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NEW GENERATION OF NATURAL ANTI-MICROBIAL PRODUCTS FOR THE PRESERVATION OF COOKED HAM

Slawomir Szczepaniak[1]*, Hubert Paelinck[1], Christophe Gyselinck[2]

[1]Katholieke Hogeschool Sint-Lieven, Belgium;
[2]DERA Food Technology N.V., Bornem, Belgium

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Cooked ham is a mild pasteurised meat product originated from hind leg of hog offered sliced and vacuum- or MA-packed for sale. Its shelf life is determined by bacterial flora surviving the cooking process and the contamination during slicing. As such hetero- and homo-fermentative Gram-positive lactic acid bacteria and *Brochothrix thermosphacta* are the main spoilage microorganisms while the prevailing Gram-negative microflora are *Serratia liquifaciens, Hafnia alvei*. Nowadays consumers demand high quality and convenient meat products, with natural flavour and taste, and the appearance of fresh processed food. The available strategies for reduction of pathogenic and spoilage bacteria tend to minimise the dosage of traditional preservatives such as sodium or potassium salts of lactic or acetic acid basing on synergistic combinations with other products derived from nature.

This research is focused on the bacteriostatic effect of ROBIN® products, a combination of long-chained organic acids of plant origin and traditional preservatives: Na-lactate (SL) and Na-diacetate (SD), against *Hafnia alvei, Brochothrix thermosphacta* and *Listeria innocua*. To this end, products of canned cooked ham, after cooking and post-thermal resting (t=24 h, T=1°C), were aseptically sliced, inoculated with known number of particular strain (2 log cfu/g), vacuum-packed and incubated at 4°C for 28 days.

First results show satisfactory growth inhibition of all bacteria by ROBIN®. The exception was the first week of incubation in the case of *Br. thermosphacta* and *L. innocua* in samples containing product R1, where the difference in growth between the control sample and the mentioned ROBIN® was not clear. The supplementation with SL and SD enhances bactericidal effect, restraining the growth completely. Prolonged shelf life of meat products by extension of bacterial lag phase was also observed in the case of all three groups of micro-organisms. Results of this study show that the dosage of traditional preservatives can be minimised or completely replaced by new products which are natural in origin.

INTRODUCTION

Cooked cured meat products, such as cooked ham, are free from heat- and halo-sensitive microorganisms. The type of the product denotes its shelf life, especially on ready-to--eat sliced meat products the spoilage appears faster than on others due to greater surface. They are usually packed in vacuum or in modified atmosphere (MAP), which supposes to maintain the shelf life of a product stored at chilled temperatures, alas the spoilage, caused by the growth of bacteria and/or enzymatic activity, frequently occurs within its shelf life. It is manifested as the development of gas, slime and/or discoloration (including greening) of the product, which is caused mostly by the presence of heterofermentative lactic acid bacteria or psychrotrophic Gram-negative *Enterobacteriaceae*. The bacterial infection is often associated with post-thermal manipulations such as slicing or packaging. Heat processing increases tenderisation or inactivates most of microflora but moderate heating may initiate germination of spore-formers as *Clostridium spp.* (pathogen), *Bacillus spp.* (mostly pathogen) or *Lactobacillus sporogenes* (probiotic bacterium with many advantages to human health) [Anonymus, 2002]. In addition, thermal treatment will not inactivate heat resistant bacteria such as *Lactobacillus vesenteroides* (*Weisella vesenteroides*) which may overgrow during storage time and contribute to spoilage [Rybka-Rodgers, 2001]. During the storage time, bacterial flora changes together with the chemical composition of a product, pH and water activity [Borch *et al*., 1996]. The predominant bacteria associated with the spoilage of refrigerated meat products are: gram-positive flora (*Brochothrix thermosphacta*), Lactic Acid Bacteria – LAB (*Lactobacillus spp., Leuconostoc spp., Carnobacterium spp*.) and psychrothrophic gram negative bacteria such as representatives of *Enterobacteriaceae* (*Shewanella putrefaciens, Serratia spp., Hafnia alvei*). Nevertheless the major bacterial group associated with the meat spoilage are hetero- and homo-fermentative lactic acid bacteria. At time of spoilage some products may contain a pure culture of only one species, while other may contain a mixture of those [Metaxopoulos *et al.,* 2002; Vermeiren *et al*., 2003; Jones, 2004; Cayre *et al*., 2005]. The addition of nitrite curing salt has many advantages such as colour development or inhibition of bacterial growth, however it exerts an impact on taste and carries risk of accumulation of carcinogenic nitrosamines in a human body [Hamasaki *et al.*, 2003; Radcliffe *et al*., 2003].

Brochothrix thermosphacta

*Author's address for correspondence: Slawomir Szczepaniak, Katholieke Hogeschool Sint-Lieven, Gebroeders Desmetstraat 1, B-9000 Ghent, Belgium; e-mail: slawomir.szczepaniak@kahosl.be

Brochothrix thermosphacta (known in literature also as *Microbacterium thermosphactum*) is a facultative anaerobic, gram-positive, catalase-positive, non-sporing, non-motile rod [Skovgaard, 1985]. The organism is closely related to genus of *Lactobacillus* and *Listeria*. *Br. thermosphacta* is predominant spoilage organism in chill-stored MA-packed and/or vacuum-packed raw as well as cooked meat products [Cayre *et al.*, 2005]. It grows in a wide range of temperatures (0°C-30°C) as well as pH (5-9), additionally its high tolerance to relatively low water activity increases its ability to overgrow many other spoilage bacteria as heterofermetative lactobacilli usually present at the same initial level [Rattanasomboon *et al.*, 1999]. *Br. thermosphacta* growing on the aerated meat products develops acetoin, acetic acid (both derived entirely from carbohydrates), iso-butyric acid (derived from valine) and isovaleric acid (derived from leucine), the major components of off odour of the spoiled product [Dainty & Hibbard, 1980]. Under anaerobic conditions mostly lactic acid is developed. It is reported that ribose is the main energy source [Grau, 1988]. Faeces of animals and/or soil are the main sources of meat contamination by *Brochothrix thermosphacta* [Skovgaard, 1985].

***Listeria* spp.**

Listeria spp. is widely distributed in environment [Grau & Vanderlinde, 1992]. *Listeria* is facultative anaerobic, gram positive psychrotrophic rods also present in coccoid forms, with good tolerance to salt. It grows good at relatively dry conditions (3% NaCl), while even at 20% NaCl may survive several weeks on food products. It is reported that *Listeria* reveals also tolerance to high levels of sodium nitrite (<1000 pm) [Aymerih *et al*., 2000]. *Listeriae* grow well at a wide spectrum of temperatures (1-45°C) and pH (may easily survive in acidic environment where the pH is below 4.5). It is reported that species of *Listeria* were recovered from meat products stored several weeks below 0°C. *L. monocytogenes* (different meat products) and *L. ivanovii* (mostly related with sheep meat) are highly pathogenic in nature [Marinsek & Grebenec, 2002].

Enterobacteriaceae

Enterobacteriaceae are small gram negative non-sporing rods capable to ferment sugars and produce acid or acid and gas. Within the family there exists further subdivision according to the ability to utilise nutrients, type of respiration, production of end product *etc*. Those bacteria able to ferment lactose are classified as coliforms. Most genera are commensals in the animal or human intestines. Some representatives of this family are able to reduce nitrates to nitrites. Inspite of the fact that *Enterobacteriaceae* are mesophilic bacteria many of them are capable to grow at chilling temperatures under vacuum as well as MA-packed products causing spoilage. It is reported that *Enerobacteriaceae* contaminate pork meat to a greater extent than other kinds of meat. *Serratia liquefaciens, Hafnia alvei, Enterobacter spp.* are able to grow at temperatures between 0 and 10°C causing spoilage of chill-stored meat products [Labadie, 1999; Borch *et al.*, 1996], at -1.5°C *Serratia spp.* and at 4°C *Hafnia alvei* dominates. *Enterobacteriaceae* produce large amounts of hydrogen sulphide, acetoin, diacetyl, 3-methyl butanol which are responsible for off odour and slime [Borch *et al.,* 1996]. *Enterobacteriaceae* are mainly related with post-producing recontamination.

The objective of research was to validate the bacteriostatic properties of two ROBIN® products, a combination of long-chained organic acids of plant origin and organic acids supplemented with traditional preservatives: Na-lactate (SL) and Na-diacetate (SD), against *Hafnia alvei, Brochothrix thermosphacta* and *Listeria innocua* in vacuum-packed sliced cooked ham products stored at 4°C up to three weeks. Experiments were commissioned by DERA Food Technology N.V., Belgium - also provider of all the products used in the study.

MATERIALS AND METHODS

Preparation of cooked ham. Pork muscles Porcine *Longissimus dorsi* of freshly slaughtered animals (24-48 h *post mortem*) were defatted, cleaned manually from any connective tissue and minced through 8 mm size pores. Afterwards meat was placed in a Stephan cutter (Stephan u. Soehne GmbH & Co, model UM12) and accurate volume of brine was applied. Brine (phosphate DERAPHOS® 0.5%; glucose syrup 0.5%; nitrite curing salts 2%; Na-ascorbate 0.1%) was prepared with 20% extension of water phase. Applying of antimicrobial combinations in appropriate concentrations preceded the canning (round-shaped cans high 50 mm, diameter 70 mm) and cooking process (T_{ext}=72°C, T_{int}=70°C). Ready products, prepared with accordance to standard Dutch recipe, were cold-deposited at 1°C for minimum 24 h before inoculation took place.

Inoculation of meat samples. All manipulations were done aseptically. Prior to the inoculation process, cans were rinsed with absolute ethanol to disinfect and were opened under the laminar airflow. Slices of cooked ham (20 g), were separately inoculated (2 log cfu/g) with particular bacterial strains (isolates from vacuum packed spoiled meat products) and closed under vacuum. Control samples and those containing antibacterial products were incubated at 4°C for up to three weeks. Three samples were taken for each analysis, additionally for statistical reason experiments were repeated three times, thus further presented results are mean values of nine measuring points.

Microbiological analyses. At regular time intervals (48 h), the samples of cooked ham were taken for microbiological analyses. Meat samples, together with adequate volume of 0.1% sterile peptone water (Merck VWR International, Belgium), were blended 60 sec in a stomacher (Seward Laboratory, model BA6021). Then bacterial supernatant was serially 10-fold diluted in 0.1% peptone water. Suspensions were thoroughly mixed at each step of dilution on Vortex (MERCK Eurolab, MELB1719). Bacterial growth was followed on selective agars, complemented with selective additives and on PCA agar (PCA agar, Merck VWR International) used for total aerobic microorganisms. McConkey agar (Merck VWR International) was used

for *Hafnia alvei*, on PALCAM-agar (Merck VWR International) was followed growth of *Listeria innocua*, *Brochothrix thermosphacta* was plated on STAA agar (Oxoid, CM0881). From each dilution 0.1 mL of bacterial suspension was spread in triplicate on each agar (n=9). In cases where bacterial concentration was below 2 $\log_{10}$ cfu/mL samples where spread with 1 mL on three plates (0.3, 0.3, 0.4 mL). Prior to spread, the plates were dried under the horizontal air flow for 30 min. Inverted Petri plates were incubated at appropriate temperatures and time.

All results were recorded as decimal logarithm of colony forming units per gram ($\log_{10}$ cfu/g) and as such were taken for statistical analyses. The means, standard deviations and error of standard deviation were computed using SPSS version 9.0 for MS Windows.

RESULTS AND DISCUSSION

The objective of research was to examine the bacteriostatic effect of synergetic combination of long-chained organic acids of plant origin (ROBIN® 1) and organic acids supplemented with minimised concentrations of traditional preservatives (ROBIN®2): Na-lactate (SL) and Na-diacetate (SD), against Gram-positive and negative spoilage bacteria as well as *Listeria innocua* as the surrogate strain of pathogenic species *L. monocytogenes*. Results showed satisfactory inhibition of all bacteria by both products. The supplementation with SL and SD (product R2) enhanced the bacteriostatic effect, restraining the growth completely.

Gram-negative bacterium *H. alvei* was effectively inhibited by both products; additionally the lag phase was extended up to 5th day of incubation in the case of R1 and 16th day in the case of R2 (Figure 1). The concentration of 7 log cfu/g, the level of spoilage, was reached on control after the first week of incubation, while for both samples containing both ROBIN® this phenomena was delayed till the fourth week.

The growth of Gram-positive *Br. thermosphacta* was completely restrained by R2 (Figure 2). The number of bacterial cells balanced on the limit of detection until 21st day of incubation. Product R1 also showed inhibitory impact on this microorganism, the difference in growth was 0.84 log cfu/g after the first week and increased to 1.83 log cfu/g after four weeks of incubation. The level of spoilage in the case of both ROBIN products was not reached during the entire period of experiment, the number of *Br. thermosphacta* viable cells was 5.45 and 4.85 log cfu/g for R1 and R2, respectively.

Both Robin® products demonstrated satisfactory antibacterial effect on *L. innocua;* additionally Robin® 2 was bactericidal. The number of bacterial cells during experiment as well as after 28 day-incubation balanced on the detection limit (Figure 3). The growth did not exceed the level of 4 log cfu/g during 4-week incubation, while on the control sample bacteria relatively fast reached 7 log cfu/g, the spoilage level. At the end of the experiment, the number of *L. innocua* colonies was 3.85 and 2.15 log cfu/g in the case of R1 and R2, resepctively.

CONCLUSIONS

The results showed that both ROBIN® products prolonged the shelf life of meat products by the extension of bacterial lag phase in the case of all three groups of microorganisms. Two of three bacteria did not reach the spoilage level, moreover the bactericidal effect on *L. innocua* was observed in the case of one product. During the experiment it was observed that a combination of pure natural components was effective enough to control growth of all bacterial strains used. Despite of that product R2, being a combination of natural components and traditional preservatives, revealed better activity, the effectiveness of R1 is

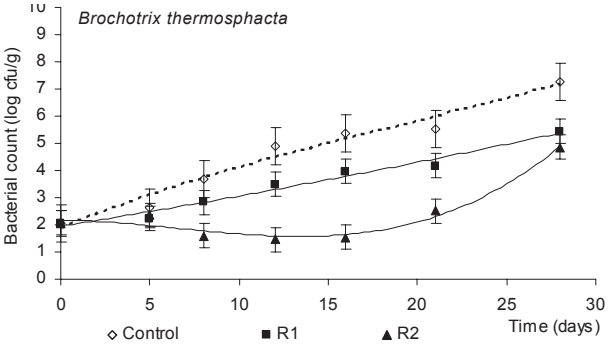

FIGURE 2. Growth kinetics of *Brochothrix thermosphacta* in samples containing antibacterial products and control.

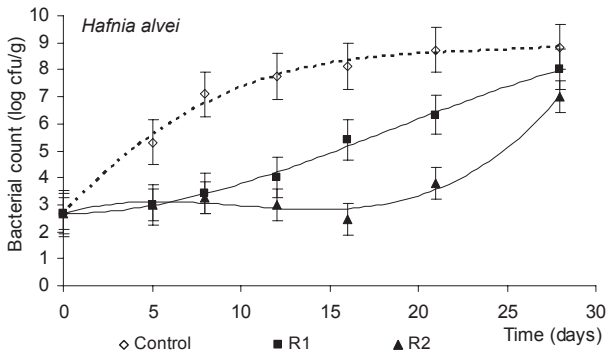

FIGURE 1. Growth kinetics of *Hafnia alvei* in samples containing antibacterial products and control.

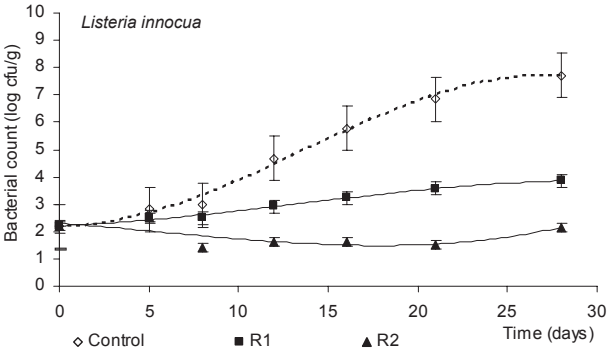

FIGURE 3. Growth kinetics of *Listeria innocua* in samples containing antibacterial products and control.

clear enough to use it as the only preservative additive in meat products. Results of this study show that the dosage of traditional preservatives can be minimised or completely replaced by natural products.

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Any further information may be obtained after contacting the authors.

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