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ABSTRACT

Innovations in seafood preservation and storage

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Abstract The increasing amount of farmed fish cannot be easily absorbed by the market as only fresh fish. The production and promotion of value-added fresh and processed fish products, which could fulfil consumers' present demands, may represent a solution to this problem. The aim of this paper is to review some of the most recent technologies, such as surface decontamination, use of "natural" additives and compounds, active packaging, used or experimented with to prolong shelf life, while ensuring the safety of fresh fish and fishery products.

Keywords Decontamination · Packaging · Predictive microbiology · Processing · Seafood

Abbreviations

AHLs	acylated homoserine lactones
LAB	Lactic Acid Bacteria
L.m.	*Listeria monocytogenes*
MAP	modified-atmosphere packaging
SSOs	Specific Spoilage Organisms
TMA	trimethylamine
TVN	total volatile nitrogen
VP	vacuum packaging

Introduction

The growing interest in a correct life style, including alimentation, and the parallel attention on food quality have contributed to orientate consumers towards fishery products which are

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considered safe, of high nutritional value and capable of influencing human health in a positive way. The development of farming of fishery products is also related to the gradual acceptance of the farmed products, which can guarantee safe foods with optimal qualitative levels when animals are bred and foods are manufactured and stored correctly.

The world expansion of acquaculture, gradually involving different fish, crustacean and mollusc species, has changed the balance of supply and demand, leading, in combination with poor marketing, to a decrease in prices of farmed fishery products. As the increasing quantity of farmed species cannot be easily absorbed if only available as fresh products, a solution to this problem could be the production and promotion of seafoods with added value. It is important, however, to give added value not only to fresh but also to processed fish, following consumers' demand for lightly preserved foods. Salting, cooking and/or pre-cooking, smoking and curing are only some examples. These methods are receiving growing attention from farmers and food manufacturers interested in broadening and/or diversifying their productions. This interest regards, both at the experimental and commercial level, not only the most common and more easily available species, but also those that have become the object of farming more recently.

The implementation of EC Regulations concerning the denomination, labelling, traceability and packaging of fish products has improved the transparency of the whole fishery food chain. Moreover, the presence on the market of a wide and increasing range of ready-to-use and ready-to-eat products, both fresh and processed (i.e.fillets, steaks, fish preparations with vegetables and flavours added) is functional to modern times and life styles, which generally require short times for domestic food preparation.

Fresh seafoods

The evolution of seafood storage and preservation techniques has to take into account the high perishability of this kind of products, related to several intrinsic and extrinsic factors. A significant proportion of spoilage agents are bacteria which are able to exploit specific metabolites of fish tissues during storage. The microbial status of seafood after the catch is closely related to environmental conditions and the microbiological quality of the water. In particular, water temperature, salt content, pollution, kind and quality of feed, methods of catch and chilling conditions may be very important (Giuffrida 2003).

Furthermore, in intensive marine aquaculture, rearing density can affect the microbial status of the water and, indirectly, the hygienic product characteristics; it also influences the stress level of fish and the hygiene of the harvesting process. Pathologies also play an important role in the bacterial contamination of farmed fish since some opportunistic bacterial pathogens are also agents of foodborne disease (*Vibrio* spp., *Aeromonas* spp.) (Balebona et al. 1998). Besides this pathogenic activity, the diffusion of the above bacteria in the water environment is well known, especially with regard to halophilic *Vibrio* spp.. However, a recent research project on the diffusion of *Vibrio* spp., *Listeria* spp. and *Aeromonas* spp. in reared *Sparus aurata* showed a higher prevalence of the genus *Aeromonas* with respect to the other ones. The frequent isolation of *A. hydrophila* and its psychrotrophic activity, represent an important food safety concern both for fresh and slightly preserved farmed seafood.

Concerning spoiling microflora, several studies have stressed the importance of seafood SSOs, such as *Pseudomonas* spp., *Shewanella* spp. and *Photobacterium* spp., which are largely distributed on the surface of the skin and gills or in the gut and are able to grow during refrigerated storage. The seafood SSOs produce ammonia, biogenic amines, organic acids and sulphur compounds from amino acids, hypoxantine from ATP degradation products, and acetate from lactate. A large amount of TMA is also produced by these bacteria capable of using TMAO in anaerobic respiration (Gram and Dalgaard 2002). Many microbial metabolites produced in seafood are similar to those observed in meat and poultry products; however, in seafood spoilage, TMA in particular contributes to the characteristic ammonia-like and 'fishy' off-flavours.

SSOs are selected primarily as a result of the physical and chemical conditions in the products; however, seafood spoilage obviously involves growth of high numbers of microorganisms to (Log >6–7 cfu/g) and the interaction (antagonism or symbiosis) between different groups of microorganisms may influence their growth and metabolism. In fact, despite the nutrient richness of fish muscle, this environment is iron-limited and siderophores are produced during bacterial growth. The high iron-binding capacity of the pseudomonad siderophores may cause this bacterial group to be favoured (Gram and Melchiorsen 1996). Furthermore, the production of siderophores may inhibit the growth of other bacterial species reducing the iron suitability. It has been shown that this mechanism affects the growth of *A. hydrophila* on *S. aurata* surfaces (skin and gills) (Giuffrida et al. 2007) as well as in in vitro experiments (Giarratana 2008).

In packed or semi-preserved seafood, LAB inhibit growth of other bacteria due to the formation of lactic acid and bacteriocins or by competition for nutrients, and this may contribute to their selection during seafood spoilage. However, a symbiosis between LAB and Enterobacteriaceae might occur since LAB may degrade arginine (to ornithine), which is then degraded by the Enterobacteriaceae to putrescine. This produces a level of putrescine higher than that produced by Enterobacteriaceae growing alone (Jørgensen et al. 2000).

A final example of the interactive properties of food spoiling bacteria is the ability of several food spoiling Gram-negative bacteria to produce chemical communication signals such as AHLs. It was shown that AHLs can be extracted from a range of foods and their concentration increases as growth of Gram-negative bacteria takes place. The role of AHLs in seafood spoilage is currently unknown, but several spoilage activities have been potentially linked to AHL regulation (Gram and Dalgaard 2002). AHLs can be extracted from spoiled fish fillets and minced fish and are produced by several important seafood spoilage bacteria.

The spoilage of some seafood is well understood and this understanding has enabled the development of preservation techniques targeted at the SSOs. An example of this is the CO_2-packaging of fresh, marine, iced fish. This inhibits the respiratory spoilage bacteria (*Shewanella* and *Pseudomonas*) and should, in principle, result in a dramatic extension of shelf life. However, because of the presence of the "CO_2-resistant", TMAO-reducing *P. phosphoreum*, the product spoils almost at the same rate as non-packaged products. Targeted inhibition of *P. phosphoreum* (e.g. by freezing or the addition of spices) reduces its growth and results in a significant extension of shelf life. Non-spoilage LAB or pure bacteriocins have been also used to extend the shelf life of brined shrimp (Einarsson and Lauzon 1995) or other semi-preserved seafood.

The possible involvement of AHLs as chemical communication signals in the regulation of the spoilage process by Gram negative bacteria also opens up a new field of food preservation. Although until recently preservation relied on the elimination (killing) or growth inhibition of spoilage organisms, AHL-regulated traits can be specifically blocked (Gram and Dalgaard 2002). This 'quorum-sensing interference' will not necessarily inhibit growth but will, in principle, only block the unwanted spoilage reactions; for example, the export of enzymes involved in the spoilage process.

All this knowledge as well as the improvement in predictive microbiology has enabled the achievement of new goals in seafood storage and preservation. In this regard the development of seafood shelf-life predictive models (Dalgaard et al. 2003) both for raw fresh seafoods and packaged ones (VP or MAP) have to be taken into account. Koutsoumanis and Nychas (2000) developed a predictive model for the growth of *Pseudomonas* spp. in *S. aurata*, showing a minimal spoilage level of Log 7 cfu/g. Rasmussen et al. (2002), studying the shelf life of Atlantic salmon portions produced for retail distribution from harvesting to the processing and storage operations, developed a stochastic mathematical model (Process Risk Model - PRM), which predicted the range of the possible shelf life for the portions under normal retail and distribution level. Furthermore, in a recent work (Giuffrida et al. 2008), the relationship among *Pseudomonas* spp., *Shewanella* spp. and Quality Index Method scores was shown.

Also in the case of bacterial agents of foodborne disease, predictive microbiology contributed to a better understanding of the seafood safety, especially with regard to the most frequently isolated pathogens in fresh and lightly preserved products, such as *L.m.* and *A. hydrophila*. Besides the well known development of primary (which describe the microbial evolution as a function of time) and secondary (which relate parameters appearing in primary modelling to environmental conditions) models, the most important innovations are those regarding modelling of pathogen behaviour in the presence of bacterial competition, since this condition frequently occurs in several seafood products. Giménez and Dalgaard (2004) modelled the simultaneous growth of *L. m.* and spoilage microflora in cold-smoked salmon by using the simple logistic equation which describes how the specific growth rate (μ) of a species is reduced when the cell concentration (N) reaches its maximum value (N_{max}). Starting from this equation, the authors inserted an additional term which takes account of the interaction between *L. m.* and spoilage microflora (LAB), so that they inhibit each other to the same extent that they inhibit their own growth. This study confirmed that growth of *L. m.* in vacuum packed cold-smoked salmon ceased when the total microflora reached their maximum population density, i.e. that a growth pattern corresponding to the Jameson effect was observed. A more complete application of this kind of competition model is represented by the Lotka-Volterra approach which enables simulation of the competition between two populations expressing several kinds of situations such as the mutual interaction and the reduction or the decline of only one population (“low or no growth”). The Lotka-Volterra model was recently used (Giuffrida et al. 2007) for the simulation of *A. hydrophila* behaviour on the skin and gills of *S. aurata* in the presence of natural spoilage bacteria during refrigerated storage, showing the competitive action of SSOs, probably due to siderophore production.

Because of the consumer demand for fresh refrigerated foods with extended shelf life, considerable research has been directed at prolonging the shelf life, while ensuring the safety of fresh fish and fishery products.

Surface decontamination

1. Acid decontamination

Since the nineties much research has been conducted to prevent the development of spoilage in fishery products using various organic acids, because of their efficiency and low cost. Sodium salts of low molecular weight and organic acids, such as acetic, lactic and citric acids, have been used to control microbial growth, improve sensory attributes and extend the shelf life of various food systems In addition to their suppressing effect on the growth of food spoilage bacteria, organic salts of sodium acetate, lactate, and citrate were shown to possess antibacterial activities against various foodborne pathogens (Khalid 2007). Furthermore, these salts are widely available, economical, and generally "recognized-as-safe".

Recently, article 3 of EC Regulation 853/2004 provided the legal basis for the use of substances other than potable water to remove surface contamination from foods of animal origin intended for human consumption. As a general consequence interest in the development and commercial applications of decontamination procedures has increased. The ability of microrganisms to adhere firmly to muscle and skin, as well as to surfaces of processing equipment, is well documented and represents an ongoing concern for the food industry. Decontamination, however, should be used only as an additional measure to further reduce the load of pathogenic microorganisms, after following good hygienic/manufacturing practices and not as a substitute.

2. Ozone decontamination

Ozone, already licensed in 2001 as antimicrobial agent for food processing and preservation by the FDA, is a highly unstable molecule with three oxygen atoms. The third atom is loosely bound and can be easily detached from the molecule. The free single oxygen atom readily reacts with other molecules and this process generates a highly reactive oxidant system which affects the structural integrity of the reactant and may lead to oxidative damage and lysis of microorganisms. The contact times and dosages for ozone disinfection are much lower and very effective compared to many other sanitizers (Fortunato et al. 1995). Korol et al. (1995) compared the efficacy of ozone and chlorine treatment of water and its antimicrobial spectrum on a variety of bacterial pathogens. It was observed that ozone, even at the dose of only 0.35 mg/L, produced the reduction of at least 5 log in populations of approximately 10^6 cells/ml of *E. coli, V. cholerae, S. typhi, Y. enterocolitica, P. aeruginosa, A. hydrophila, L. m. and S. aureus*. Recent GRAS (Generally Recognized As Safe) status has encouraged food processors to use ozone for sanitation. The effect of treatment with gaseous ozone on the shelf-life of different fish species has been investigated. Results showed an extension of some days at refrigeration temperature when ozonizated ice and/or gaseous ozone were used. Some freshness indicies (TVN and TMA) were lower than the control ones and no increase in oxidation of unsaturated fatty acids was observed. The combination of ozone pre-treatment with storage at refrigeration temperatures appears to be a feasible means to prolong the storage life of different fish species (Fabbrocile et al. 2005).

3. Naturally-occurring antimicrobial systems

In addition to emerging systems, there is a growing demand for milder and more natural means for food preservation as consumers look at foods as a basic tool for managing healthy purposes. This has brought attention to the wide range of naturally-occurring antimicrobial systems, employed by plants, animals and microorganisms, with the aim of exploiting some of them in seafoods.

Spices and essential oils are rich in phenolic compounds, such as flavonoids and phenolic acids, which exhibit a wide range of biological effects, including antioxidant and antimicrobial properties. The aromatic herb Rosmarinus officinalis, used to give special aromas or flavours to foods, is known to have also the abovementioned properties. Several studies have shown the effectiveness of rosemary extract in slowing the growth of a number of bacteria, such as *E . coli* and *S. aureus,* that are involved in food spoilage, thus extending the shelf life of meat. Limited data, however, are available on its effect on the quality of fish and fish fillets/steaks.

The combined effect of minced and sterilized rosemary has been investigated on vacuum packaged swordfish steaks as this appreciated species has also been part of the Mediterranean diet for many years. Results demonstrated that rosmarinus antioxidants have the potential for suppressing oxidation in vacuum packaged swordfish, even if the extension of shelf life was limited to only two days (Anastasio et al. 2003).

4. Packaging

In parallel with the use of natural or chemical preservatives, commercialisation strategies included traditional vacuum packaging (VP) and MAP systems, experimenting with the use of different film properties and different gas and gas mixtures.

VP, combined with the use of oxygen-limiting packaging materials, may slow or inhibit the growth of aerobic psychrophlic bacteria involved in the microbial spoilage of refrigerated foods, but selective pressure conditions for the proliferation of anaerobic and microaerophilic bacteria may arise. More recently, the usefulness of vacuum-skin packaging (VSP) techniques, that allow a tight disposition of the packaging material over the food surface, has been reported.

Vacuum packaging seems to prevent the development of oxidative off-flavours and the loss of flavour volatiles, thus improving flavour. However, owing to the physico-chemical features of fish muscle (composition, pH, water content etc.) the shelf life extension of fishery products is not as long as that of meat and meat products, even if GMPs are adopted along the whole food chain.

This statement has been confirmed by research conducted on lots of sea bass and sea bream fillets packaged in different ways (Cortesi 2006). The farmed fish were killed in water and ice and immediately transported to a processing plant, where they were filleted, both manually and mechanically, and packaged following four technologies: two MAPs with different gas mixtures and packaging (i.e. CO_2 70%, N_2 25% and O_2 5% using polystyrol trays with holes and pads on the bottom and Cryovac LID 2050 and CO_2 63%, N_2 22% and O_2 15%, using a rigid tray Cryovac UBRT 1621 without a pad and the same LID 2050); Darfresh (DF) (Cryovac Darfresh TH 300), a process that creates a vacuum package with an upper film resembling a second skin, and the Steam cooking system (SC), a MAP packaging providing an alternative microwave solution for steam cooking thanks to the combination of a proprietary PP tray (Cryovac trays SCT 1621), a built-in water reservoir and a Multiflex EOP“M” lid. Sensorial characteristics were judged excellent until 10 d from catch, also for some additional days for SC and MAP. On the same days spoilage microrganisms were at acceptable levels. On the whole microbiological analysis showed good hygienic conditions during storage which was monitored up to 26 d from fish capture. Pathogens were never detected, with the exception of *L.m.* which was isolated from both sea bream and sea bass fillets mainly belonging to the same processing lot. It should be considered, however, that no limit has been established for this microorganism for foods destined to be consumed after cooking. Physical and chemical parameters, with the exception of DF packaged sea bream fillets, showed superimposed values for all the packaging typologies until 14 d from catch. TVN levels showed a poor correlation with sensorial analysis, since, at the end of the trial, they were under the limits reported in the literature for judging a product unfit for human consumption.

More recently, newer and very promising packaging systems, consisting of films containing antimicrobial or antioxidant agents designed to be released slowly during storage, have been reported to improve the shelf life of animal foods. Antimicrobial packaging materials could be a potential alternative solution to prevent the development of spoilage and pathogenic microorganisms. Instead of mixing antimicrobial compounds directly with food, incorporating them in films allows the functional effect to occur on the food surface where microbial growth mostly occurs. Antimicrobial packaging may include systems such as adding a sachet into the package, dispersing bioactive agents in the packaging, coating bioactive agents on the surface of the packaging material or utilizing antimicrobial macromolecules with film forming properties or edible matrices. In 2004, with Regulation (EC) No 1935/2004, materials or articles intended to come into either direct or indirect contact with food must no longer be sufficiently inert to preclude substances from being transferred to food. New types of materials and articles designed to actively maintain or improve the condition of the food (active food contact materials and articles) or to monitor the condition of the food (intelligent food contact materials and articles) may be allowed even if, of course, the main requirements for their use are to be established to include positive lists of authorised substances. A document has been published recently to give guidance to petitioners wishing to present an application for the safety assessment of a substance prior to its authorisation (Guidance Document 2008).

Processed foods

The demand for minimally processed foods is steadily rising. This trend also involves fishery foods, resulting in the creation of innovative products obtained by means of new technologies or processes similar but often milder than those used in the past. The fish species interested in this trend are also farmed fish.

Since the presence of bluefin tuna (Thunnus thynnus) is limited to some fishing areas and the species undergoes a high fishing pressure the commercial answers to this situation might be the development of farming techniques and/or the diversification of the commercial offer. The commercial value of this species, especially in the oriental market, has prompted research aiming at fully exploiting the potential of this species through the production of innovative tuna based products.

Three kinds of sliceable smoked tuna products were manufactured using both whole muscles and retails from fresh and frozen subjects. The products were tuna “salami” (dry fermented sausages ripened for different times); tuna “salted” products (dry-salted and brine-salted seasoned products obtained from dorsal and ventral whole muscles) and cooked tuna products (muscle salted in brine and cooked in casings or in the metal containers used for pig hams). The raw seafoods obtained from Thunnus thynnus were smoked traditionally with beech tree shavings, for the cooked ones smoke flavours were used. All products (the whole products and the sliced ones) were vacuum packaged at the end of processing, stored at +4°C and analyzed for their microbiological, sensorial and chemical (biogenic amines and PAHs included) parameters. The shelf life was different according to the processing method but on the whole was long enough to justify each kind of production (about 30 d for cooked products, up to 3 months or more for the seasoned ones).

On the whole results demonstrated the safety of the processing methods used, even if it was necessary in some instances to adopt some corrective actions. No significant correlations were observed between sensory characteristics and physico-chemical parameters (Cortesi 2007).

Among processed products, more ancient and simple methods should also still be considered, such as cooking or pre-cooking, associated to vacuum packaging prior or post processing. “REPFEDS” (Refrigerated Processed Food of Extended Durability) have a potential which at present has not been entirely exploited. The product range includes cooked fish with few or no added ingredients and more complex fish preparations.

Conclusions

Producers should aim at the diversification of their productions and develop collateral activities of the different food chain steps, such as a) the application of innovative packaging systems, with positive consequences of added value for the products and of improved quality and innovation for the producers; b) the automation and rationalization of the production methods, including those allowing an easier product manipulation during transport and storage.

Research, at both the experimental and commercial level, are indispensable steps to reach the abovementioned goals. Moreover, research represents a preliminary stage for the definition and application of specific brands, a goal which is presently aimed at by almost all producers. The presence on the market of foods with a distinguished brand, whose characteristics are regularly in line with consumers’ expectations, leads in fact to “brand awareness”. Safety, hygiene, shelf life and food presentation are other important characteristics capable of appealing to consumers’ attention.

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