Biopreservation of lightly preserved seafood products

by **Françoise Leroi**

Consumer trends indicate an increasing demand for natural, minimally processed convenience seafood with few additives. Biopreservation techniques developed by researchers in France to control *Listeria monocytogenes* and to extend shelf life of mildly preserved seafood have been commercialised.

The numerous changes in culinary habits in the last decades of the past century and current consumer demands have guided the food industry to produce a great variety of convenience food products, many of them ready-to-eat and minimally processed. In seafood, the market for raw fish with or without ingredients added (*sushi*, *sashimi*) and lightly preserved products such as cold-smoked fish, marinated products (*ceviche*, gravad salmon, *carpaccio etc*), mildly cooked peeled crustaceans, brined shrimp, ready to prepare desalted cod, *etc* has steadily increased.

All those products are highly perishable and susceptible to growth of undesirable microorganisms such as spoilage and pathogenic bacteria. The sources of contamination can be the raw material itself but recontamination can also occur during the handling, processing and packaging of the product. The treatments are usually not sufficient to totally eliminate micro-organisms. As several of these products are eaten raw, minimising their presence and preventing their growth, besides maintaining hygienic processing conditions, are essential to ensure product quality and safety.

Stabilising minimally processed seafood products

The microbial safety and stability of most food products are based on an application of preservative factors called hurdles. Each hurdle implies putting microorganisms in a hostile environment, which inhibits their growth or causes their death. Some of these hurdles have been empirically used for years to stabilise meat, fish, milk and vegetables. This sometimes leads to a completely different product with its own new taste characteristics. Examples of conventional hurdles in marine products are temperature (cooking and/or storage at chilled temperature), salt, smoke, organic acids, and vacuum and modified atmosphere packaging.

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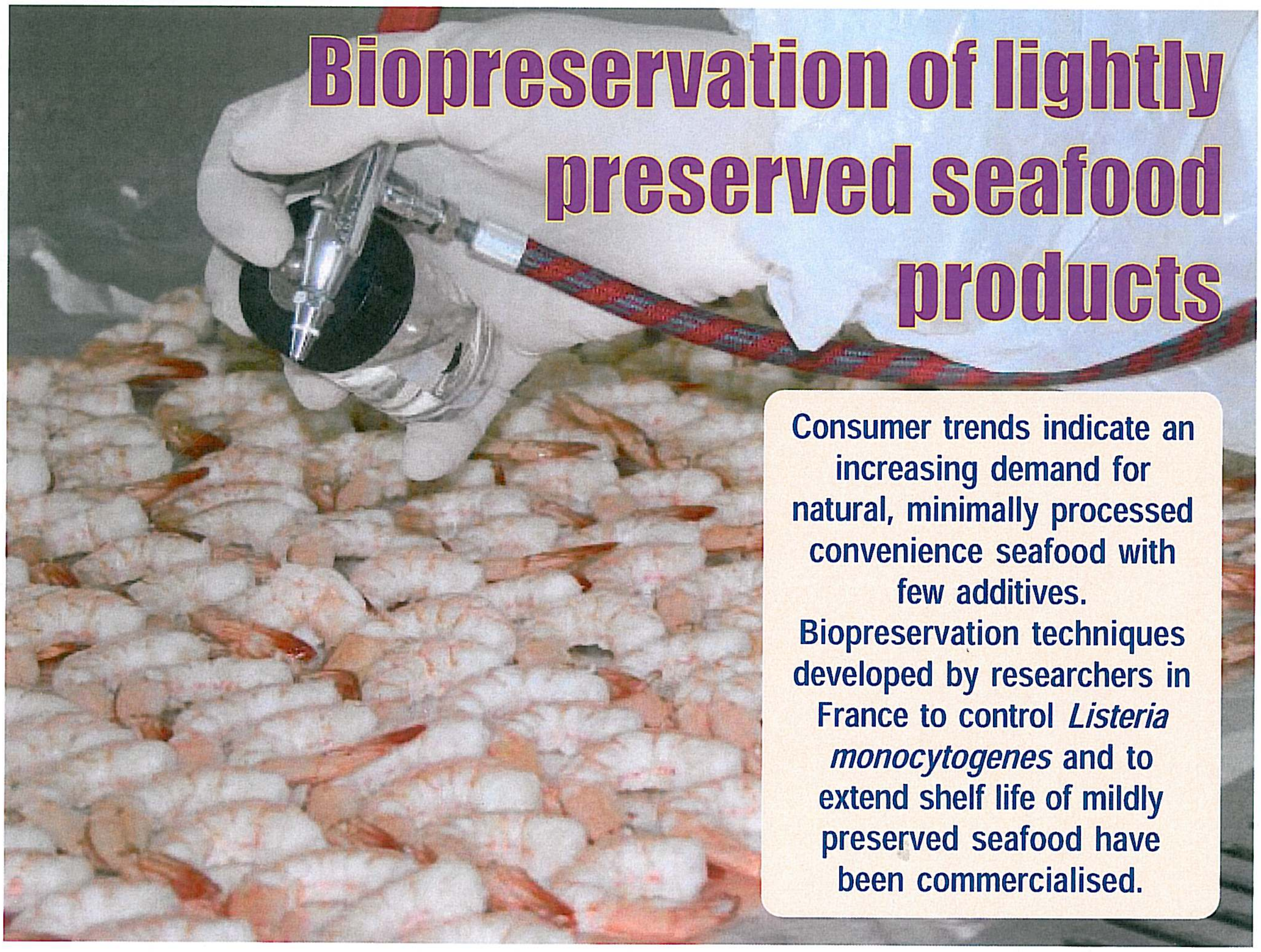
Many potential hurdles for food have already been described including bacteriocins, chitosan, nitrate, lactoperoxidase and essential oils as well as novel decontamination technologies such as high



Treatment of peeled cooked tropical shrimp with bioprotective bacteria has extended its shelf life to 28 days.

Left to right: salmon smoking; cold smoked salmon; and salmon salting.

pressure, pulsed light, electrolysed NaCl water, ozone *etc.* All these technologies are not always applicable in fisheries either because they are not totally efficient in fish and/or they change the characteristics of the product. The consumers want minimally treated natural products with preservatives at the lowest level as possible. Also more and more consumers take nutritional aspects into consideration.

Biopreservation

Biopreservation is an alternative natural technology used to extend the shelf life and/or control the growth of endogenous pathogenic bacteria in refrigerated products. It involves inoculating protective bacteria selected for their inhibition properties towards undesirable micro-organisms. Lactic acid bacteria (LAB) are usually chosen for these applications as they produce a wide range of inhibitory compounds such as organic acids, hydrogen peroxide, diacetyl and bacteriocins or compete with other micro-organisms by nutrient depletion. In addition, they are associated with traditional fermented products and thus have the GRAS (Generally Recognized As Safe) status granted by the US Food and Drug Administration.

LAB also benefit from a healthy image associated with dairy products. LAB are widespread in nature and are commonly found in many food products as well as in the genital, intestinal and oral cavities of animals and humans. Human beings have empirically used endogenous LAB for natural fermentation of milk, meat, vegetables and fruits for thousands of years that has resulted in new stabilised products due to acidification. The LAB species traditionally used have been selected and produced as commercial lyophilised starter cultures which can be added to the food and allow better control of the fermentation.

LAB have long been disregarded for seafood preservation because they are thought to be not present in fresh fish flesh whose characteristics favour marine gram-negative bacteria. In the nineties, presence of LAB in high quantity has been highlighted in lightly preserved seafood products and studies on the selection of bacteria with antimicrobial properties have intensified. However, very few commercial applications have currently appeared in seafood products.

A major hurdle is that these products are not fermented and the selected LAB strains should not change their delicate organoleptic qualities. Many bacteria that gave promising results in liquid medium proved to be ineffective in products, either because they were poorly established in the environmental conditions or because they produced unpleasant odours. A more recent strategy involves selecting LAB naturally present in seafood products, in order to ensure their good growth in the marine matrix stored at chilled temperature.

Microbial risk

It has been clearly shown that organoleptic spoilage is mainly due to microbial activity. For a very long time, however, the mechanisms of microbial spoilage of lightly preserved fish products were poorly understood. Indeed, the microbiota of these products is very complex and varies according to the origin of the raw material, the hygienic conditions in the factory, the process itself and the storage conditions. It is commonly admitted now that many species are present and that some LAB, *Enterobacteriacea*, *Brochothrix thermosphacta* and typical marine gram-negative bacteria are often implicated in sensory deterioration in a very complex manner with species interacting within each other.

Concerning pathogens, bacteria from human or processing origin can be found (*Salmonella*, *Escherichia coli*, *Staphylococcus aureus*) and may constitute a risk, even at low concentration. Good hygienic practices are, therefore, necessary to prevent the products from post-process contamination. However, endogenous pathogenic bacteria naturally present in the marine environment, and thus in the fish, may also cause problems (*eg Vibrio cholerae, V parahaemolyticcus, Clostridium botulinum* type E, histamine-producing bacteria, *Listeria monocytogenes*) and it is necessary to strictly prevent their growth. Many of them can adequately be controlled by strictly respected chilled storage and/or sufficient preservatives (*eg* salt, smoke *etc*).

However, *L monocytogenes* still remains a major microbial risk associated with lightly preserved seafood as it can grow at low temperature (0°C), low pH (4.5) and low water activity (0.92) and in aerobic or anaerobic conditions. *L monocytogenes* is a pathogenic bacteria responsible for listeriosis, primarily a food-borne disease generally associated with high mortality (20-40%). The prevalence of *L monocytogenes* in mildly processed seafood such as cold-smoked salmon, trout or peeled and cooked brined shrimp stored under modified atmosphere packaging is highly variable but quite elevated. Initial contamination is commonly below 1 CFU/g but *L monocytogenes* can multiply during storage and sometimes exceed the European tolerated limit of 100 CFU/g (EC 1441/2007) at the end of the shelf-life. Although few cases of listeriosis due to consumption of seafood products have been reported, it is vital to control its growth and risk.

Control of food safety with biopreservation

Most of the work on fisheries biopreservation has been conducted on the pathogenic *L monocytogenes* bacteria. As it is not always easy to work with this class II pathogen, many studies have been performed with the surrogate *L innocua* and results have rarely been validated for *L monocytogenes*. Here, we will only present examples of results obtained with *L monocytogenes*.

Many authors have selected several strains of *Carnobacterium maltaromaticum*, *C divergens* and *Lactobacillus sakei* that successfully prevented the growth of *L monocytogenes* in cold-smoked salmon stored under vacuum, in *surimi* and in cooked peeled shrimp stored under modified atmosphere packaging. A strain of *Lactococcus piscium* was also selected for its effect on *L monocytogenes* and *Staphylococcus aureus* in shrimp. Most of the time, the effect is due to production of bacteriocins but, in some cases, competition for glucose was the presumptive reason for inhibition. However, all these tests are rarely repeated on different strains of *L monocytogenes* and the consequences of

Use of bioprotective bacteria can inhibit the growth of *Listeria monocytogenes* in cold-smoked salmon without affecting the organoleptic properties.

adding a protective culture on the microbial ecosystem and on the sensory characteristics of the final product are not always taken into account.

Currently, very promising results have been achieved by two French institutions IFREMER and ONIRIS in Nantes, France. They screened three strains of *C divergens* and *C maltaromaticum* for their antilisterial activity against a collection of 60 *L monocytogenes* strains selected from the smoked salmon industry using an agar spot test. All the *Listeria* strains were inhibited but three different groups could be distinguished differing in sensitivity to the three *Carnobacterium* strains. The antilisterial capacity was then tested in cold-smoked salmon blocks co-inoculated with the *Carnobacterium* and mixtures of *L monocytogenes* strains. In all cases, excellent results were obtained and one of the strains allowed maintaining the level of *L monocytogenes* at <50 CFU/g (initial contamination of 100 *L monocytogenes*/g) during four weeks of vacuum-packed storage at 4 and 8°C, even for the less sensitive *L monocytogenes*. In addition, it was noticed that the strain did not acidify the product and did not change its microbial ecosystem (except for *Listeria*). A trained panel of fifteen judges tasted the inoculated products and confirmed that there was no organoleptic modification compared with the non-inoculated product. The concept is now being commercialised by the Italian company *Sacco Srl*.

Improving quality, extending shelf life with biopreservation

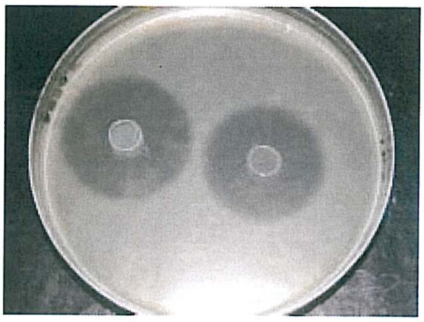
Another important challenge is to reduce food loss, which very often is due to development of spoiling microorganisms. However, there have been very few conclusive attempts to control the spoilage microbiota with protective culture, the reason being the complexity of the microbial ecosystem present in mildly processed seafood. Most of the tests done with *Carnobacterium* have proven their ineffectiveness in delaying seafood spoilage.

Recently, two strains of *Leuconostoc gelidum* and two strains of *Lactobacillus piscium* selected by IFREMER and ONIRIS have shown very promising results in delaying the spoilage of naturally contaminated tropical shrimp and vacuum-packed cold-smoked salmon. These strains are exceptionally well adapted to the fish matrix and to chilled storage conditions, characteristics not commonly found for LAB. Uninoculated shrimp or cold-smoked salmon slices were totally spoiled after 28 days but kept their freshness characteristics when inoculated with these bacterial strains. However, the mechanism of action is still not understood and results seem to be dependent on the bacterial strains and the seafood products. Even though any correlation with the classical quality indices could not be found, an *in situ* inhibition of *B thermosphacta* (a major spoilage bacteria) recently shown by *Lactobacillus piscium* could partially explain the protective effect observed in shrimp.

Safety aspects of bioprotective bacteria and regulation

Safety aspects of the bioprotective bacteria have to be taken into consideration for food application. The production of histamine must be checked, as it is a regulated compound in fish rich in histidine, leading to allergy-like syndromes. In seafood the production of histamine linked to *C maltaromaticum*, *C divergens*, *Leuconostoc gelidum* and *Lactobacillus piscium* has never been reported. These promising species of LAB considered for seafood preservation did not exhibit any problematic resistance to antibiotics nor have cytotoxicity potential.

With the exception of those encompassed by the Novel Food Regulation (1997),



Inhibition of *Listeria monocytogenes* by *Carnobacterium maltaromaticum* in agar plate.

microorganisms used for bioprotection of food are not subject to regulation in Europe. On 19 November 2007, the European Food Safety Authority (EFSA) adopted guidelines for Qualified Presumption of Safety (QPS) that can be considered as the European equivalent of the American GRAS status in terms of risk assessment. However, this QPS status is only requested for feed application and there is a real gap in regulation for food application. A list of microorganisms judged suitable for QPS status has been published in the *EFSA Journal* (2008). It contains 73 species of microorganisms, among them 47 LAB species belonging to genus *Bifidobacterium, Lactobacillus, Lactococcus, Streptococcus, Leuconostoc, Pediococcus* and *Propionibacterium*. The strains that could be used in seafood, such as the previously described *Carnobacterium* sp, *Lactobacillus* sp, *Lactococcus* sp or *Leuconostoc* sp, have frequently been found in marine products at high level but humans have not traditionally used them for preservation of fish. Their presence in seafood and their potential advantages for management of quality and safety have been discovered only in the last 10-20 years.

Although not necessary for a food application, it can be anticipated that the same guidelines in terms of risk assessment for bacterial strains will be requested in the future. *C maltaromaticum, C divergens, Lactobacillus piscium* or *Leuconostoc gelidum*, pointed out as potential bioprotective bacteria for seafood products, are not included in the QPS list but exclusion does not necessarily imply any risk associated with their use. Many microorganisms commonly encountered in food production were not considered because they are not presently the subject of pre-market authorisations and so would not be notified to EFSA. The QPS list is supposed to be annually updated. With proof of their beneficial effect, precise taxonomic data and strong evidence of safety, it is reasonably conceivable that many new bioprotective strains for marine products will obtain QPS status if notified to EFSA.

Conclusion

Despite the promising results obtained on *L monocytogenes* reduction and shelf-life extension, the use of preservative bacterial cultures in the fish industry is uncommon compared to dairy and meat products. Most of the biggest companies producing microbial starters do not sell LAB for a specific seafood application because no adequate strains have been found. However, things are moving quickly. Concerning safety, more than 20 years of fundamental and applicative research on *C maltaromaticum* and *C divergens* strains allowed us to obtain precise molecular taxonomic data, proof of safety, strong evidence of their beneficial effect for mastering *L monocytogenes* risk in lightly preserved seafood products without any negative effect on their sensory characteristics and a deep comprehension of the mechanism involved. The company *Sacco Srl* (Cadorago, Italy) has licensed the IFREMER/ONIRIS development for commercialisation.

Improving quality of seafood still remains a challenge as the spoilage mechanism is so different from one product to another that the selection of protective microorganisms has to be adapted to each matrix and each processing plant. However, strains are already available for some applications. A patented starter culture LLO from a French company (*Bioceane*, Nantes, France) is commercialised for specific preservation of seafood such as cooked peeled shrimp.

An increasing number of studies is aiming to exploit the ability of marine LAB to control the quality and the safety of marine products. This technology has to be used as an additional hurdle to prevent growth of undesirable microorganisms and cannot substitute for good hygienic practices.

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