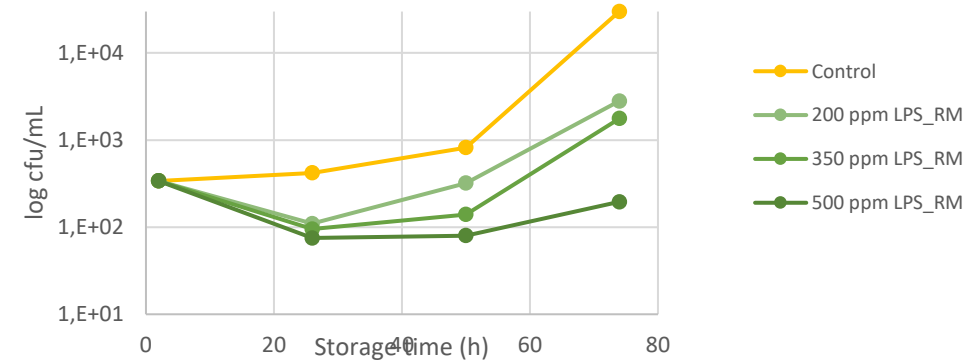
INDUSTRIAL TEST M

- 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).

Development of aerobic psychrotrophics in raw milk at 7°C - Effect of LPS_RM

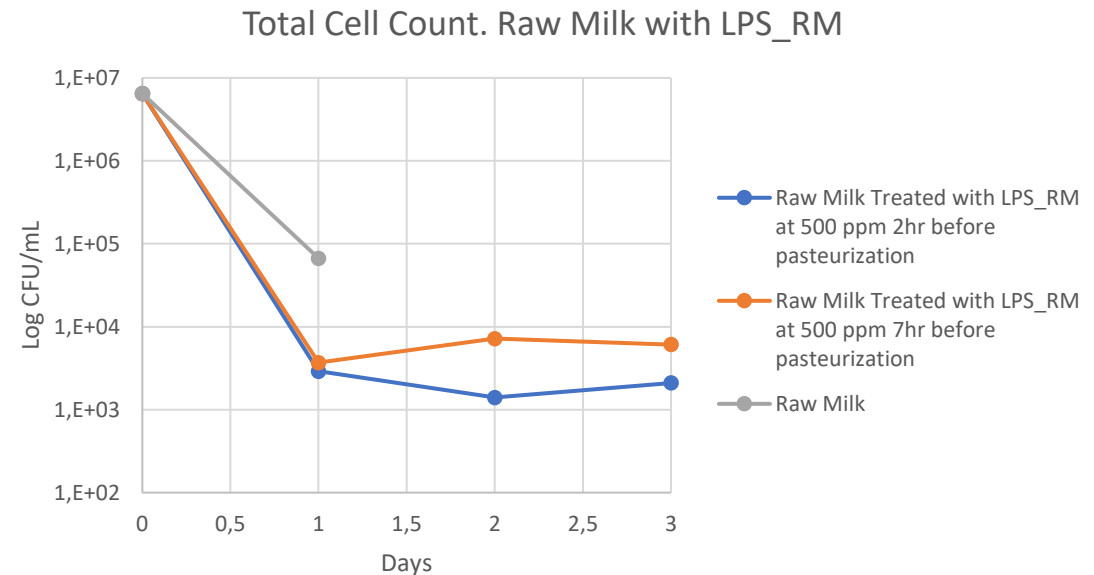


Development of coliforms in raw milk at 7°C - Effect of LPS_RM



Industrial Test Z

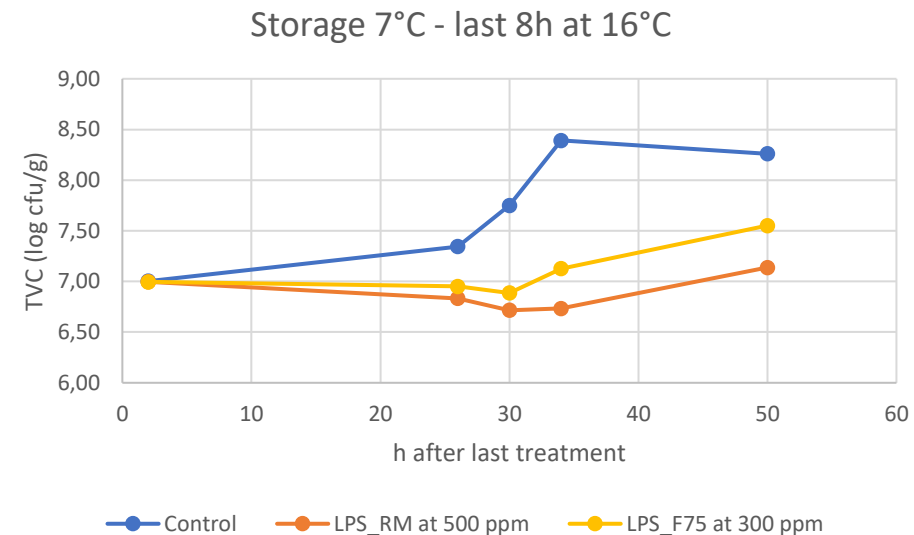
- LPS_F75 was added in raw milk.
- The samples were stored at 7°C for 3 days.
- When using LPS in the raw milk, we observe a growth delay (longer lag phase and slower growth rate).





Industrial Test 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).





Questions?

THE SMART NATURAL SOLUTION



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Thank you