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MICROFLORA OF LOW-SALT HERRING I. THE EFFECT OF SORT OF PACKAGING ON MICROFLORA OF HERRING A`LA MATIAS

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ABSTRACT

Results of qualitative and quantitative analyses of microflora of traditionally and vacuum-packed herring a`la Matias are presented. Forty fillet samples of one producer were bought in randomly chosen local shops and tested. It was stated that their microflora depended on sort of packaging. Gram-positive rods: *Brevibacterium spp.*, Gram-negative rods: *Pseudomonas spp.*, and Gram-positive cocci: *Micrococcus spp.* were dominant in vacuum-packed samples. Microflora of products in traditional packaging was much more varied. Yeast analysis also showed greater diversity within samples packaged in traditional way as they contained *Saccharomyces spp.*, *Zygosaccharomyces spp.* and *Candida spp.* Only *Candida spp.* was found in vacuum-packed samples.

Key words: bacteria, yeasts, salt herring, packaging

INTRODUCTION

There are two basic methods of salting: low and strong. Originally, only traditional strong salting was applied. It served a twin purpose - providing a product with characteristic, desired set of organoleptic properties and protecting it against spoiling. Such a product had storage stability at 10°C-12°C for 10 months or over 12 months if kept at 0°C-5°C. Microflora of salt herring depends on microbiological condition of raw material and processes occurring during maturation and storage of the product (Sikorski 1980). Research on microflora of strong-salt herring was carried out mostly in the interwar years. Part of them was continued in the fifties and concluded by Shewan (1961). It was observed that the increase of the number of bacteria up to 10^5-10^6 CFU/g occurred during storage of the product at 20°C and 37°C for the first 15 days. Then, it had been decreasing steadily over 3 logarithms for 5 months of storage. At these temperatures product was stable for 3-4 months and the total number of human pathogens in brine was 10^2 CFU/ml. Traditional strong salting eliminates most micro-organisms pathogenic for human beings. Microflora of salt and raw fish is different. It changes qualitatively and quantitatively during fish processing due to contacts with hands of staff, surfaces of barrels, tubs and salt. Halophobic microflora declines during maturation. Typical microflora consists of halophylic cocci, yeasts, moulds, sporing bacteria and few Gram-negative rods. Characteristic representatives of microflora of fresh fish: *Pseudomonas spp.* and *Achromobacter spp.* are not detected (Shewan 1961).

Nowadays low salting is the most frequently employed method. It recommends fillet salting as opposed to strong salting based on carcass processing. Brine at 10-15% of sodium chloride concentration with or without sugar and spices is used. The important additive is also an enzymatic compound which provides the desired set of organoleptic properties. Products preserved by application of low salting are not as stable as if strong salting was applied. For this reason, preservaties and vacuum packaging are the additional ways to ensuring low-salt product safety. Unfortunately, there were few accompanying microbiological analyses preceding a promotion of such products on the market. Therefore, the aim of this study was to analyse microflora of traditionally and vacuum-packed herring a`la Matias qualitatively and quantitatively.

MATERIALS AND METHODS

Forty samples of traditionally and vacuum-packed low-salt herring fillets a'la Matias of one producer, bought in randomly chosen local shops, were tested. At the same time only 2-3 samples of product were bought at regular intervals.

Decimal dilutions were prepared by sterile collecting of twenty-gram non-skinned meat samples. According to Polish Standard (PN-89 A-86730) 180 ml of dilution liquid (pH 7.2-7.4), which consists of pepton tryptone (Difco)-1.0g, NaCl-8.5g and distilled water-1000 ml, was added to the samples.

Samples were homogenized for 3 minutes. Subsequent decimal dilutions were prepared from the homogenized material. 0.1ml of diluted homogenate was cultured on appropriate medium to analyse micro-organisms quantitatively.

The number of psychrophiles and mesophiles was estimated. Cultures were conducted on nutrient agar (BTL, Poland). To estimate the number of psychrofiles they were incubated at 10°C for 7 days. The incubation at 30°C lasted 72 hours to estimate the number of mezophiles. Gram-stained microscopic slides were prepared for each colony grown on solid medium.

Species and genus identification of Gram-negative rods was confirmed by ID32 GN test (bioMerieux).

ApiCoryne and ID 32 STAPH tests (bioMerieux) were used for species and genus identification of Gram-positive rods and Gram-positive cocci, respectively.

The number of yeasts was estimated by culturing diluted material on solid medium (pH 6.6) (PrItPN-ISO 7954) for quantitative analysis of yeasts and moulds. It consists of:

Yeast extract	5.0g
Glucose	20.0g
Chloramphenicol	0.1g
Agar	15g
Distilled water	1000ml

Cultures were incubated at ambient temperature for 3-5 days and identified with ID 32 C test (bioMerieux). All tests were carried out on VITEK System ATB Expression (bioMerieux).

The amount of micro-organisms expressing particular biochemical activity, i.e. proteolytic, lipolytic, amylolytic activity and reduction of the –SH groups to H_2S (hydrogen sulphide) was estimated.

Proteolytic activity was confirmed on Frazier medium (Duszkiewicz-Reinhard et al.,1996). Colonies were treated with a particular reagent (mercury chloride - 15g, HCl - 20 ml, distilled water - 1000 ml).

Lipolytic activity was tested on agar medium supplemented with Tween 80 (Duszkiewicz-Reinhard et al., 1996).

Amylolytic enzymes expression was tested on starch agar medium treated with iodine plus potassium iodide (Burbianka et al., 1993).

Colonies with clear rims were regarded as positive strains expressing particular enzymatic activity.

Hydrogen sulphide production was detected on Lead Acetate Agar (LAA) (pH 7.0):

Beef extract	3.0g
Yeast extract	3.0g
Bacto peptone	5.0g
Bacto tryptone	15.0g
Ferric citrate	0.3g
Cysteine HCl	0.4g
NaCl	5.0g
Sodium thiosulphate	0.5g
Agar	20.0g
Distilled water	1000ml

Black colonies were regarded as H_2S-producing.

Statistical analyses i.e. the mean, standard deviation and significance by Scheffe test were preformed on Statistica PL software.

RESULTS

The number of psychrophiles and yeasts isolated from vacuum-packed low-salt herring fillets was significantly higher than found in traditionally packed fillets ($p \leq 0.01$). The number of mesophilic micro-organisms in both kinds of product was comparable. Results are presented in Table 1.

Table 1. The number of bacteria and yeasts in traditionally and vacuum-packed herring

Micro-organisms	Traditionally packed samples CFU/g/ml × 10^5	Vacuum-packed samples CFU/g/ml × 10^5
Psychrophiles	2.15** ± 2.57	10.12** ± 7.86
Mesophiles	14.46 ± 27.61	13.43 ± 13.08
Yeasts	5.53** ± 8.21	30.43** ± 16.11

****- statistically significant differences at $p \leq 0.01$**

Statistically significant differences of enzymatic activity were only found in a group of amylolytic bacteria ($p \leq 0.01$). Results are presented in Table 2. No H_2S-producing bacteria were detected in tested samples.

Species identification was also carried out. Our research showed that dominant genera in traditionally packed herring were *Pseudomonas spp*. (24% of total microflora), *Cellulomonas spp.* (24%) and *Micrococcus spp.* (21%). Additionally, *Corynobacterium spp*. (15%), *Xantomonas spp*. (14%) and *Staphylococcus spp*. (2%) were detected.

Table 2. The number of micro-organisms expressing particular enzymatic activity isolated from traditionally and vacuum-packed low-salt herring

Micro-organisms	Traditionally packed samples CFU/g/ml × 10^4	Vacuum-packed samples CFU/g/ml × 10^4
Amylolytic	4.05* ± 4.54	0.85* ± 0.71
Proteolytic	13.15 ± 24.72	0.95 ± 0.93
Lipolytic	45.50 ± 86.45	26.20 ± 24.82

***- statistically significant differences at $p \leq 0.05$**

Microflora of vacuum-packed herring was slightly different. *Cellulomonas spp*. was not detected whereas *Brevibacterium spp*. comprised 40% of total microflora. *Pseudomonas spp*. and *Micrococcus spp*. composed 34% and 16%, respectively. Traces of *Escherichia spp.* and *Xanthomonas spp.* making up 5% of total microflora were detected (Table 3).

Table 3. Classification of micro-organisms isolated from traditionally and vacuum-packed low-salt herring

Species	Traditionally packed samples		Vacuum-packed samples	
	CFU/g/ml × 10^4	[%]	CFU/g/ml × 10^4	[%]
Micrococcus spp.	35	21	38	16
Staphylococcus hominis	3	2	0	0
Gram-positive rods:				
Corynobacterium accoleus	2	1	0	0
Corynobacterium propinquum	20	12	0	0
Corynobacterium glucu-semi	3	2	0	0
Cellulomonas spp. Microbacter spp.	40	24	0	0
Brevibacterium spp.	0	0	94	40
Gram-negative rods:				
Pseudomonas putida	40	24	80	34
Escherichia coli	0	0	12	5
Xanthomonas maltophilia	23	14	12	5
Total	**166**	**100**	**236**	**100**

Species analysis of isolated yeasts showed that dominant *Candida zeylanoides* (51%) and different species of *Saccharomyces spp.* (38%) and *Zygosaccharomyces spp.* (11%) composed a total amount of micro-organisms in traditionally packed herring. Only *Candida spp*. was found in vacuum-packed herring. *Candida famata* was a dominant species (88%). *Candida glabrata* (6%) and *Candida dattila* (6%) were also detected. These relationships are presented in Table 4.

Table 4. Classification of yeasts isolated from traditionally and vacuum-packed low-salt herring

Species	Traditionally packed samples		Vacuum-packed samples	
	CFU/g/ml × 10^4	[%]	CFU/g/ml × 10^4	[%]
Saccharomyces kluyverii	21	38	0	0
Zygosaccharomyces spp.	6	11	0	0
Candida zeylanoides	28	51	0	0
Candida glabrata	0	0	18	6
Candida dattila	0	0	18	6
Candida famata	0	0	268	88
Total	**55**	**100**	**304**	**100**

RESULTS AND DISCUSSION

Our research covered qualitative and quantitative analyses of microflora of traditionally and vacuum-packed low-salt herring fillets. Smaller amount of psychrophiles and yeasts was found in traditionally packed samples than in vacuum-packed proving the difference between tested products. Results point to that even if vacuum-packed products have prolonged expiry dates, not only they do not protect products against the outgrowth of micro-organisms but also they promote it to a greater extent than traditional packaging. As no H_2S producing bacteria were found, it supplies a proof that such a microflora probably does not play any important role in food spoilage.

Gram-positive cocci were represented by *Micrococcus spp*. in traditionally and vacuum-packed products. Traces of *Staphylococcus spp*. were additionally isolated from traditional packaging. Gram-positive rods: *Corynobacterium spp.* and *Celullomonas spp.* were found in traditionally packed herring whereas *Brevibacterium spp*. was detected in vacuum-packed samples. Gram-negative rods were represented by *Pseudomonas spp.* and *Xanthomonas spp*. They were found in traditionally packed samples as well as in vacuum-packed. In the latter *Escherichia spp.* was also found (Tables 3).

Knochel and Huss (1984) showed that microflora of salt herring regardless the fishing place consisted of 70% of moderate halophilic Gram-negative rods, 20% of Gram-positive cocci and 3% of yeasts. Their results differed significantly from the results of our studies. We found that Gram-positive rods and cocci were very important components of microflora of traditionally packed herring and dominant in vacuum-packed samples. We showed that dominant micro-organisms in herring products were Gram-negative rods (data not published). According to Roberts (1998) preservation of product causes substitution of Gram-negative microflora of a raw product with Gram-positive micro-organisms in a processed product. As opposed to Knochel and Huss (1984) we stated that low salting caused substitution of Gram-negative rods by Gram-positive rods. They probably belonged to lactic acid bacteria which may form microflora of food spoilage. This thesis was proved by Gancel et. al. (1997) who isolated 78 strains of bacteria from smoked and vacuum-packed salt herring fillets. All strains were classified as *Lactobacillus spp*. In our research small amounts of *Pseudomonas spp*. were found in traditionally and vacuum-packed herring. *Shewanella putrefaciens* was not detected. Jay (1996) suggests that it is a significant component of microflora of raw and processed fish products. If such products are stored for a long time at 2°C – 15°C it multiplies and becomes dominant. Lack of *Shewanella putrefaciens* in our samples might have been caused by testing them before their expiration date as either micro-organisms have not multiplies yet or they were incubated by a sort of packaging. Jay did not analyze typical microflora of salt fish spoilage.

Pathogenic bacteria e.g.: *Vibrio spp., Listeria monocytogenes, Salmonella spp.*, were not detected in analyzed products. Low salting promotes occurrence and multiplication of *Listeria monocytogenes*. It can grow at 10% of salt concentration and even 16% of NaCl is not a lethal dose (Holt et. al. 1994). It is found in salt and fresh water and sediment (Arvanitidou et.al. 1997; Bremer et.al. 1998; Colburn et. al. 1990; Sikorski 1996). It is also frequently isolated from raw and processed fish (Hatermink and Geordsson 1991; Jemmi 1990; Dąbrowski et. al. 2000). As selective media were not applied in our research *Listeria spp.* was not detected. In our previous work we stated that 10% of salt herring samples in the retail trade was contaminated with *Listeria* using a typical procedure for its isolation (Dąbrowski et. al. 2000).

As shown in Table 2 the dominant microflora of traditionally and vacuum-packed low-salt herring fillets was lipolytic micro-organisms. It may explain the characteristic rancid smell of spoiled products or products after their expiration date. As mentioned above fish microflora reflects microflora of fisheries. Recently the presence of yeasts in sea water is of particular interest. In the northern Pacific Ocean *Rhodotorula spp., Torulopsis spp., Cryptococcus spp., Saccharomyces spp., Sporobolomyces spp.* and *Candida spp*. are found and *Rhodotorula spp*. is dominant. Yeast species are also detected in the North Sea and Black Sea. Samples collected from the strait of Long Island contained *Candida spp., Cryptococcus spp., Hansenula spp., Rhodotorula spp.* and *Saccharomyces spp.* (Meyers 1967; Meyers 1967a; Reinheimer 1977; Roth 1962, Zaleski 1987).

Our results showed that yeasts were a very important contaminant of salt herring. Their number was significantly higher in vacuum-packed samples than in traditional packaging. Interesting differences were observed in both sorts of packaging. *Saccharomyces spp., Zygosaccharomyces spp.* and *Candida zelanoides* were found in traditionally packed herring. In vacuum-packed products *Saccharomyces spp.* and *Zygosaccharomyces spp.* were replaced by *Candida spp*. This phenomenon is a classical example of ecological succession of micro-organisms caused by environmental changes. Our research showed that a sort of packaging does not exert a profound influence on the number of micro-organisms but it modifies their species variety.

As a conclusion we can state that low-salt herring should be precisely analyzed by microbiologists. Our research concentrated on qualitative and quantitative micrcoflora of herring. Application of particular packaging eliminates distinct groups of micro-organisms and promotes the others which colonizes and occupies the niche in the absence of normal occupant.

CONCLUSIONS

1. Microflora of traditionally packed low-salt herring differs significantly from microflora of vacuum–packed low-salt herring.
2. Gram positive rods: *Brevibacterium spp*. and Gram-negative rods: *Pseudomonas spp*. are dominant in vacuum-packed low-salt herring, whereas microflora of traditionally packed low-salt herring is more varied.
3. In vacuum-packed products the number of *Candida spp*. increases while the number of *Saccharomyces spp.* and *Zygosaccharomyces spp*. decreases.

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