Edible antimicrobial film with a mixture of Essential Oils against Listeria monocytogenes on seafood products

XXXIV CYCLE

PhD: Stefania Camellini

Tutor: Prof.ssa Patrizia Messi

Co-Tutor: Prof.ssa Carla Sabia

University of Modena and Reggio Emilia

Listeria monocytogenes is a foodborne pathogen with a high mortality rate in humans.

The vulnerable groups are:

- pregnant women
- fetuses
- immunocompromised individuals

Listeria infections were most reported in the age group over 64 years and particularly in the age group over 84 years.

The overall EU case fatality was high (17.6%) and cause 92,1% of hospitalization.

Table 2: Reported hospitalisations and case fatalities due to zoonoses in confirmed human cases in the EU, 2019

Disease	Number of confirmed human cases	Hospitalisation				Deaths			
		Status available (%)	Number of reporting MS[b]	Reported hospitalised cases	Proportion hospitalised (%)	Outcome available (%)	Number of reporting MS[b]	Reported deaths	Case fatality (%)
Campylobacteriosis	220,682	29.1	16	20,432	31.8	78.0	17	47	0.03
Salmonellosis	87,923	44.5	15	16,628	42.5	71.8	17	140	0.22
STEC infections	7,775	37.3	18	1,100	37.9	61.0	20	10	0.21
Yersiniosis	6,961	27.4	15	648	33.9	57.0	14	2	0.05
Listeriosis	2,621	51.1	19	1,234	92.1	65.1	20	300	17.6
Tularaemia	1,280	22.8	12	149	51.0	21.6	13	1	0.36
Echinococcosis	739	33.3	14	109	44.3	31.4	14	2	0.86
Q fever	950	NA[c]	NA	NA	NA	67.3	13	4	0.63
West Nile virus infection[a]	443	83.7	9	347	93.5	99.3	11	52	11.8
Brucellosis	310	44.5	11	98	71.0	36.8	12	2	1.75
Trichinellosis	96	16.7	5	6	37.5	25.0	7	1	4.20
Rabies	4	NA[c]	NA	NA	NA	75.0	3	3	100.0

Smoked salmon withdrawn; the Ministry of Health has announced that a batch branded "Wild Sockeye smoked salmon" presents a microbiological risk for *Listeria monocytogenes* (May 2021).

Shrimp cocktail is considered among the potential food sources when cases of invasive listeriosis are being traced.

The increasing interest and resaerch activity in edible packaging have been motivated by:

- increasing consumer demand for safe, convenient and stable foods

- Awereness of the negative enviromental impacts of non-bioegradable packaging waste

“Edible coating are defined as a thin layer of material which can be consumed and provides a barrier to moisture, oxygen and solute movement for the food. The material can be a complete food coating or can be disposed as a continuous layer between food components (Guilbert, 1986)”

Functional compositions	Materials
Coating forming materials	**Protein**: Collagen, gelatin, casein, whey protein, corn zein, wheat gluten, soy protein, egg white protein, fish myofibrillar protein, sorghum protein, pea protein, rice bran protein, cottonseed protein, peanut protein and keratin
	Polysaccharides: Starch, modified starch, modified cellulose(CMC, MC, HPC, HPMC), alginate, carrageenan, pectin, chitosan, gellan, gum and xanthan gum.
	Lipids: waxes (beeswax, paraffin, carnauba wax, candelilla wax, rice bran wax), resins (shellac, terpene) and acetoglycerides
	Composite : Bi-layer composite film, Emulsion composite film
	Plasticized: glycerin, propylene glycol, sorbitol, sucrose, polyethylene glycol, corn syrup, water
	Functional additives: Antioxidants, antimicrobials, nutrients, nutraceuticals, pharmaceuticals, flavors and colours
	Other additives: emulsifiers (lecithin, tweens, spans) lipid emulsions(edible waxes, fatty acids)

Why use the Edible Coating?

- They can be consumed with the packaged products
- Increase the Shelf-life of the product
- Carriers for natural antimicrobial and antioxidant agents (Essential oils and Bacteriocins)
- Improve organoleptic qualities
- Can be used as product separators
- Increase waterproofing
- Toxicity-free → GRAS
- Completely biodegradable
- Easy of use: immersion, spray or spraying of the product
- Low cost

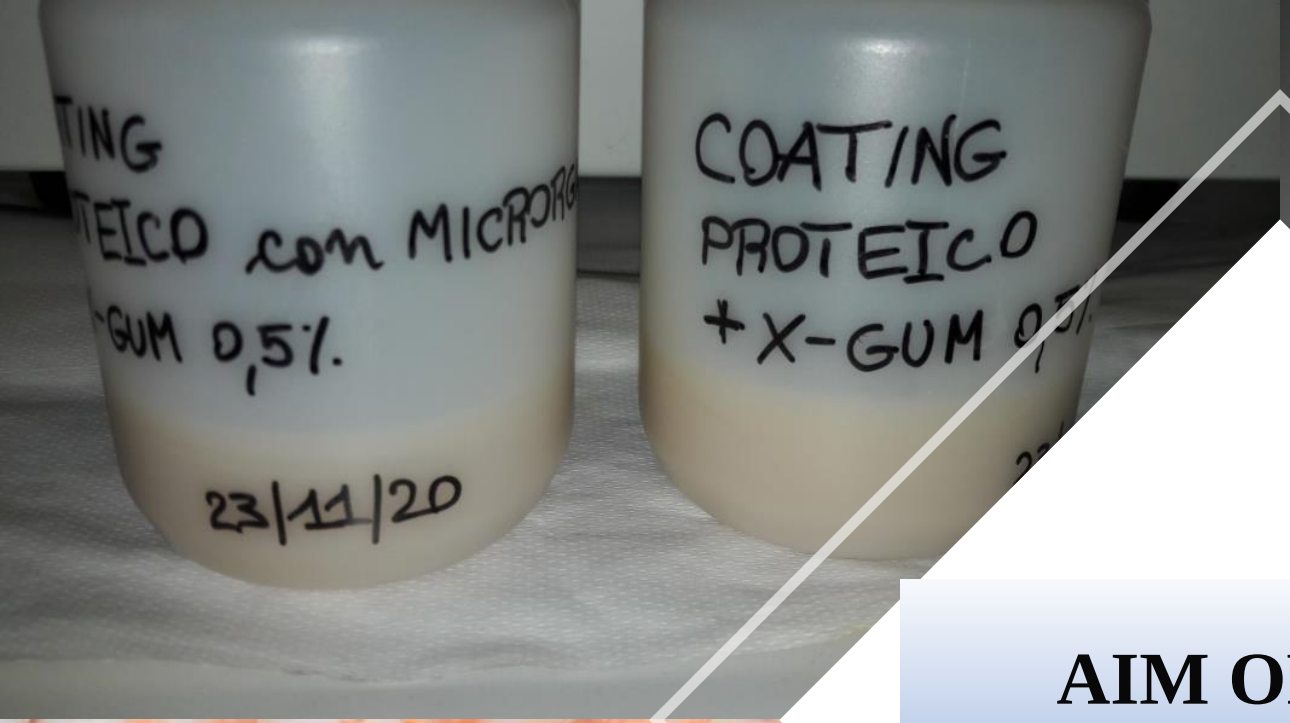
Australian Company Plantic created a corn starch bioplastic with vegetable dyes

Essential oils (EOs) are natural bioactive compounds obtained from plant materials (leaves, buds, fruits, flowers, herbs, twigs, bark, wood, roots and seeds).

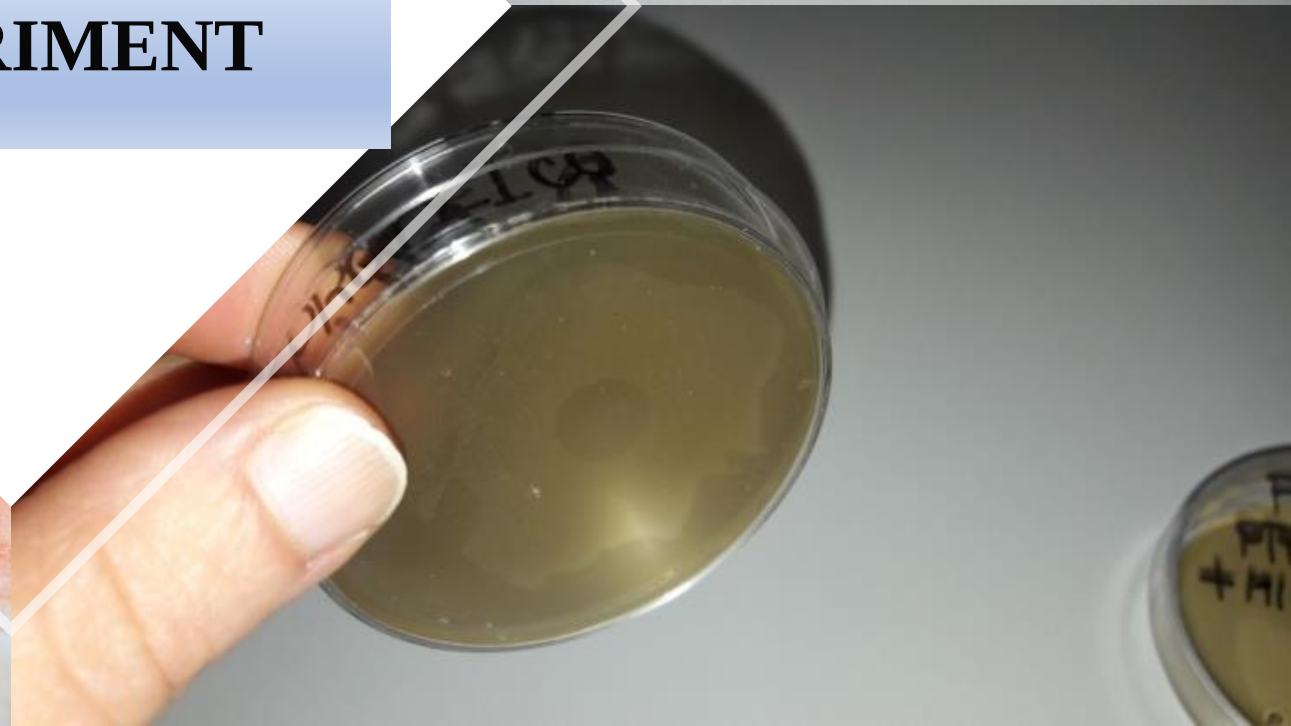
EOs have been reported to possess significant antiseptic, antibacterial, antiviral, antioxidant, anti-parasitic, antifungal, and insecticidal activities.

Health and Human Services Public Health Services have recognized essential oils as safe substances and some essential oils contain compounds that can be used as antibacterial additives.

As food additives, essential oils are regulated by the Food and Drug Administration (FDA). A ceiling concentration limited to less than 300 ppm makes toxicity through ingestion of flavored foodstuffs unlikely, although allergic reactions are still possible



AIM OF THE EXPERIMENT



METHODS:

- **Materials and strain**
- **Anti-Listeria activity determination**
- **Minimal inhibitory concentration (MIC) and the Fractional Inhibitory Concentration Index (FICI) determination**
- **Shrimps contamination and coating**
- **Anti-Listeria activity determination**

Stomacher 700

Results

Bacterial strain	***Salvia officinalis***	***Mentha piperita***	***Thymus vulgaris***	***Citrus limon***
Listeria monocytogenes **NCTC 10888**	**128 µL**	**32 µL**	**8 µL**	**32 µL**

Bacterial strain	***S. officinalis*** **+ *M. piperita***	***S. officinalis*** **+ *T. vulgaris***	***S. officinalis*** **+ *C. limon***	***M. piperita*** **+ *T. vulgaris***	***M. piperita*** **+ *C. limon***	***T. vulgaris*** **+ *C. limon***
Listeria monocytogenes **NCTC 10888**	6 µL +1,5 µL	6 µL +0,8 µL	6 µL +1,5 µL	3 µL +0,8 µL	3 µL +3 µL	0,8 µL +3 µL

Results

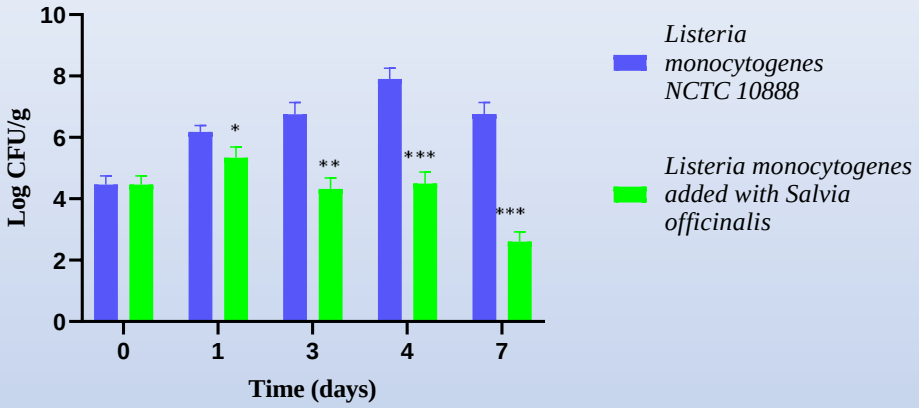


Figure 1. Antibacterial activity of *S. officinalis* against *Listeria monocytogenes* NCTC 10888

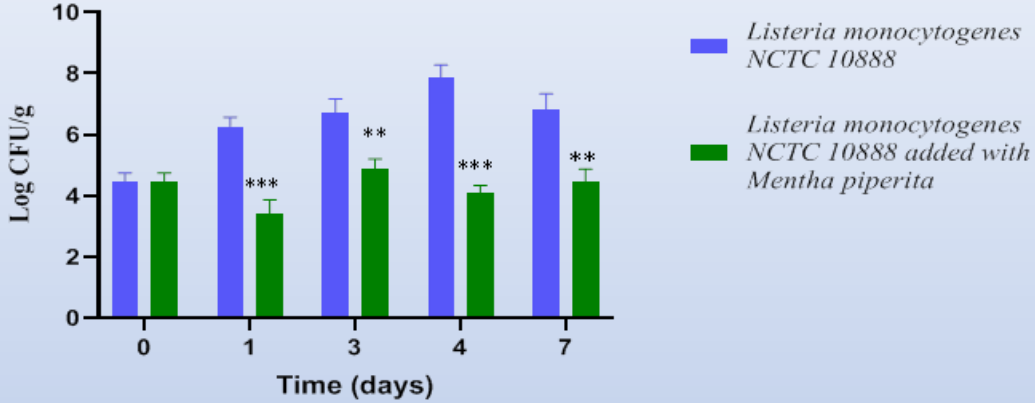


Figure 2: Antibacterial activity of *M. piperita* against *Listeria monocytogenes* NCTC 10888

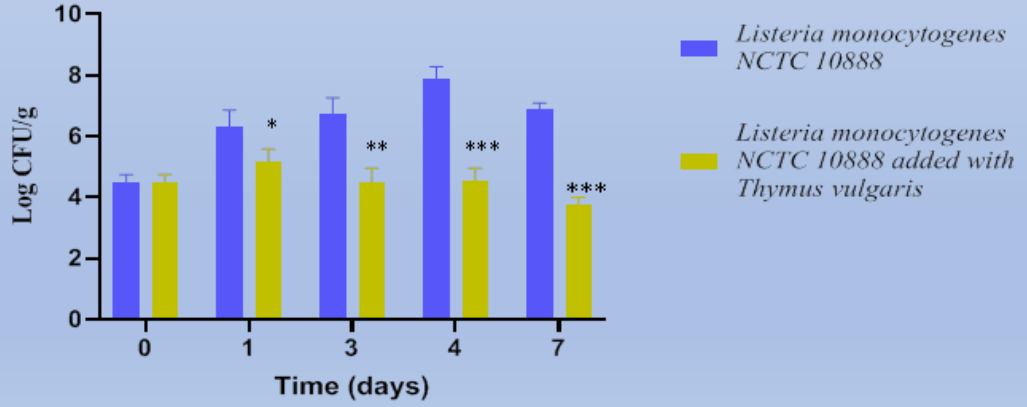


Figure 3. Antibacterial activity of *T.vulgaris* against *Listeria monocytogenes* NCTC 10888

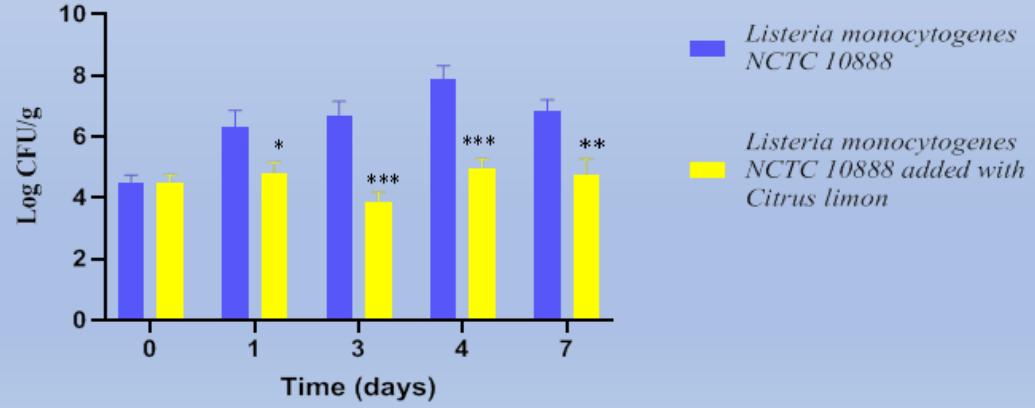
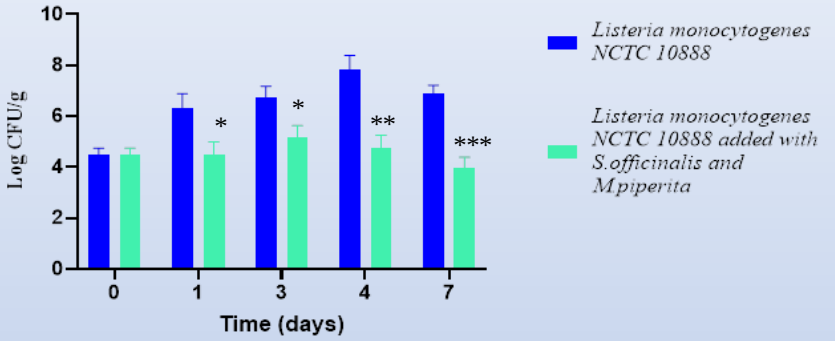
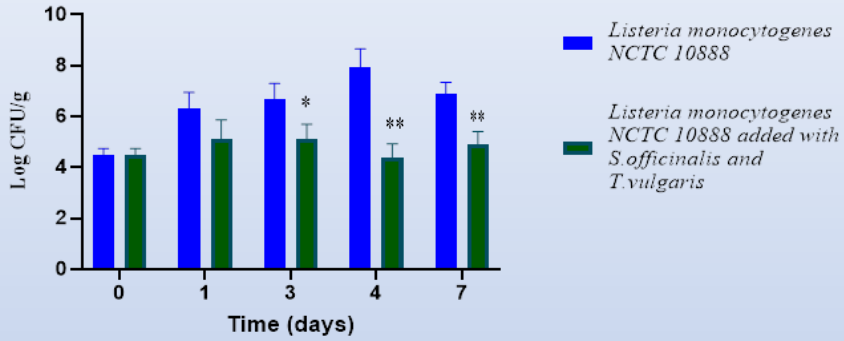


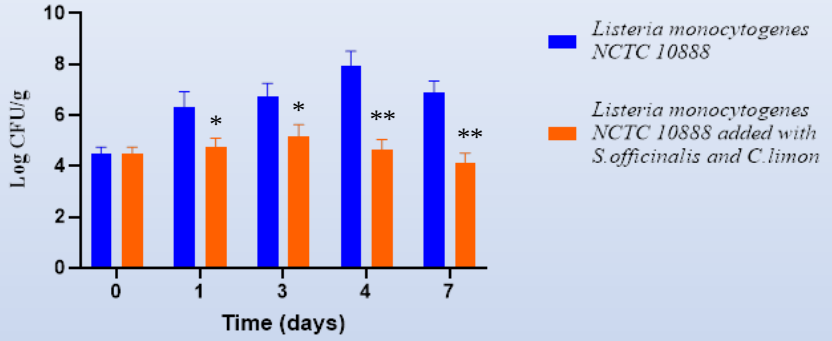
Figure 4. Antibacterial activity of *C. limon* against *Listeria monocytogenes* NCTC 10888

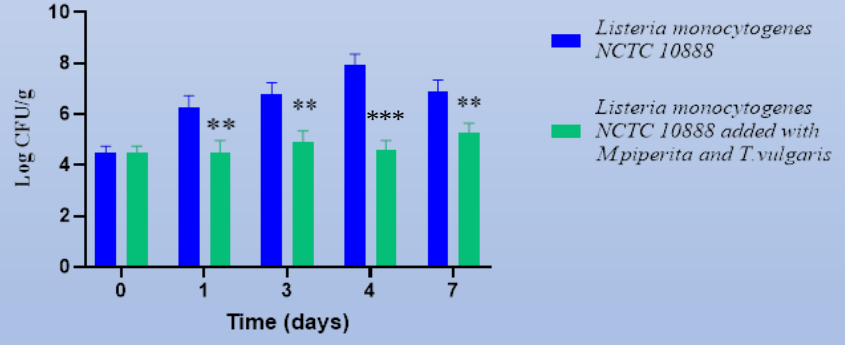
Each experiment was replicated three times. p-values of < 0.05 (*), $p < 0.01$ (**), $p < 0.001$ (***) and $p < 0.0001$ (****) were considered significant by t-test and ANOVA.

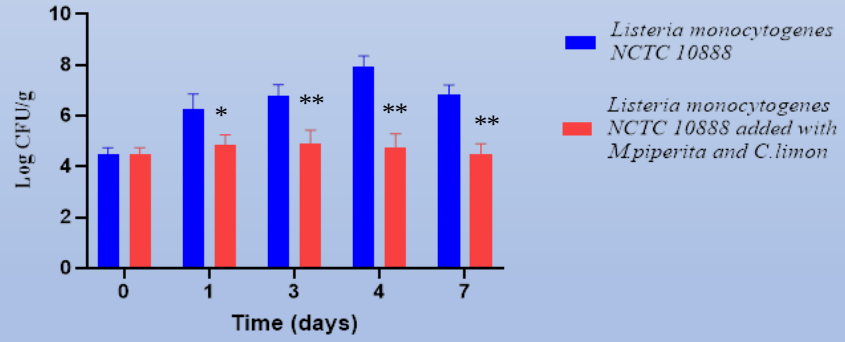
Results

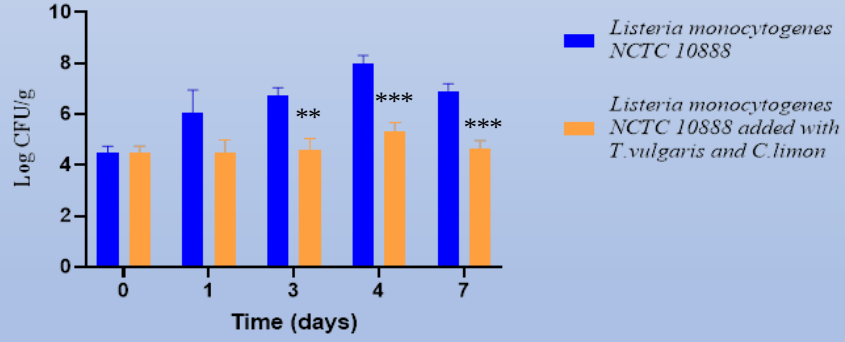












Each experiment was replicated three times. p-values of < 0.05 (*), $p < 0.01$ (**), $p < 0.001$ (***) and $p < 0.0001$ (****) were considered significant by t-test and ANOVA.

Conclusion

- The inclusion of the EOs within the coating not only ensures the anti-listerial activity increasing the shelf-life of the food products, but it also can improve the sensory properties. It is also important underline that the use of edible coating obtained from food by-products is a great advantage for the environmen because it is biocompatible and eco-friendly.

- Further studies will be however necessary to improve the perspectives of active edible coatings for future applications in the food industry.

SAFE

NATURAL

GREEN

Greater knowledge on the spatial distribution and interactions of microbial species in food

Applications of Film and Coating with bacteriocins and essential oils

Discovery of new bacteriocins

Studies on bacteriocins and essential oils

References:

- Ganiari, S.; Choulitoudi, E.; Oreopoulou, V. Edible and Active Films and Coatings as Carriers of Natural Antioxidant for Lipid Food. Trends Food Sci. Technol. 2017, 68, 70–82.
- Nychas G.J.E. Natural Antimicrobials from Plants. In: Gould G.W., editor. New Methods of Food Preservation. Blackie Academic Professional; London, UK: 1995. pp. 58–89.
- Jami M, Ghanbari M, Zunabovic M, Domig KJ, and Kneifel W. Listeria monocytogenes in Aquatic Food Products—A Review Comprehensive Reviews inFood Science and Food Safety 2014 Vol.13, pag 798-813 doi: 10.1111/1541-4337.12092

THANKS FOR ATTENTION