Bacteriocins in food: evaluation of the factors affecting their effectiveness

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Bacteriocins are ribosomally synthesized antibacterial peptides or proteins secreted mainly by lactic acid bacteria. They exhibit activity against food-borne pathogens and are generally recognized as safe compounds. Use of bacteriocins in food preservation offers several benefits, among them: it reduces the use of chemical preservatives and decreases the extension of thermal treatments. Bacteriocins can be produced *in situ* by the inoculation of the bacteriocin producer strain or can be produced *ex situ* and added to the food as antimicrobial additives. Each form presents advantages and disadvantages. Moreover, the composition of the food and the interaction with other preservation factors affect bacteriocin production and its antimicrobial activity. The main objective of this chapter is to review the bibliography concerning the factors affecting the *in situ* production of bacteriocins and its effectiveness. Also, the efficiency of bacteriocins added as antimicrobials will be discussed. Meat, meat products and fish will be covered with special emphasis. This information will help to choose the conditions that assure the effectiveness of bacteriocins and contribute to improve the safety and quality of food products.

Keywords bacteriocin; effectiveness in food

1. Introduction

Bacteriocins are ribosomally synthesized peptides or proteins with antibacterial activity produced by different bacteria. In particular, bacteriocins produced by lactic acid bacteria possess many advantages: they are generally recognized as safe substances (GRAS); are not toxic to eukaryotic cells; are inactivated by proteases during digestion process; are active against food borne pathogens and food spoilage bacteria [1]. In general, bacteriocins exhibited a narrow spectrum of activity being common its ability to inhibit *Listeria monocytogenes*. They are classified according to their genetic, structural and biochemical characteristics into several groups: i) Class I, small peptides (molecular weight < 10 kD) containing unusual amino acids like lanthionine. This group is further divided into subgroups A and B based on their mode of killing; ii) Class II, small heat stable non-lantibiotic containing peptides and usually positive charge at neutral pH, this group is divided into four subgroups (IIa, IIb, IIc and IId); iii) Class III, heat labile proteins and iv) Cyclic peptides that required lipid or carbohydrate moieties for their activity [2].Even though, a large number of bacteriocins have been isolated and characterized, their use as food preservatives is still very limited. Few bacteriocins are commercially produced, being the main nisin and pediocin. However, nisin is the only approved as food preservative in many countries [3]. Bacteriocins have been mainly isolated from dairy, vegetables and meat products and a few were obtained from fish products [2].

Use of bacteriocins in food preservation offers several benefits, among them: they are safe and natural agents; its use reduces the need of chemical preservatives and can decrease the extension of thermal treatments. Since bacteriocins do not possess a wide antimicrobial spectrum and it is not possible to use them as a single conservation factors, they are used in combination with other stress factors as part of the hurdle technology [4].

2. Bacteriocins in food

2.1. Forms of incorporation

Bacteriocins can be incorporated to food systems as *ex situ* produced bacteriocins preparations or by the inoculation with the bacteriocin producer strain. Each one of these forms presented advantages and disadvantages [4]. *Ex situ* preparations are obtained by growing the producer strain at industrial scale and then concentration and purifications processes are needed to obtain a pure form of the bacteriocin. Mentioned processes are expensive and time consuming being difficult to obtain good recoveries. Moreover, the compound obtained must be approved as preservative prior to its use in a food product. Furthermore, the antibacterial activity have to be checked in a first step *in vitro* and then in the food product since it is common to observe less activity in the food than in model systems. This trend is due to the fact that the bacteriocin can loss its activity due to enzymatic degradation and interactions with food components such as proteins and lipids [3, 4, 5]. One strategy to overcome this problem is to apply the bacteriocin in the form of an immobilized preparation using a carrier which acts as a reservoir that releases the bacteriocin. Different methods have been used for the immobilization: adsorption to the producer cells [6, 7], incorporation into coatings and films [8], and inclusion into starch particles [9].

In situ bacteriocin production does not require a specific legislation approval, it has low cost of production, but has other requirements: the strain has to be well adapted to the food environment being able to grow and produce the bacteriocin in the concentration required to inhibit the target bacteria [4]. Bacteriocin strains can be used as protective culture for non-fermented foods, as starter cultures or as co-cultures for fermented foods. In fermented foods, the strain must carry out the fermentation process and at the same time to produce the bacteriocin. In co-cultures fermented foods, the strain must not interfere with the starter function. In non-fermented foods, the growth of the protective culture do not modify the sensory properties of the food and if a temperature abuse takes place, the strain must become the dominant spoiler preventing in this way the development of pathogens [4]. It has to be present that in many cases the bacteria spontaneously loss the ability to produce the bacteriocin, the producer strain can be infected by bacteriophages, or the indigenous biota can inhibit the bacteria. Finally, some safety aspects have to be taken into account when using the bacteriocin producing strain. In seafood products, the production of histamine must be checked, as it is regulated in fish rich in hystidine for the allergic problems that it can caused [10]. Since the genus *Enterococcus* is sometimes associated with pathogenicity [11], the presence of virulence factors and the possibility of multiple antibiotic resistance have to be discarded prior to its use as biopreservative.

2.2. Factors affecting the effectiveness

The effectiveness of bacteriocins in food will depend on many factors that can be summarized in: the interaction with the food matrix and or with the target bacteria and the action of the microbiota present in the food [4]. As an example, addition of divalent cations such as Ca^{2+}, or Mg^{2+} led to a reduction of nisin activity since they were bounded to anionic phospholipids making the cytoplasmic membrane more rigid and thus reducing the affinity of the bacteriocin towards the cytoplasmic membrane [12]. Regarding the action of the microbiota present, it can be mentioned that the presence of competing microorganisms can be an environmental factor stimulating bacteriocin production such as divercin [13]. In other cases, microbiota can compete for nutrients with the bacteriocin producing strain inhibiting its growth and therefore bacteriocin production [4].

Factors affecting the efficiency of bacteriocins in several food matrices will be discussed in the next section

3. Food matrices

3.1. Emulsions

The traditional definition states that an emulsion consists of two immiscible liquids (usually oil and water), with one of the liquids dispersed as small spherical droplets in the other [14], which can be expanded saying that emulsions can be conveniently classified according to the distribution of the oil and aqueous phases: i) a system which consists of oil droplets dispersed in an aqueous phase is called an oil-in-water or O/W emulsion (e.g., mayonnaise, milk, cream, soups, and sauces); ii) a system which consists of water droplets dispersed in an oil phase is called a water-in-oil or W/O emulsion (e.g., margarine, butter, and spreads). But emulsions are more than two phases, they are thermodynamically unstable, and hence, a stabilizer is needed to form emulsions that are kinetically stable for a practical period of time (a few days, weeks, months, or years) by including substances known as emulsifiers and/or thickening agents prior to homogenization. On the one hand, emulsifiers are surface-active molecules which adsorb to the surface of freshly formed droplets during homogenization, forming a protective membrane which prevents the droplets from coming close enough together to aggregate. Most emulsifiers are amphiphilic molecules (i.e., they have polar and nonpolar regions on the same molecule), being the most common ones: proteins, small-molecule surfactants, and phospholipids. On the other hand, thickening agents are ingredients which are used to increase the viscosity of the continuous phase of emulsions, enhancing emulsion stability by retarding the movement of the droplets. The most common thickening agents used in the food industry are polysaccharides (starchs and gums, among others). From a microbiological point of view, the preservative agent chosen to keep food emulsions safe and healthy will depend on many environmental and structural factors inherent to the food composition, shelf-life, storage type and target microorganisms. Nowadays there is a variety of emulsions being preserved by means of bacteriocins, mainly nisin which has been used as a food preservative in processed cheese, baby foods, mayonnaise, various pasteurized dairy, liquid egg products, and salad dressings [15].

Whether a bacteriocin will be functional in an emulsion or not will depend on the interactions that this polypeptide exerts within the different phases of the emulsion, being the aqueous phase a key factor in this matter. There is where microbial growth takes place and its structural characteristics become relevant as long as they influence microbial growth. Brocklehurst and coworkers [16] had established that the way in which microorganisms grow (colonies, films or planktonic cells) in emulsions depended on the average droplet size of the dispersed phase. Therefore, a comprehensive approach to the functionality of a bacteriocin in an emulsion should consider not only the antimicrobial effectiveness *per se* but also every component of the food matrix. Many reports had described that nisin activity was less effective when the fat content of food emulsions was increased [17, 18, 19, 20]. The influence of stabilizers on nisin efficacy had also been described. Emulsifiers, such as non-ionic surfactants, were shown to stimulate nisin activity

[21, 22, 20, 23] whereas zwietterionic ones were antagonistic [24]. Thickening agents, such as gums or corn starch, were presented as potential conditioners of bacteriocins antimicrobial activity [25, 26]. Besides, other environmental factors, namely pH, NaCl, divalent cations, were found to reduce bacteriocin activity in model systems [5, 12].
Several studies have shown that bacteriocin activity is diminished in foods that contain fat. How fat or lipids interfere with nisin activity could be related to its mode of action. Henning and coworkers [27] indicated that bacterial cytoplasmic membrane is the major target and is disrupted by nisin's interaction with its phospholipid components. It was further suggested that the electrostatic attraction between nisin molecules and the negatively charged phospholipids is involved in the antibacterial effects [28]. Moreover, Ming and Daeschel [29] observed low antilisterial activity of nisin in one of two strains of *L. monocytogenes* Scott A which had the lowest phospholipid content of both strains. As the nisin resistant cells were found to bind less nisin and release less phospholipids than the sensitive cells (when treated with the same concentrations of nisin), the authors attributed the effect to the decreased phospholipid component on the membrane of the resistant cells, and concluded that nisin reacts with the phospholipids, and that cellular damage depends on the amount of membrane phospholipid present. Taking into consideration that the reported phospholipid content in bovine whole milk is higher than skimmed milk, Bhatti et al. [18] postulated that the phospholipids present in ~2% fat market milk were responsible for binding a large portion of the added nisin, resulting in reduced nisin available to react with the cell membrane of the target microorganism. Thereby, antimicrobial activity had been reduced. On the contrary, that was not the case in skim milk, where similar nisin concentrations had been sufficient to cause disruption of the listerial cell membrane. Earlier studies from Jung et al. [20] had shown 33% of reduction in nisin activity when it was added to skim milk and 88% when added to milk containing 12.9% fat.

In spite of the fact that the work from Bhatti et al. [18] suggested an excellent explanation supporting the idea of phospholipid-mediated interactions, many are the questions which had arisen when the use of emulsifiers and other preservatives was proposed to counteract decreases of nisin activity in food emulsions. Castro and coworkers [17] found that the increase of oil level in the presence of nisin produced different effects which depended on system composition. In the presence of nisin alone, a decrease of the death rate constant of *Lactobacillus fructivorans* in salad dressings was observed when oil concentration increased. But this tendency changed in the presence of potassium sorbate and/or Tween 20 (Tw). In those emulsions containing nisin+potassium sorbate (PS) and nisin+PS+Tw, a slight increase in oil level determined a significant increase in death rate constants, while a further increase of the oil content determined a decrease in the values of death rate constants. These results had led to the idea that the antagonistic effect of fat on nisin activity was concentration dependent and could have been attributed to: I) Nisin being less effective as oil level was increased, as it had been mentioned above. II) The dissimilar rheological properties observed for the studied emulsions, which could have affected the effectiveness of the different preservatives in two divergent ways: i) an increase in oil level could have led to a higher death rate constant due to the reduction of the nutrient availability given by the stronger solid character of the emulsion matrix; ii) an increase in oil level could have led to a lower death rate constant because it hindered the meeting of the preservatives with the microorganisms as a result of the stronger solid character of the system. III) An increase of the oil phase determined an increase in the external droplet surface (as long as droplets had kept their size) leading to an increase of the interfacial surface which enabled more nisin to be at the interface. Although this situation would change bacteriocin effectiveness, it would allow the meeting of the preservative and the microorganism at the oil droplet surface (or at the interface), enhancing nisin effectiveness against microbial growth. This scenario was proposed by Kurup, Wan, and Chan [30, 31] and it was reported by Brocklehurst et al. [16]. Hence, two possibilities could be expected: i) nisin effectiveness decreased as a consequence for not being at the water phase which would lead to a reduction of the death rate constant; ii) nisin effectiveness increased because of the fact that the interface gave like a ''meeting point'' where nisin encountered microorganisms, which would augment the death rate constant. Consequently, the reported nisin activity in these emulsions had been the result of an intriguing combination of factors where the concentration of the oil phase and the presence of a surfactant reigned.

According to McClements [14], the adsorption of a polypeptide, like nisin, to the oil–water interface formed in an emulsion can be counteracted by the use of emulsifiers, like the Tween family or lecithin, since they displace bacteriocins from the interface by changing its thermodynamic environment. An enhancement effect on nisin activity in milk by the emulsifier Tween 80 was reported by Jung et al. [20] and Bhatti et al. [18]. Monolaurin was used in combination with nisin to inhibit *Bacillus licheniformis* spore growth and *Bacillus* sp. vegetative cells in milk [21 and 22], respectively). Effects of Tween 80 and lecithin on the activity of sakacin C2 against *Escherichia coli* ATCC 25922 in pasteurized and homogenized whole milk were studied by Gao et al. [32]. The formation of stable complex between sakacin C2 and zwitterionic phospholipids of lecithin had a negative effect on bacteriocin antimicrobial activity against *E. coli.* In this study, the milk fat significantly had decreased the effect of sakacin C2 against *E. coli* in milk but Tween 80 helped to offset this effect by decreasing the amount of protein bound to milk fat droplets. Conversely, a study by Rogers and Montville [26] showed that increasing concentrations of phospholipids (lecithin) may increase the loss of nisin effectiveness against *Clostridium botulinum 56A* in a model food system.

The influence of several gums (xanthan gum, λ-carrageenan, arabic gum and tragacanth gum) on the growth and the production of a bacteriocin-like substance from *Lactobacillus curvatus/sakei* ACU-1 was assessed by Castro et al. [25] in model systems using MRS broth. Xanthan and arabic gum did not significantly influence bacterial growth rate while tragacanth and λ-carrageenan promoted it. The effect of the presence of gums on bacteriocin production was dependent

upon the type of gum, i.e. compared to the control system, arabic gum diminished it, and the rest of the gums showed an enhancing effect. Arabic gum interfered with bacteriocin activity, meaning that its use in food products would be conditioned. Nevertheless, Rogers and Montville [26] found that carrageenan, guar gum, soluble starch or xanthan gum did not modify nisin antimicrobial activity in a model system.
On the whole, food emulsions are really complex systems which become unique for every particular formulation. Hence, it should be expected that the effectiveness of bacteriocin activity would be associated to each specific formula not necessarily following any presumption from previous data.

3.2. Meat and meat products

Changes in the food chain create opportunities for the emergence of new diseases and the re-emergence of old ones. Since pathogens do not recognize national boundaries, the rapidity with which individual microorganisms can circumnavigate the globe spreading infections makes the control of communicable diseases an enormous challenge for governments as well as for the public and primary health care systems. The manufacture of an increasing range of novel meat products as functional foods and the inclusion of ingredients considered beneficial for health [33] may also pose additional dangers with respect to safety. Additionally, the presence in meat products of chemical additives and residues of agrochemical and veterinary drugs is also perceived by consumers as a health risk. Even when the level of these residues seldom exceeds the regulatory limits in meat products [34], the use of antibiotics in intensive animal production poses the additional risk of bacterial resistance, which constitutes a microbiological hazard rather than a strictly chemical residue one. Nitrates are commonly used to prevent clostridial growth in meat; however, safety concerns regarding the presence of nitrites have prompted the food industry to look for alternative methods of preservation [35]. On these bases, the need for solutions concerning the hygienic quality of meat products has been increased along these last decades. Consumers demand food that is free from pathogens, with minimal processing and fewer preservatives and additives but with an unimpaired sensorial quality. As a response to these conflicting demands, current trends in the food industry include the investigation of alternative inhibitors to ensure food safety. Biopreservation has gained increasing attention as means of naturally controlling the shelf life and safety of meat products. The application of bioprotective cultures to ensure the hygienic quality is a promising tool although, as pointed out by Holzapfel et al [36], it should be considered only as an additional measure to good manufacturing, processing, storage and distribution practices, foods should not be preserved by bacteriocins alone but rather as part of a system with multiple hurdles.

Some microorganisms commonly associated with meats have proved to be antagonistic towards pathogenic and spoilage bacteria. As it has been previously said, lactic acid bacteria (LAB) have a major potential for use in biopreservation because they are safe for human consumption (GRAS status) and are the prevalent microflora during storage in many foods [37]. Bacteriocins of lactic acid bacteria have the potential to prevent microbial food spoilage and to inhibit growth of pathogens such as *L. monocytogenes*, fact that have attracted considerable interest in recent years and makes them prone to be used as natural food preservatives.

L. monocytogenes is hard to control because it can grow over a wide range of temperatures and pH values and because of its tolerance to high levels of sodium chloride and sodium nitrite [38], agents commonly used by the meat industry to control pathogens and to enhance water retention, meat particle cohesion, fat binding and colour preservation [39]. *L. monocytogenes* may be a problem in fermented meat products that are subjected to a very short drying process resulting in products that have a relatively high water activity and produce insufficient acid to inhibit its growth. Comminuted cured pork (German-type fresh mettwurst) exemplifies such a quickly ripened product in which the multiplication of *L. monocytogenes* may occur under certain conditions of elevated temperatures and slow acid production [40].

In the manufacture of fermented meat products the bacterial starter cultures used are essentially, lactic acid bacteria from genera *Lactobacillus* [41] and *Pediococcus* [42] and *Micrococcaceae* from genera *Micrococcus* [43] and *Staphylococcus* [44, 45]. Yet a number of factors must be taken into consideration when choosing a bacteriocinogenic starter culture for meat fermentations, such as the ability of the strain to grow and produce the bacteriocin *in situ*, diffusion of the bacteriocin through the meat [46], its binding to food compounds like proteins and fat [5], the influence of specific meat ingredients such as salt and nitrite, and conditions such as proteolytic degradation that destabilize the biological activity of the bacteriocin [47, 48].

Although nisin is the only commercially exploited lantibiotic to date, efforts are being made to develop applications for other lantibiotics. Lacticin 3147, a two-peptide lantibiotic produced by *L. lactis subsp. Lactis* DPC3147 isolated from Irish kefir grains, exhibits a bactericidal mode of action against food spoilage and pathogenic bacteria [49]. The high heat stability and broad pH range of lacticin 3147 make it attractive for use in the food industry. Even though most lactococcal bacteriocins were isolated from dairy and vegetable products, several nisin-producing *L. lactis* strains were isolated from fermented sausages, indicating the potential use of lactococci in meat fermentation. Nisin-producing *L. lactis* strains from Spanish fermented sausages [50] and from traditional Thai fermented sausage [51] were effective in inhibiting closely related LAB, *L. monocytogenes*, *C. perfringes*, *Bacillus cereus* and *S. aureus*. Moreover, *Lactobacillus sakei* L45 isolated from Norwegian dry sausages and *Lb. sakei* 148 from Spanish fermented sausages secrete lactocin S, a lantibiotic whose moderate spectrum of activity comprises LAB and *Clostridium* [52]. The

abundance of LAB strains producing lantibiotic bacteriocins evidences the significance of these substances in fermented products. These bacteriocins are present in different products and geographical environments [37].

Nisin or its combination with lower levels of nitrate can prevent the growth of Clostridia. Though some researchers concluded that nisin is not effective in meat applications due to high pH [53], inability to uniformly distribute nisin, and interference by meat components such as phospholipids [54], other found contradictory results. Moreover, it was verified that nisin is inactivated by glutathione in a reaction catalyzed by glutathione S-transferase. Glutathione is found in raw meat, and the reaction greatly diminishes the activity of nisin [35].

In pork meat, nitrite has only a slight negative effect on the growth of *L. monocytogenes* [55]. It is therefore necessary to take additional prevention measures such as the use of a starter culture having the ability to grow and produce bacteriocin *in situ*. Kouakou et al. [56] had tested the antilisterial action and the pH modification of *L. curvatus* strain CWBIB28wt in various pork meat systems during storage at 4ºC, relating observations to the level of bacteriocin activity and pH measured in these systems at each sampling. The work led to the following observations: i) there was no significant ($p > 0.05$) change in the pH in any inoculated product; ii) the *L. curvatus* strains CWBI-B28wt and CWBI-B28mt grew better in fat-rich than in lean meat, probably due to the high water activity of fat-rich meat; iii) the protection conferred by the bacteriocinogenic *L. curvatus* strain was not complete, the inability of a bacteriocinogenic starter to completely prevent *L. monocytogenes* growth in food systems over a long period of refrigerated storage had been reported previously for *Lactobacillus sake*, *Lactobacillus casei*, *Lactobacillus bavaricus*, *Pediococcus acidilactici*, and *Lactococcus lactis*, regrowth observed reflects proteolytic degradation of bacteriocin at the end of the culture [56]; iv) nitrite antagonized the antilisterial effect of *L. curvatus,* this is in keeping with the observations of many investigators, but different mechanisms would appear to be involved in different situations. One reported mechanism is slower growth of the bacteriocinogenic strain in the presence of nitrites, leading to reduced volumetric bacteriocin production [57]. Another is nitrite-triggered repression of bacteriocin production [58]. Chumchalová et al. [59] and Hornbæk et al. [60] had also observed decreased production of bacteriocins by lactic acid bacteria in the presence of sodium nitrite. It had been suggested that the decrease in bacteriocin production in the presence of salt is due to interference of sodium chloride molecules with binding of the induction factor, which is essential for bacteriocin production, to its receptor [61]. Other mechanisms described were direct interference of nitrite with the action of bacteriocin through binding to its pathogen-targeting pole and enhancement of Listeria resistance through nitrite-induced stress [62]. A high-fat content antagonized the antilisterial effect of bacteriocinogenic *L. curvatus*. As mentioned above, *L. curvatus* grew significantly better in high-fat than in low-fat meat. One might thus expect bacteriocin production to be high under these conditions. Yet in the absence of nitrite, the level of antilisterial protection conferred by the bacteriocinogenic *L. curvatus* strain was much lower in high-fat meat than in lean meat. Several authors [59, 63, 64] thought that the unsatisfactory effect of bacteriocin-producing strains *in situ* is due to hydrophobic interactions of the bacteriocins with fat. Several factors in the food model system may interfere with bacteriocin activity. Sakacin P, produced by the studied strain, may adsorb to meat and fat particles and this may result in its inactivation [65]. Some bacteriocins, like nisin, have a stabilising effect on the fat–water interface; their association with fat is readily reversible and does not prevent their antilisterial action. Other bacteriocins, like sakacin P, bind tightly to lipids in the food matrix [5]. They may remain trapped, unable to interact with the target pathogen. This could also make them hard to recover from the matrix.

A commonly examined system is sausage, since its spoilage is often attributable to lactic acid bacteria that can be inhibited by bacteriocins. Davies et al. [63] examined the influence of fat content and phosphate emulsifier on the effectiveness of nisin in sausage and found that lower fat contents correlate with higher nisin activity in the system. Other studies [66] had used nisin in combination with lactic acid to show an increased effect when the preservatives were used together to inhibit Gram negative organisms. No advantage to this combination was seen when used to inhibit *L. monocytogenes* Scott A or *Lactobacillus* spp. Nisin was also effective at inhibiting *Brochothrix thermosphacta* when incorporated in a cold meat-binding system [35].

Lb. curvatus CRL705 isolated from artisanal Argentinean sausages [67] produces Lactocin 705, a bacteriocin whose activity depends on the complementary action of two peptides (lac705a and lac705b). The bactericidal action of this two-component bacteriocin relies on the complementary action of two peptides which form poration complexes in the cytoplasmic membrane, thereby dissipating ion gradients and resulting in inhibition of the growth of target microorganisms. These events are strongly stimulated in the presence of glucose and adversely affected by Ca^{2+} ions; thus, these components together with other food parameters can have a significant influence on bacteriocin activity [37]. The shelf life of refrigerated vacuum-packaged meat is dependent on the growth of psychrotrophic bacteria including lactobacilli, leuconostoc, carnobacteria and *Brochothrix thermosphacta*. Rozbeh et al. [68] reported an immediate bactericidal effect of nisin and also of pediocin AcH, a bacteriocin produced by *P. acidilactici* AcH, on *L. mesenteroides* that had been inoculated into vacuum-packaged beef.

With respect to the application of bacteriocins in fat rich foods, many results suggest that bacteriocins will be more efficient when applied to a meat surface, than in liquid food and forcemeat, where the fat, water and the bacteriocins are mixed, such as in homogenized whole milk, sausages, etc. As it was described in the previous section, several studies on use of bacteriocins in milk had shown reduced efficiency with increasing fat content and may support this hypothesis [20, 59, 19]. Studies on the effect of fat in meat or other muscle foods are more ambiguous. Examples are the results

reported by Davies et al. [63], who observed that nisin was more efficient in sausages with low fat content than with high fat content, and Aymerich et al. [69], who demonstrated better effect of enterocin against *L. innocua* in paté with 20% fat than in cooked ham with 3.5% fat.

Regarding practical application of bacteriocins, the important issue is not whether the bacteriocin is bound or not, but if it is active. Few studies have addressed this problem. Aasen et al. [5] found that more than 80% of the added bacteriocin was adsorbed to the muscle protein, but the activity of the protein-bound bacteriocin could not be assessed. Proteolytic activity caused degradation of the studied bacteriocin, sakacin P, and probably other bacteriocins in not heat-treated foods could suffer from the same reaction, but these authors suggested that losses could be compensated by increased dosages of bacteriocins.

3.3. Fish and fish products

Fish and fish products are very perishable since they contain relatively large quantities of free amino acids and volatile nitrogenous bases. During storage, fish quality is quickly reduced being chemical and enzymatic reactions the cause of the initial loss of freshness, while microbial spoilage produces the end of the shelf life [70]. The increasing demand for high quality fresh fish has intensified the search for new methods and technologies for preservation. Many natural antimicrobials are proposed to be used for increasing shelf life of fish products and inhibiting pathogenic flora. It is of special interest the inhibition of *L. monocytogenes.* The growth of this bacterium is difficult to control in foods, especially in ready to eat (RTE) ones [71]. As an example, about 1600 cases of listeriosis are reported each year in the United States. Ready to eat fishery products containing *L. monocytogenes* represents 6.6% of the total, sampled at the processing stage. In particular*, L. monocytogenes* may be found in cold smoked salmon since there is no listericidal step during production. Bacteriocins can act against Listeria in smoked salmon and several authors have applied different bacteriocins to inhibit this pathogen [6-8, 72-78]. A compilation of the use of bacteriocins to preserve fish and fish products is shown in Table 1.

Table 1 Compilation of some uses of bacteriocins for the preservation of fish and fish product

Bacteriocin-form of application	**Additional hurdle**	**Fish – target microrganisms**	**Reference**
Nisin Z, carnocin U149, bavaricin A. Crude & purified preparations	Refrigeration, benzoate, sorbate, brine solution	Brined Shrimp, spoilage flora	Einarsson & Lauzon [87]
Carnobacterial strains, *in situ* production	Refrigeration	Simulated cold-smoked salmon, *Listeria monocytogenes*	Duffes et al. [72]
Nisin (commercial form)	Heat (60 or 65°C) Moderate heat	Cold-pack lobster meat	Budu-Amoako [92]
Sakacin P (purified) and or *Lactobacillus sakei*, *in situ* production	Refrigeration	Cold-smoked salmon, *Listeria monocytogenes*	Katla et al. [73]
Nisin (commercial form)	Sodium lactate and refrigeration	Cold-smoked rainbow trout	Nykänen et al.[81]
Nisin (commercial form)	Chitosan films containing sodium lactate, sodium diacetate, potassium sorbate, sodium benzoate	Cold-smoked salmon	Ye et al. [8]
Lactococcus lactis PSY2 *in situ* production	Refrigeration	Reef cod fillets, pathogenic and spoilage flora	Sarika et al. [88]
Carnobacterium maltaromaticum in situ production	Hydroalcoholic extract of alecrim pimenta, vacuum packed, refrigeration	Surubim fish, *Listeria monocytogenes*	Dos Reis et al. [83]
Divergicin M35 Purified and crude preparations or *C. divergens* M35, *in situ* production	Refrigeration	Cold-smoked salmon *Listeria monocytogenes*	Tahiri et al. [6]
Nisin (commercial form)	Calcium alginate coating and lysozyme	Smoked salmon, *Listeria monocytogenes and Salmonella anatum*	Datta et al [74]

Enterococcus faecium ET105, Lactobacillus curvatus ET106, L. Curvatus ET30, L. Delbruekki ET32, Pediococcus acidiliactici ET34, *in situ* production.	Vacuum and refrigeration	Cold-smoked salmon *Listeria innocua*	Tomé et al. [75]
Lactobacillus curvatus CWBI-B28, purified preparations and *in situ* production	Refrigeration	Cold-smoked salmon *Listeria monocytogenes*	Ghalfi et al. [7]
Carnobacterium piscicola CS526, in situ production	Refrigeration	Cold-smoked salmon *Listeria monocytogenes*	Yamazaki et al.[76]
Carnobacterium divergens V41, in situ production	Refrigeration	Cold-smoked salmon *Listeria monocytogenes*	Brillet et al.[77]
Carnobacterium divergens V41, in situ production or Culture supernatant	Refrigeration	*Listeria innocua*	Vaz-Velho et al. [78]
Nisin	Zataria multiflora boiss Essential oil and refrigeration	salted fish fillets *Vibrio parahaemolyticus* and *Listeria monocytogenes*	Ekhtiarzadeh et al. [80]
Nisin	Cinnamon, edta, alginate calcium coating refrigeration	Snakehead fish fillets Indigenous bacteria	Lua et al. [79]

Lactic acid bacteria of different genus such as *Lactococcus, Enterococcus* and *Carnobacterium* have been used as protective cultures. In particular, the *Carnobacterium* has been reported as a good choice since strains from this genus are able to grow and produce bacteriocin at low temperature and high sodium chloride concentrations. Also, some *Carnobacterium* species have the ability to grow in foods with low carbohydrate content such as fish and have low acidifying potential, as a consequence no changes in sensory properties are expected [6]. Use of bacteriocin producing strains of *C. piscicola*, [72, 76] and *C. divergens* [6, 77, 72] were reported for the control of *Listeria* in smoked salmon. The efficacy for listerial inhibition depended mainly on the adequate selection of: i) bacteriocin or the bacteriocinogenic strain, in the case of *in situ* production; ii) the form of application, iii) the interaction with food components and/or other conservation factors.

The comparison of the activity of different bacteriocins or their producer strains has been studied as an example, Tome et al., [75] evaluated the ability of five bacteriocin producing strains, *Enterococcus faecium* ET *ET105, Lactobacillus curvatus ET106, L. Curvatus ET30, L. Delbruekki ET32* and *Pediococcus acidiliactici* ET34, selected by their capacity to grow and produce the inhibition of *Listeria* in the conditions applied for salmon processing. The bacteriocin producing strains were added to salmon fillets by immersion and subjected to cold smoked process. Results obtained showed that ET105 was the best biopreservative for controllong *Listeria* while ET106 and ET34 exerted a bacteriostatic action.

The influence of the form of bacteriocin application on its effectiveness has also been evaluated in several studies. Ghalfi et al. [7] analyzed different strategies for the addition of the bacteriocin producing strain *Lactobacillus curvatus* CWBI-B28: *in situ* production, spraying with partially purified bacteriocin, packaging in bacteriocin coated plastic film, and immobilization onto the producer cell. All the approaches were useful for the inactivation, but the efficacy was diverse. Samples treated with either partially purified or *in situ* bacteriocin production promoted a decline in Listeria counts to 0.7 log $CFU.cm^{-2}$ within the first week but one log cycle increase was observed after day 14. The bioactive packaging produced a slower inactivation but no increase in the population was determined during storage. Immobilization onto the producer cell was the most effective treatment since a complete inactivation was verified within 3 days at 4ºC and no increase in *Listeria* growth was observed up to 22 days of storage. The adsorption of the bacteriocin to the producing strain does not need premarket approval since it can be regarded as a conditioning method of the lactic acid bacteria which has the status of generally recognized as safe. Tahiri et al. [6] also studied different application strategies -they used *C. divergens*, a strain that produced divergicin M35, purified divergicin and culture supernatants of *C. divergens*- for *Listeria* inhibition in cold-smoked salmon. Different patterns of inhibition were obtained depending on whether the producer strain, the purified bacteriocin or the culture supernatants were used. The application of the bacteriocinogenic strain promoted a slower but more pronounced reduction on Listeria counts than the one observed for the purified bacteriocin or the culture supernatant. The latter produced a more rapid and persistent inactivation than divergicin M35. This trend is linked with the possible degradation of divergicin M35 by endogenous proteases or by molecular interaction with the food matrix. However, in other studies the purified form was more effective than the producer strain [2, 78]. Mentioned trends stressed that stability of purified bacteriocin in the food matrix played a key role on effectiveness.

Bacteriocins are used in combination with other stress factors which can exert different effects on the activity of bacteriocins. There are many reports about the use of nisin in combination with other stress factors such as the use of edible films [79] and other antimicrobial agents such as essential oils [80], sodium lactate, or chitosan [81, 8, 82].

Ekhtiarzadeh [80] found a synergistic action of multiflora boiss essential oil and nisin on *Listeria monocytogenes* and *Vibrio parahaemolyticus* inhibition in refrigerated salted fish fillets. Moreover, Nykänen [81] reported that the combined used of nisin and sodium lactate exerted a synergic action on Listeria inhibition. Mentioned results suggest that nisin in combination with other antimicrobials is useful for controlling *Listeria* growth in fish.
The evaluation of the effect of other antimicrobials on the activity of bacteriocins produced *in situ* was scarcely evaluated. Dos Reis et al. [83] used the bacteriocinogenic *Carnobacterium maltaromaticum* C2 and the hydroalcoholic extract of *Lippia sidoides* cham. to control *Listeria monocytogenes* growth in Surubim, a South American tropical fish during storage at 5ºC. In this case, no synergistic effect of the combined used of the hydroalcoholic plant extract and carnobacteria was observed. The plant extract was able to inhibit *Listeria* but it could also inhibit bacteriocin production. These results emphasize the need to carefully consider the interaction between the stress factors in order to optimize bacteriocin effectiveness.

In addition to the listericidal action, bacteriocins can also be useful to control spoilage flora. This task is probably more difficult than controlling Listeria because spoilage is the consequence of a complex ecosystem composed of different species. For this reason the search of bacteriocinogenic strains with wide antimicrobial spectrum is a must. In this field, several bacteriocinogenic LAB strains have been isolated from sea products which are active on many spoiling and pathogenic fish flora [84, 85, 86]. Moreover, few studies were done with the purpose of controlling spoilage flora by using bacteriocins [87,88]. Sarika et al. [88] evaluated the ability of the *Lactococcus lactis* bacteriocinogenic strain PSY2 isolated from the body surface of marine perch to inhibit spoilage bacteria at different storage temperature. For that purpose, red cod fillets were sprayed with the bacteriocin, wrapped in aluminum foil and stored at 4, 0 and -18ºC for storage and at selected times the evolution of the indigenous flora was evaluated. Viable counts of psychrophilous in bacteriocin treated samples were lower than 10^7 CFU.g^{-1} being acceptable for consumption according to the International Commission of Microbiological Standards for Foods (ICMSF) [89] until the third week of storage at 4ºC while control samples became unacceptable before the second week. Einarssson et al. [87] evaluated the ability of purified and crude Nisin Z, carnocin U149 and bavaricin A to extend the shelf life of brined shrimp in comparison with a control free of preservatives. Results obtained showed that carnocin U149 did not extend the shelf life, while crude bavaricin A extended the shelf life from 10 days (control system) to 16 days. Nisin in the crude or purified form was able to extend shelf life to 31 days. Results commented demonstrated that bacteriocins are useful for extending the shelf life of fish at refrigerated storage and their effectiveness depends on many factors among them, bacteriocin structure, its application form and the interactions with other preservation factors.
Bacteriocingenic lactic acid bacteria can be used as starter culture and also to improve the safety of fermented fish, as an example, Diop et al., [90] added the nisin producing *Lactococcus lactis* subsp. lactis strain CWB 1410 to aid with the spontaneous fermentation that takes place during the preparation of traditional Senegalese fermented fish. It was found that nisin was produced during fish fermentation and counts of enteric bacteria populations was lower than those found in fermented fish without the lactic acid bacteria. These data suggest that use of nisin producing strain can enhance the safety of fermented fish. Fish and fish products can be preserved with the aid of bacteriocins as it was commented. The growth and bacteriocin production is a must in the biopreservation process. The use of bacteriocinogenic strains isolated from fish may improve the probabilities of adaptation. In this sense, there are several reports about bacteriocin producing strains isolated from fish [85, 86, 86, 91].The search of these strains is of great interest to improve fish biopreservation [10].

4. Conclusions

Bacteriocins in combination with other stress factors are a valuable tool to control the growth of pathogenic and spoilage bacteria in foods. Since the efficacy of mentioned antimicrobials can be influenced by the chemical composition and the physical conditions of foods it is necessary to validate the antimicrobial activity in each particular food system to establish the effective concentration and the most adequate combination of additives or preservative treatments to be applied with the antimicrobial.

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