ASSESSMENT OF THE USABILITY OF CHEMICAL INDICATORS ASSOCIATED WITH GROWTH OF LACTIC ACID BACTERIA IN A PROCESSED MEAT PRODUCT

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Keywords: processed pork meat, microbiology, *Carnobacterium*, *Lactobacillus*, *Leuconostoc*

Introduction

It has previously proven extremely difficult to apply conventional total or selective microbial counts for the prediction/indexing of 'shelf-life' as defined by sensory/consumer assessment for many types of food products (Anonymous 2000, McMullen and Stiles 1989). Particularly, in the case of processed meat products such as the sliced Danish pork meat product, "rullepølse", the use of such microbial counts has proven highly ineffective in the prediction of the limit of sensory defined shelf-life. This is largely because lactic acid bacteria (LAB) which are not prominent spoilage organisms, constitute the predominant micro flora in vacuum or modified atmosphere packaged products. Rullepølse (pronounced in English as rule-purls) consist of a large flat piece of boned pork belly with skin removed, spread with herbs and seasoning, and then rolled up. It is cooked and shaped into a rectangle, cooled and thinly sliced as a cold cut to be placed on an open-faced sandwich (Danish: smørrebrød) frequently garnished with raw onion rings. This product will on the one hand exhibit a comparatively long shelf life but on the other hand the exact sensory shelf life is very difficult to predict as the numbers of bacteria tend to plateau and ultimately decrease over storage time (Figure 1). Thus, a correlation to consistently decreasing sensory quality is not data analytically viable.
There is a pressing need for the development and evaluation of new methods for the prediction and indexing of decreasing consumer/sensory based shelf-life quality in processed meat products. The development of such an index will ultimately enable the shelf-life of these products to be controlled efficiently and ultimately, we postulate, may allow shelf-life extension from a sensory perspective, via more efficient control of these products during production. This research project proposal is aimed at developing a methodology whereby sensory deterioration to a 'rational rejection point' (a products 'shelf-life') can be established through monitoring chemically the changes in concentrations of bacterial metabolites by the natural micro flora (see Figure 1). Thus, a quality indexing method can be established giving a sound chemical methodology for the prediction of 'sensory based product shelf-life'. This will enable improvement in product quality, potentially enhance designated shelf-life, and certainly reduce product waste. Table 1 gives an overview over potential LAB chemical indicators in rullepølse.

Table 1: Potential lactic acid bacteria chemical indicators of rullepølse quality included in this study.

Indicator	Producer organisms	Present in sterile meat	Usability
D-lactic acid	*Leuconostoc*, some *Lactobacillus*	-	++[a]
L-lactic acid	All lactic acid bacteria	+	+[b]
Acetic acid	*Carnobacterium*, *Leuconostoc*, some *Lactobacillus*	-	+++
Tyramine	*Carnobacterium*	-	++[a]
Arginine/Ornithine	*Carnobacterium*, some *Lactobacillus*	-	+++[a]

[a]Producer organism is not consistently present in products which limit the application
[b]The fact that the compound is also a result of post-mortem glycolysis complicates its use as an indicator

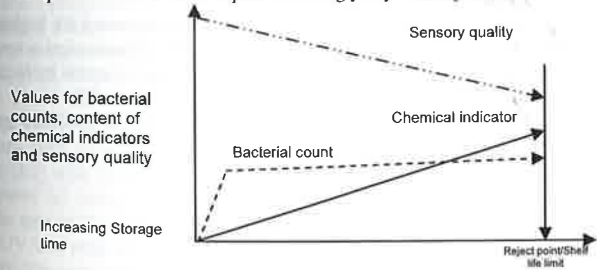

Figure 1: Chemical indicators as a quality index for processed meat products.

Materials and Methods

Bacteriological standard methods included PCA for aerobic counts and NAP agar for LAB counts. Tyramine, arginine and ornithine contents were analyzed by use of a HPLC post column derivatization method (Leisner *et al.* 1994). Total contents of lactic and acetic acid were analysed by a standard HPLC procedure and contents of D and L-isomers of lactic acid were determined enzymically. A meat extract experiment was conducted by using sterile-filtered meat juice inoculated with pools of LAB strains previously isolated from rullepølse products. The same raw material was used for meat juice as is being used for manufacturing rullepølse

Results and Discussion

A preliminary survey examined the content of aerobic mesophilic bacteria, LAB, pH and contents of biogenic amines and the amino acids arginine and ornithine in a range of different rullepølse products (Table 2). Among the LAB only a few different species, *Carnobacterium divergens*, *Lactobacillus sakei* and *Leuconostoc carnosum* were encountered and then only in a selection of the products. Only *C. divergens* produces tyramine and tyramine producers were therefore unevenly distributed in Rullepølse products. This limits the application of this chemical indicator (Table 1). It is therefore of interest to note that both carnobacteria and *Lb. sakei* were able to convert the arginine into ornithine (results not shown). Thus, the arginine/ornithine contents may offer an alternative to tyramine for establishing a rational rejection point.

With this point in mind we conducted a meat juice experiment. Both carnobacteria and *Leuc. carnosum* grew rapidly in this medium whereas *Lb. sakei* showed biphasic growth probably due to exhaustion of a substrate, *e.g.* glucose. Neither *Lb. sakei* nor *Leuc. carnosum* produced any tyramine whereas *C. divergens*_produced tyramine starting at the middle exponential phase and continuing into the stationary phase (results not shown). These results confirms that tyramine production by carnobacteria, if present, appear to be a candidate as an indicator of "the microbial age" of a product. We also analysed the metabolism of arginine into ornithine and both *C. divergens* and *Lb. sakei* but not *Leuc. carnosum* were able to conduct this reaction (results not shown). Finally, the content of organic acids was analysed by use of HPLC and for some samples the contents of the D and L-isomers of lactic acid were determined. The implications of these findings will be discussed.

Table 2: Characterisation of rullepølse products (MAP or VP[a], pH 5.7-6.6) by aerobic counts (PCA), Lactic Acid Bacteria (LAB) counts, identification (ID) of microflora and analyses of tyramine, arginine and ornithine.

Product	PCA Log (cfu/g)	LAB Log (cfu/g)	ID (NP)[b]	ID (PCA)[b]	Tyramine (µg/ml)	Arginine (µg/ml)	Ornithine (µg/ml)
A	5.4 ± 0.2	5.5 ± 0.1	*Ls*	*Ls*:	<0.5		
B	7.3 ± 0.1	7.3 ± 0.0	*Ls*[#] , *Cd*[§]	*Ls*[#]:, *Cd*[§]	<0.5		
C	3.9± 0.1	4.1 ± 0.0	*Cd*, *Lc*	*Cd*, *Lc*	0.4	20.5	1.7
D	7.0 ± 0.2	6.8 ± 0.1	*Ls*[#] , *Lc*[¤]	*Ls*[#], *Lc*[¤]	<0.5		
E	4.7 ± 0.1	4.7 ± 0.2	*Ls*, *Lc*	*Ls*, *Lc*	0.3	28.9	3.1
F	4.2 ± 0.1	4.2 ± 0.1	*Lc*[¤], *Ls*[#]	*Lc*[¤], *Ls*[#], NI	<0.5		
G	8.2 ± 0.1	8.2 ± 0.1	*Lc*[¤], NI	*Lc*[¤], NI	1.9	10.5	9.5

[a]MAP=Modified Atmosphere Packaged, VP=Vacuum Packaged

[b]*Cd*: *Carnobacterium divergens* [§]or other species, *Lc*: *Leuconostoc carnosum* [¤]or other species, *Ls*: *Lactobacillus sakei* [#]or other species. NI=LAB not identified to species level.

Conclusions

Tyramine producers (*i.e. Carnobacterium* spp.) are unevenly distributed in various products and batches of Rullepølse. This may limit the application of this chemical indicator. An alternative is to monitor the content of free arginine and ornithine as carnobacteria and lactobacilli isolates from rullepølse are able to convert arginine into ornithine by the arginine deiminase pathway. We are currently investigating this issue further.

References

Anon. (2000). ATV-rapport (In Danish): Micro organisms in our food – A focussed approach for higher quality. Final report from the ATV-comittee on microbiological criteria for foods. Academy for technological sciences, ATV.

Leisner, J.J., Millan, J.C., Huss, H.H., Larsen, L.M. (1994). Production of histamine and tyramine by lactic acid bacteria isolated from vacuum-packed sugar-salted fish. Journal of Applied Bacteriology, 76:417-423.

McMullen, L.M., Stiles, M.E. (1989). Storage life of selected meat sandwiches at 4°C in modified gas atmosphere Journal of Food Protection, 52:792-798