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NEW STRATEGIES FOR THE PRESERVATION OF COOKED HAM

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At a nearly constant consumption of meat products the consumption of high quality cooked ham – sliced and prepackaged – increases steadily the last years. The ban of nearly all technological aids and organic preservatives, together with the inevitability of (re)contamination during slicing makes these hams difficult to prepare. The prohibition to use phosphates is managed by the use of raw hams with superior technological qualities. The prohibition to use lactates and (di)acetates in such "clean label" products obliges to use natural preservatives. These chemicals derived from herbs/spices (coriander, origanum, thyme, *etc*.), vegetables (garlic, onion, horse radish, *etc*.) or fruits are able to delay bacterial growth, even to inhibit growth completely when applied in synergetic combinations. Also bacteriocins as nisin, pediocin, lactocin and curvacin are able to inhibit particular groups of bacteria. Their direct application to meat products is rigorously restricted and in most cases prohibited. Otherwise bacteriocins can be supplied indirectly by the use of protective cultures. But for cooked ham this is far from evident. On the other hand, the storage time can be prolongated for months by cold-pasteurisation techniques as pascalisation. Recently high pressure equipment has been developed that can destruct the spoilage and pathogenic microflora leaving colour and flavour unaltered. These pascalisers can generate from 2000 to 7000 bars of pressure. This technology is already in common use in Japan, Spain and the United States of America.

INTRODUCTION

By the absence of recent food scandals the consumption of meat products remained remarkable stable for many years in Belgium. Annually an average of € 170 are spent for meat products, which corresponds with a consumption of 16-16.5 kg per capita. Nearly 24 percent of it is cooked ham. In this group of meat products the consumption of normal quality ham decreases for an increase of high quality labelled cooked ham, the so-called *Meesterlyke* ham. The ban of nearly all technological aids, among which phosphates, makes these hams difficult to prepare. To avoid negative effects as deficient waterbinding, cracks, holes and poor slicebility, the raw hams are selected by pH, colour and/or conductivity. An important part (nearly 55 percent in 2004) of this type of cooked ham is sold sliced and prepackaged in gas atmosphere (20% carbon dioxide + 80% nitrogen). Such a distributed meat product requires a shelf life of 3 to 4 weeks at 7°C.

Despite stringent hygienic conditions during finishing, handling and slicing of the cooked ham it is almost impossible to avoid minor contaminations. From this general contamination, it will be mainly lactic acid bacteria that preferably develop under the anaerobic circumstances in the sliced prepackaged cooked ham at temperatures below 7°C. Above numbers of ten million colonies per gram, these bacteria can be responsible for sensory deviations like souring and gas formation.

TRADITIONAL PRESERVATIVE PRODUCTS

Nitrites and nitrates

The main reasons of applying nitrite and/or nitrate in meat products are (1) the colour formation, (2) the curing taste, (3) the prevention of the outgrowth of spoilage as well as pathogenic bacteria and (4) the protection against the oxidation of fatty acids.

The antimicrobial properties of nitrites strongly depend on pH. At pH = 5- 6 nitrite is bacteriostatic while at pH=7 it is not at all. In acidic environment bacteriostatic properties of nitrite increase ten-fold if pH decreases by one unit [Pezacki, 1981]. According to Wirth [1989], the minimum inhibitory concentration of nitrite against food poisoning pathogens like *Clostridium* spp, *Staphylococcus* spp. and *Salmonella* spp. is 80-150 ppm. Other researchers report that 50 ppm is sufficient to inhibit Enterobacteriaceae, while at 150 ppm LAB will grow undisturbed [Gonzalez, 2002]. On the other hand, 10 ppm inhibits *Lactobacillus curvatus*, a strain which produces a listericidal bacteriocin [Verluyten, 2003].

Lactic acid and lactates

Lactic acid is secreted by numerous bacteria. Lactic acid and its derivatives are Generally Recognized As Safe (GRAS) food additives proven to inhibit bacterial species in food products, *e.g.* meat products. Besides its bacteriostatic activity, lactic acid acts as flavour enhancer and antioxidant preventing fat oxidation by decreasing pro-oxidative effect of NaCl.

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It is commonly reported that 3% Na-lactate (SL) can extend the shelf life of vacuum-packaged ground beef below 7°C by significant inhibition of total psychrotrophic count [Sallam & Samejima 2004]. Houstma *et al.* [1993] examined the influence of SL on food pathogens in laboratory media. They report similarities in inhibition between Gram-positive and Gram-negative bacteria. Generally the gram-positives are more sensitive for SL; the growth inhibition started from 268 mmol/L for *Staphylococcus aureus* while G(-) bacteria were inhibited from 714 mmol/L (*Yersinia enterocolitica, Salmonella spp.*). The antilisterial activity of SL was reported to be in the range of 804-892 mmol/L. 625 mmol/L was sufficient to inhibit growth of *Bacillus* strains. Oh and Marshal [1993] reported that 0.5% of SL has a slight impact on *Listeria monocytogenes* in tryptic soy broth. Sodium lactate at a concentration of 1.5-3.5% is also reported to inhibit spore germination and delay toxin production of *Clostridium botulinum* [Maas *et al.*, 1989; Houstma *et al.,* 1994].

The bacteriostatic activity of lactates can be improved by the combination with other food additives. The most common are the combinations with sodium chloride or/and sodium (di)acetate. Many researchers report that those combinations can extend the shelf life of meat products and protect them against growth of spoilage as well as pathogenic bacteria [Choi & Chin, 2003; Stekelenburg *et al.,* 2001; Mbandi & Shelef, 2002; Sallam & Samejima, 2004; Tan & Shelef, 2002]. The 2%+2% combination of SL and NaCl can significantly extend the shelf life of vacuum packaged ground beef stored at 2°C by completely inhibition of Enterobacteriaceae after 21-day storage and significantly reduce the growth of LAB [Sallam & Samejima, 2004]. The antilisterial activity of SL with sodium diacetate is confirmed by many researchers. Mbandi and Shelef [2002] reported that a combination of 2.5% SL with 0.2% Na-diacetate is sufficient to completely inhibit the growth of *L. monocytogenes* in beef bologna stored at 5°C for 45 days.

Bacteriocins

Biopreservation by utilizing microorganisms to stress or inhibit other bacteria is being discussed nowadays in the scientific literature to a greater extent than ever before. Bacteria which are involved in competitions for the habitat for undisturbed growth are able to produce many biologically active molecules including bacteriocins. This constant-lasting competition has been noticed by men and used in the protection of foodstuffs (*i.e.* meat products) against bacterial spoilage.

Bacteriocins such as nisin, pediocin, lactocin, curvacin or others are small ribosomally synthesized polypeptides or proteins which are secreted in high diversity by many bacterial strains. Generally, lactic acid bacteria are considered as reservoirs of antibacterial peptides with potential food apply. Among all LAB the most comprehensively exploited strains are *Lactococcus spp., Lactobacillus spp., Leuconstoc spp.*. The biologically active molecules, they secrete, are mostly heat stable and are proven to inhibit even the most dangerous food-borne pathogens. Their specific activity involves permeabilizing of cell membrane, inhibition of cell wall synthesis, inhibition of RNase or DNase, *etc*. [Scannel *et al.*, 2000]. The application of bacteriocins to meat products is rigorously restricted and in most cases prohibited. As an alternative, the bacteriocins can be introduced in the meat products by the use of the so-called "protective cultures". Such (bio)preservation can be performed in direct and indirect way [Hugas, 1998]. The indirect (bio)preservation can be performed by utilizing a pure culture of the bacteriocin-producers. This is also possible by adding a mesophilic strain of lactic acid bacteria whose concentration will be kept at an initial low level in chilling conditions but the growth, and so the production of bacteriocins, will start when the temperature increases. The addition of a non-purified fermentation liquid obtained by growing the bacteriocin-producers is in fact one of the direct (bio)preservation methods.

Whereas protective cultures are applied succesfully in fermented (meat) products for many years their use in cooked ham production is still under investigation.

NATURAL PRESERVATIVE PRODUCTS

Phytochemicals

Nature contains phytomolecules which may be considered as potential antibacterial food additives. Many of them possess, besides antioxidant, also bacteriostatic activity, which makes them suitable for the preservation of meat products. Many of those phytochemicals act specifically to targets in bacteria by blocking certain mechanisms within the cell. Generally, herbs and spices are considered to have such active molecules, while other *Flora* representatives, *e.g.* flowers and fruits, are less exploited. Coriander (*Coriandrum sativum*), minth (*Mentha*), oregano (*origanum vulgare*), thyme (*thymus vulgares*), clove (*Syzygium aromaticum)* or rosemary (*Rosmarinus offinicalis*) are the most studied herbs and spices possessing bacteriostatic properties. D-limonene and carvon, applied at concentrations of 1000-3000 ppm satisfactory inhibit food-borne pathogens as *Escherichia coli, Salmonella typhi, L. monocytogenes* and *St. aureus* [Delaquis *et al.* 2002]. Thymol, carvacrol and p-cymene, being a precursor of carvacrol, are commonly reported to possess bactericidal activity if applied in synergetic combinations [Delaquis *et al.*, 2002; Aligannis *et al.,* 2001]. These molecules damage membrane integrity by increasing its permeability followed by affecting pH homeostasis and equilibrium of inorganic ions as well as dissipation of internal pH gradient [Lambert *et al.,* 2001]. Moreover, p-cymene is reported to incorporate in the lipid bilayer of bacterial cell to facilitate transport of carvacrol across the cytoplasmic membrane [Ultee *et al.,* 1999]. It is reported that thymol as well as carvacrol act listericidal at a concentration of around 200 ppm, additionally their activity may be enhanced by a combination with anti-microbial molecules of other plants [Lin *et al.,* 2004]. (+)-Borneol reveals listericidal activity at a concentration of 390 ppm [Mourey & Canillac, 2002].

Organic acids

The inhibitory activities of different organic acids have been appreciated by men and used for preserving food products. Their effectiveness and mode of action depends on the number and the sort of functional groups as well as the length of the carbon chain. Straight long-chained and phenolic acids have many advantages and great antibacterial potential in acting on cells. Especially their similarities in structure with the bacterial cell wall components facilitate the bacteriostatic activity. Those acids can attach to cell wall or incorporate in its structure causing negative consequences to the functionality of such a "modified" bacterial cell. Weak acids have greater inhibitory properties at acidic environment where the pH is lower than their pKa and the molecule is in its undissociated form. Bacteria have mechanism to compensate the low environmental pH and will not pass the charged molecule to get inside the cell. Only uncharged molecules of undissociated acids may cross bacterial wall and dissociate at higher internal pH. Disturbed electron potential results in the inactivation of essential enzymes, which leads to the inhibition of bacterial growth. Phenolic acids such as cinnamic ($C_9H_8O_2$), p-coumaric ($C_9H_8O_3$), ferulic ($C_{10}H_{10}O_4$) or caffeic ($C_9H_8O_4$) are reported to show strong antilisterial activity [Wen *et al.,* 2003]. Caproic ($C_6H_{12}O_2$) and caprylic ($C_8H_{16}O_2$) acids are reported to be highly effective in the inhibition of *Listeria innocua* and *L. ivanovii* as well as *Oenococcus oeni.* Both *Listeria* were inhibited despite their general acid tolerance [Nakai & Siebert, 2004]. *Pseudomonas aeruginosa*, a common spoilage bacteria of meat stored under oxygen rich conditions, is reported to be sensitive to acetic ($C_2H_4O_2$), caproic, formic (CH_2O_2) and lactic acids ($C_3H_6O_3$) [Wen *et al.,* 2003]. Gram positive sporeformers *Bacillus cereus* and *B. subtilis* are reported to be sensitive to caprylic, benzoic ($C_7H_6O_2$) and butyric ($C_4H_8O_2$) acids [Hsiao & Siebert, 1999].

Pascalisation

High hydrostatic pressure (HHP) represents a non-thermal technique for manufacturing all kinds of meat products without post-processing contamination, which is highly important in the cases of sliced ready-to-eat packaged meat products. To improve bacterial control HHP can be supported by natural food additives such as bacteriocins, phytochemicals or others. HPP is also a powerful tool to minimize hazard associated with food-borne pathogens in all kinds of meat products [Hugas *et al*., 2002]. It is postulated that the HPP treatment can extend the shelf life of meat products by controlling the growth of both spoilage and pathogenic bacteria [Yuste *et al*., 1999]. It can be stated that a treatment of vacuum-packaged meat products with high pressure (~10^8 Pa) is sufficient to reduce significantly the bacterial count and not to exert any negative impact on the products' quality [Garriga *et al*., 2002]. After such treatment most of the bacterial vegetative cells are destroyed. Also many enzymes are inactivated. The inactivation of bacterial cells depends on many factors as pH, temperature, time of exposure, extent of pressure *etc*. and also phenotypic diversity of organisms. Generally it may be affirmed that the bacteriostatic effect increases with pressure augment. The main targets of HHP in cell are probably wall (increased permeability caused by precipitation of wall components), enzymes (inactivation due to energy crisis), disturbance in active transport and DNA formation, *etc*. Microorganisms are probably not immediately inactivated but their death takes place as a natural consequence of sublethal cell damages [Doona *et al*., 2005]. Some bacteria are partially resistant to HHP. Gram positive bacteria are less susceptible to high pressure treatment than the Gram negative ones. The shape of the bacterial cell has also important consequences in survival; cocci are more difficult to destroy than rods. In addition, the age of bacterial cells and exposure to crude physical conditions are not without significance. Older cells of the stationary growing phase or those which were subjected to temperature shock are more resistant to HHP [Archer, 1996]. The presence of high amounts of nutrients makes microorganisms less potent to pressure inactivation also lessened fluidity of cell by low temperature treatment declines the bacteriostatic efficacy of HHP probably due to decreasing the length of the membrane fatty acids. Lower water activity has the same effect [Wemekamp-Kamphuis *et al.,* 2002; Smelt, 1998].

The physical damage aspects of meat components start at pressure close to 10^8 Pa. At 1.5E+8 Pa colour of meat turns to that of cooked meat, meat tenderisation increases and autolytic activity of lysosomes starts to break down. Higher pressure (~5.0E+8Pa) results in degradation of calpains and inactivation of cathepsins.

CONCLUSIONS

Despite the most strigent hygienic conditions during the preparation and slicing one cannot avoid contaminations of the finished meat product. To prolongate the shelf life of these semi-preserved food products precautions have to be taken against the outgrowth of pathogenic and spoilage bacteria. The increased demand to more natural meat products can be satisfied by the use of antimicrobials of natural origin. Making the use of mutual synergetic effects these chemicals can be supplied to lower the required amounts of traditional preservatives as lactates and (di)acetates. In figured out combinations they can be effective on their own; at least again the most common pathogen *L. monocytogenes* and most spoilage bacteria.

Whether protective cultures can be used in this kind of heated meat product remain subject of current research.

On the other hand one has proved that nearly sterile end products can be obtained by making use of pascalisation, which gives large perspectives for distribution of sliced MA-packaged cooked ham under unfavourable conditions; *e.g.* regions with a hot climate and poor refrigeration capacities.

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