MICROBIAL ECOLOGY OF MARINATED MEAT PRODUCTS

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Introduction

The consumption of marinated meat has been steadily rising in Finland. Marinating is especially used for poultry products. It has been estimated by the Finnish meat processing industry that about 80% of poultry sold at the retail level is marinated. These products are variable containing skinned meat strips and fillets and skin-on leg and breast fillet cuts. For consumers they are convenient in meal preparing since only heat treatment is need without further handling or spicing of the meat. As an additional advantage of easy handling, although *Salmonella* carriage in Finnish poultry is scarce, reduced risk of food cross contamination with pathogenic bacteria should be mentioned.

The word "marinate" comes probably from the Latin word "marine" to Italian, Spanish and French languages referring to soaking/pickling in salt brine. What is being meant by marinating today varies a great deal between different countries. Sometimes salting, adding phosphates and some spices is considered as marinating. Some marinating procedures have been targeted to tenderize poorer quality meat (Young and Lyon 1997a and b). In Finland, marinades are nowadays complex sauces which have a great effect on product appearance and taste. They are water-oil emulsions typically containing salt, sugar and acids (acetic, citric), rheology-improving additives (like xanthan gum and guar gum), antimicrobial agents (like sorbate and benzoate) spices and aroma strengtheners. The pH of these marinades is usually acidic, less than 5, so sugar is needed to cut the edge of the acidic taste. Basic flavor is often obtained using pepper, onion and tomato base together with other added spices. There is a big selection of different flavors, including curry, Chinese-type, Italian-type, honey and barbecue marinades. The amount of marinade added on meat is variable among product types, 20 to 30% (wt/wt) being quite typical for meat strips.

Aims of packaging and marinating technology

Marinated Finnish meat products are usually packaged under modified atmospheres (MA) to prevent the growth of aerobic spoilage organisms. Modified atmosphere packaging (MAP) of meat usually results in the dominance of psychrotrophic lactic acid bacteria (LAB) if proper cold-storing is applied to control the growth of *Enterobacteriaceae* (Borch et al. 1996, Smolander et al. 2003). In addition to the LAB, *Brochothrix thermosphacta* has also been associated with spoilage in some countries (Borch et al. 1996) but this species has not been detected to form a major part of the spoilage population in Finnish MAP marinated broiler meat strips (Susiluoto et al. 2003) .

The main aims of marinating have been considered to be tenderizing, flavoring and enhancing safety and shelf life of meat products due to inhibition of microbial growth. The growth of microbes has been considered to be suppressed by the acidic pH and the use of sorbates and benzoates in the marinades. In the period 1997 to 2000 the National Technology Agency of Finland supported in the first study program (Uudistuva elintarvike tutkimusohjelma) a project associated with meat marinating technology. During these studies, marinating was surprisingly not found to increase the shelf life of these products and also the effect on tenderizing was not as good as anticipated. It was actually found that there were higher bacterial levels in the marinated products than in the corresponding non-marinated products. In 1997, severe spoilage problems

were noticed in certain marinated poultry products. This prompted our interest to study and identify the specific spoilage organisms in the Finnish products. During the same time, the group of Professor Hänninen (Department of Food and Environmental Hygiene, University of Helsnki) started to analyze the effect of marinating on *Campylobacter*. Since then these studies have been carried out in collaboration with the Finnish meat industry and with support obtained from the Academy of Finland and National Technology Agency. Our work has targeted beef, pork and poultry products. Currently we have the best knowledge of the microbial ecology associated with marinated poultry products. Some of these studies are presented in the following paragraphs in order to elucidate the microbial complexity associated with marinating.

Microbial ecology in marinated poultry products

Specific spoilage LAB in marinated poultry

In our first study (Björkroth et al. 2000), LAB associated with gaseous spoilage of MAP, raw, tomato-marinated broiler meat strips were identified. We use a 16+23S rDNA RFLP (ribotyping) database for LAB identification since the psychrotrophic LAB species cannot be identified using traditional phenotypical tests. A mixed LAB population dominated by a *Leuconostoc* species resembling *L. gelidum* was found to cause the spoilage of the product. *Lactobacillus sakei, Lactobacillus curvatus* and a Gram-positive rod phenotypically similar to heterofermentative *Lactobacillus* species were the other main organisms detected in this spoilage population. Increase in pH together with the extreme bulging of packages was considered to suggest a rare LAB spoilage type called "protein swell". This spoilage is characterized by excessive production of gas due to amino acid decarboxylation and the rise of pH is attributed to the subsequent deamination of amino acids. However, in recent studies (not yet published) we have shown that these LAB are not able to decarboxylate the major meat-associated amino acids. The rise in pH values is likely to result from the buffering capacity of the meat. A polyphasic taxonomy approach, including classical phenotyping, whole-cell protein electrophoresis, 16+23S rDNA RFLP, 16S rDNA sequence analysis and DNA-DNA reassociation analysis, was used for the identification of the dominating *Leuconostoc* species. 16S rDNA gene sequences of two spoilage strains possessed 98.8 and 99.0% sequence similarity with the *L. gelidum* type strain. DNA-DNA reassociation, however, clearly distinguished these two species. These same strains showed only 22% and 34% hybridization with the *L. gelidum* type strain. Based on these results, a separate species status was proposed for these *Leuconostoc* strains, and the name *Leuconostoc gasicomitatum* was given to this spoilage-associated species. Recently also the unidentified Gram-positive rod has been shown to be a new psychrotrophic *Lactobacillus* species, *Lactobacillus oligofermentans* (Koort et al. 2004b).

In another study (Susiluoto et al. 2003) we wanted to evaluate if LAB in retail, MAP, marinated broiler meat strips on sell-by day commonly harbor *L. gasicomitatum.* A total of 32 packages, 3 to 5 packages of 7 differently marinated broiler meat products, were studied at the end of the producer-defined shelf life (at 6ºC, 7 to 9 days depending on the manufacturer). Bacteria were cultured on MRS and Tomato Juice Agar (TJA), Rogosa SL agar (SLA), Plate Count Agar (PCA) and Streptomycin Thallium Acetate Agar (STAA) for the enumeration of LAB, lactobacilli, total bacterial count and *B. thermosphacta*, respectively. The average CFU/g of the 32 packages was 2.3×10^8 on PCA. The highest bacterial average, 3.1×10^8, was recovered on TJA, the corresponding CFU/g averages on MRS and SLA being 2.3×10^8 and 1.3×10^8, respectively. Despite the high LAB numbers detected, radical spoilage changes such as unpleasant odor, slime production and formation of gas were not seen. *B. thermosphacta* did not form a significant part of the bacterial population since none of the levels exceeded the spoilage threshold level of 10^5 CFU/g reported in previous studies for this organism. In order to characterize the dominating LAB population, 85, 85 and 88 colonies from MRS, TJA and SLA, respectively, were randomly picked, culture purified and identified to species level. Fifty-six of the 170 isolates picked from the non-selective LAB media (MRS and TJA) were identified as *L. gasicomitatum,* followed by *Carnobacterium divergens* (41 isolates), *L. sakei* (31 isolates) and *L. curvatus* (20 isolates) species. SLA proved not to be completely selective for lactobacilli because the growth of *Leuconostoc* spp. was not inhibited, *Carnobacterium* spp. were the only species not detected on SLA. This study confirmed that *L. gasicomitatum* commonly occurs in MAP, marinated poultry products.

Initially contaminating and spoilage LAB in the products and processing environment

In order to show which of the initial LAB contaminants are also causing spoilage of a MAP, marinated broiler leg product at 6ºC, LAB were enumerated and identified on the 2nd and 17th day following

manufacture. A total of 8 fresh and 13 spoiled packages were studied for LAB levels. In addition, aerobic mesophilic bacteria and *Enterobacteriaceae* were determined. The average CFU/g values in the 8 fresh packages were 1.3×10^3, 9.8×10^3 and 2.6×10^2 on MRS, PCA and Violet Red Bile Glucose agars (VRBG), respectively. The commercial shelf life for the product had been set as 12 days and all packages analyzed on the 17th day were deemed unfit for human consumption by the sensory analysis. The corresponding CFU/g averages in the spoiled product were 1.4×10^9, 1.1×10^9 and 3.9×10^7 on MRS, PCA and VRBG agars, respectively. For characterization of the LAB population, 104 colonies originating from the fresh and 144 colonies from the spoiled packages were randomly picked, purified and identified to species level. The results showed that enterococci (35.7% of the LAB population) were dominating in the fresh product whereas carnobacteria (59.7%) dominated among the spoilage LAB. *Enterococcus faecalis*, *Carnobacterium piscicola* and *Carnobacterium divergens* were the main species detected. In general, when the initial LAB population is compared to the spoilage LAB, a shift from homofermentative cocci towards carnobacteria, *L. sakei/curvatus* and heterofermentative rods (*L. oligofermentans*) was seen in this marinated product. A novel *Enterococcus* species, *E. hermannienesis* (Koort et al. 2004a), was also described from the enterococci present in the initial population.

After the three studies showing many novel LAB species in these marinated products we wanted to study what is the role of the broiler chicken as a source of these spoilage LAB. We made four slaughterhouse visits during which carcasses and air of the slaughterhouse and adjacent processing facilities were sampled. These results showed that the broiler skin or mucous membranes were not major sources of the spoilage LAB but they are commonly found in the air in the processing rooms suggesting environmental contamination of the processing facilities (Vihavainen et al. 2004). We think that the cold processing facilities favour the adaptation of the psychrotrophic strains in the processing facilities because the marinades themselves have not been found to harbour spoilage LAB.

Survival of Campylobacter spp. in marinated poultry products
Marinating has been considered to increase safety of poultry products. Perko-Mäkelä et al. (2000) studied the survival of *Campylobacter jejuni* in plain marinade and in both marinated and non-marinated drumsticks and sliced breast meat strips during storage at 4ºC. The marinade had a pH of 4.5 and NaCl content of 5.9% (wt/wt). Inocula consisted of a cocktail of 7 *C. jejuni* strains. In the plain marinade, the *C. jejuni* inocolum level was initially log 5.4 CFU/ml and within 24 hours a log 2.4 CFU/ml decrease in *C. jejuni* level was noticed. After 48 hours no *Campylobacter* were detectable in the marinade. However, the fate of *Campylobacter* in both marinated and non-marinated meat products was quite different in comparison to the situation in the plain marinade. Surprisingly there was no difference between the marinated and non-marinated products. When an inoculum of log4 to log5 CFU/ml was added to 300g product, the organisms were detectable at least for 9 days. With a smaller inoculum level of log1 to log2 CFU/ml, *Campylobacter* were detected for at least five days. These results show that marinating may not necessarily have an effect on the survival of enteric pathogens, like *Campylobacter*. This may also be due to the buffering capability of meat quickly neutralizing the pH of the acidic marinade. The change in the acidic pH towards neutrality results in dissociation of the lipophilic acids making their antimicrobial effect nonexistent.

Conclusions and further studies

Marinating is changing the spoilage LAB population initially present in the product. We have detected many novel species in these products. Currently we think that addition of marinades is favoring the growth of some psychrotrophic LAB which are rarely detected in non-marinated meat products. *L. gasicomitatum* has also been detected from carrots and a spoiled fish product (Lyhs et al. 2003) but not from the skin or membranes of broiler chicken. As a psychrotrophic organism it does not survive in gastro intestinal tract and we currently consider this species as an environmental bacterium well-adapted in the cold processing facilities. The use of MAP and marinating is favoring the growth of this bacterium and to lesser extent also *L. oligofermentans*. In order to control *L. gasicomitatum* and *L. oligofermentans* growth in these products we should understand the metabolism of these species better.

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