<https://helda.helsinki.fi>

Developing microbial spoilage population in vacuum-packaged charcoal-broiled European river lamprey (*Lampetra fluviatilis*)

Merivirta, Lauri

Elsevier

2005

International journal of food microbiology. 2005. 101(2): 145-152.

<http://hdl.handle.net/1975/519>

<http://dx.doi.org/10.1016/j.ijfoodmicro.2004.10.046>

Downloaded from Helda, University of Helsinki institutional repository.

This is an electronic reprint of the original article.

This reprint may differ from the original in pagination and typographic detail.

Please cite the original version.

1
2
3
4
5
6
7
8 **Developing Microbial Spoilage Population in Vacuum-packaged**
9 **Charcoal-broiled European River Lamprey (*Lampetra***
10 ***fluviatilis*)**
11

12 LAURI O. MERIVIRTA^{1,2*}, JOANNA M. K. KOORT¹, MEIJA KIVISAARI², HANNU
13 KORKEALA¹ and K. JOHANNA BJÖRKROTH¹
14
15

16 ¹Department of Food and Environmental Hygiene,
17 Faculty of Veterinary Medicine,
18 P.O. Box 57; FIN-00014 University of Helsinki, Finland ;

19 ²Porilab, Tiedepuisto 4, FIN-28600 Pori, Finland
20
21
22
23

---

* Corresponding author, Tel: +358-2-6213324; Fax: +358-2-6213333;
Email: lauri.merivirta@finnet.fi

ABSTRACT

Microbiological and sensory changes in vacuum-packaged charcoal-broiled river lampreys from three lamprey processing plants were monitored as a function of time at 8°C. The lampreys were examined every 7 days up to 8 weeks for aerobic plate count (APC) and lactic acid bacteria (LAB). The highest mean APC and LAB were 6.01 log CFU/g and 4.86 log CFU/g, respectively. Only 6 out of 15 lots reached an APC value of 7.0 log CFU/g during storage. The sensory scores remained at the baseline levels after 8 weeks' storage. Twenty-seven isolates were randomly picked from MRS agar and identified to species level using a 16S and 23S rDNA *Hind*III RFLP (ribotyping) database and sequencing of the 16S rRNA gene if no database match was obtained. Twelve of the 27 isolates were identified as *Lactobacillus curvatus* subsp. *curvatus*, and two *Leuconostoc mesenteroides* and one *Weissella halotolerans* strain were also detected. Twelve isolates were not identified by the LAB database. However, they possessed very high (99.9%) 16S gene sequence similarity with either *Staphylococcus warneri* or *Staphylococcus pasteurii* type strains. The LAB detected, with the exception of *W. halotolerans*, have commonly been associated with spoilage of fishery products, but in these vacuum-packaged lampreys they were not the dominant organisms within the developing spoilage population.

Key words: River lamprey; Sensory property; Spoilage; Vacuum-packaging; Aerobic plate count; Lactic acid bacteria; *Staphylococcus*; Ribotyping; 16S ribosomal gene sequencing

1. Introduction

European river lamprey (*Lampetra fluviatilis*) is a rare food item in the Baltic Sea region where the charcoal-broiling on burning alder is the traditional way of processing lampreys. The annual catch in Finnish coastal rivers of 1 500 000 – 2 000 000 lampreys is used for human consumption only (Ikonen et al., 1982; Tuunainen et al., 1983). Lampreys feed on fatty fish such as Baltic herring and sprat. Therefore the fat deposits may account up to 47% of the dry weight. The lampreys stop feeding in the beginning of the spawning migration and fishing season in August (Ikonen et al., 1982) and their intestinal flora is reduced thereafter. The fishing season continues to December. The amount of lampreys brought to the market in Finland was 25 000 kg in 1997 (Finnish Game and Fisheries Research Institute, 1999). Lampreys are often sold in open-air booths, where temperature changes are possible. They are normally eaten without any further heating. Because the product is perishable and normally brought to the market without chilling, it was important to investigate the microbiological and sensory changes in vacuum-packaged, chilled product in order to develop the hygienic quality of the product.

Merivirta et al. (2003) studied the development of microbial levels in charcoal-broiled river lampreys stored at 22°C and 3°C. They found that on the production day the mean aerobic plate count (APC) in broiled lampreys was between 1.67 and 2.29 log CFU/g depending on the manufacturer. At 22°C, the mean APCs of samples increased markedly within 4 days, and after 6 days they were between 5.04 and 6.23 log CFU/g. Chilling and storing at 3°C improved the shelf-life of the product. The mean APC did not exceed 2.82 log CFU/g during 24 days of storage. However, neither the effect of vacuum-packaging nor sensory changes were studied.

Lactic acid bacteria (LAB) often form the main bacterial group in vacuum-packaged slightly preserved fishery products stored at chilled temperatures. This has been observed in many different types of fish products, including cold-smoked herring (Magnússon and Traustadóttir, 1982) and vacuum-packaged, cold-smoked salmon and rainbow trout (Shimasaki et al., 1994; Civera et al., 1995; Truelstrup Hansen, 1995; Leroi et al., 1998; Lyhs et al., 1998). The lamprey product differs from those being actually not smoked but charcoal broiled. To the authors knowledge no data exists on the microbiological development in charred vacuum-packaged lamprey. We therefore set out to compare changes in products of three manufacturers (A, B and C) with respect to developing LAB and to identify the dominant species in these products. Further aims were to determine the shelf-life of vacuum-packaged charcoal-broiled river lampreys and to observe sensory changes in products during storage.

2. Materials and methods

2.1. Processing of lampreys

The three plants used a traditional charcoal broiling procedure originating from centuries past. The methods used to produce charred lamprey are transferred from father to son. Lampreys are rubbed, in the round, with salt in a motorized, stainless steel apparatus resembling a concrete mixer. The process is carried out at room temperature.

Manufacturer A added 1,3 kg (one litre) of crystalline salt (NaCl) for every 40 kg of lampreys, and manufacturer B used about 1 kg of salt for 50 kg of product, although this

99 depended on the consistency and the origin of the lampreys. The amount of salt used in
100 plant C was 1 kg for every 50 kg of product. The mixing time in the running apparatus
101 varied from 1 h to 1 h 30 min depending on the size, colour and origin of the lampreys.
102 After salting, the lampreys were rinsed under running water for 5 min. The water used was
103 tap water controlled by municipal authorities. After a waiting period of 12 h, the lampreys
104 were broiled in ovens over charcoal made from alder at the temperature of 300°C in
105 average. The broiling time varied depending on the person in charge, with total time
106 ranging from 20 to 30 min. The chilling occurred at room temperature, and the product was
107 delivered to the market with no further processing.

109 2.2. *Sampling*

111 A total of 450 lampreys were collected from five different production lots of each of the
112 three plants. Each of these 15 samples was divided into eight subsamples, which were then
113 vacuum-packaged using a VAC STAR 6500 ST packaging machine (Vac Star, Kerzens,
114 Switzerland) in polyamide-polyethylene film (Suomen Union Verpackungs, Östersundom,
115 Finland) with an oxygen permeability of 25 ml O₂/m²/24h/atm (23°C, 75% RH) and a
116 water vapour permeability of 2.5 g/m²/24 h (23°C, 85 RH). This resulted in 120 packages,
117 40 from each plant, which underwent sensory and microbiological analyses associated with
118 different storage time points at 8°C.

120 Every week, after 1-8 weeks' storage, one package from each plant was opened and a
121 representative 10-g sample of lamprey was placed in a sterile stomacher bag (Seward Ltd.,
122 London, UK) with 90 ml of saline-peptone solution (0.85% NaCl, 0.1% peptone)
123 (Maximal Recovery Diluent, Lab M Ltd., Bury, England) and homogenized with a

stomacher (Lab-Blender 400, London, UK). The homogenized sample was serially diluted using saline-peptone solution (Lab M Ltd.). Each dilution was plated onto the appropriate media using a poured or spread plate technique depending on the method.

2.3. Sensory evaluation, salt content and water activity

Sensory evaluation was performed once a week to determine when the samples were spoiled. The sensory evaluation panel consisted of three trained judges who were familiar with off-flavour problems typical of lampreys. The samples were evaluated for aroma and taste using the method described by Amerine et al. (1965). All samples were coded by number and were presented to the panel in randomized order. The scale for taste was 0-10, and arithmetic means were calculated from individual scores. The salt (chloride) concentration, expressed as %NaCl, was determined by potentiometric titration. In addition we made a separate experiment to determine the a_w as a general indication of product composition.

2.4. Enumeration of APC and LAB and selection of LAB strains for species identification

Plate count agar (Oxoid Ltd., Hampshire, UK) was used for the enumeration of APC according to the ISO method (1991). LAB were enumerated by plating of the samples on MRS Agar (Oxoid Ltd., Basingstoke, Hampshire, England). The medium was inoculated using the spread plate technique, and the plates were incubated under anaerobic CO₂ atmosphere (Anaerogen, Oxoid Ltd.) at 20°C for 5 days. Duplicate plates were incubated under aerobic conditions. From each sample, randomly picked colonies were pure-cultured using MRS broth and MRS agar (Oxoid Ltd.). Altogether 27 colonies were selected from

the MRS plates and subjected to species-level identification. The colonies were selected from the plates with the two highest dilutions showing growth.

For DNA extraction, MRS broth was inoculated and the tubes were grown at 25°C for 1-2 days depending on the growth rate. After incubation, cells harvested from 1.5 ml of MRS broth were used for DNA extraction. If needed, the isolates were stored in MRS broth at -70°C.

2.5. 16S and 23S rDNA HindIII RFLP, Ribotyping

DNA was isolated using a modified (Björkroth and Korkeala, 1996a) guanidium thiocyanate method of Pitcher et al. (1989). In this modification, the cell lysis solution contains mutanolysin (250 U/ml, Sigma, St. Louis, MO, USA) in addition to lysozyme (25 mg/ml, Sigma). Restriction endonuclease treatment of 8 µg of DNA was done using *HindIII* restriction enzyme (New England Biolabs, Beverly, MA, USA) as specified by the manufacturer. *HindIII* was chosen because it has been found to provide species-specific patterns for various spoilage LAB (Björkroth and Korkeala, 1996b, 1997; Björkroth et al., 1998, 2000). Restriction endonuclease analysis (REA) was performed as described earlier (Björkroth and Korkeala, 1996a). Genomic blots were made using a vacuum device (Vacugene, Pharmacia, Uppsala, Sweden), and the rDNA probe for ribotyping was labelled by reverse transcription (AMV-RT, Promega, Madison, WI, USA) using a Dig DNA Labelling Kit (Roche Molecular Biochemicals, Mannheim, Germany) as previously described by Blumberg et al. (1991). Membranes were hybridized overnight at 58°C, and detection of the digoxigenin label was performed as recommended by Roche Molecular Biochemicals.

174

175   2.6. Numerical analysis of *HindIII* ribopatterns

176

177   The membranes were scanned with a Hewlett-Packard ScanJet 4c/T tabletop scanner
178   (Boise, ID, USA). Numerical analysis of the ribopatterns was performed using the
179   Bionumerics 3.0 software package (Applied Maths, Sint-Martens-Latem, Belgium). Based
180   on internal controls, 1.5% position tolerance and 0.5% optimization was allowed for
181   bands/patterns. Similarity between all pairs was expressed by Dice coefficient correlations,
182   and the unweighted pair-group method with arithmetic averages (UPGMA) was used for
183   construction of the dendrogram. The ribopatterns were compared with the corresponding
184   patterns in the LAB database at the Department of Food and Environmental Hygiene,
185   University of Helsinki, Finland. This database includes patterns of all relevant spoilage
186   LAB in the genera of *Carnobacterium*, *Lactobacillus*, *Leuconostoc*, *Enterococcus* and
187   *Weissella* (Björkroth and Korkeala, 1996b, 1997; Björkroth et al., 1998, 2000).

188   Identification of the isolates was done based on locations of the type strains in the clusters.

189

190   2.7. Sequencing of 16S rRNA gene

191

192   When ribotyping database analysis did not result in clear clusters, representative strains
193   from these clusters were subjected to 16S rRNA gene sequence analysis. The nearly
194   complete 16S rRNA gene was amplified by PCR with universal primer pair F8-27 (5'-
195   AGAGTTTGATCCTGGCTGAG-3') and R1541-1522 (5'-
196   AAGGAGGTGATCCAGCCGCA-3'). Sequencing of the purified (QIAquick PCR
197   Purification Kit, Qiagen) PCR product was performed by Sanger's dideoxynucleotide
198   chain termination method using two long (primers F19-38 [5'-

CTGGCTCAGGAYGAACGCTG-3'] and R1541-1522) and two shorter (primers F926 [5'-AACTCAAAGGAATTGACGG-3'] and R519 [5'-GTATTACCGCGGCTGCTG-3']) reactions. Samples were run in a Global IR2 sequencing device with e-Seq 1.1 software (LiCor, NE, USA) according to the manufacturer's instructions. The similarity between these sequences and sequences in GenBank was determined using BLASTN 2.2.6 software (Altschul et al., 1997).

2.8. Statistical analysis

Student's t-test was used to calculate differences between plants in APC, LAB and sensory scores. When growth was under 3.0 log CFU/g, the number used in statistical calculations was 2.70 log CFU/g.

3. Results

APC results are presented in Table 1. After one week of storage, APC was under 3.0 log CFU/g in all samples. After 2-8 weeks' storage, the mean APC increased in samples from plants A and C to 6.01 and 4.87 log CFU/g, respectively, but remained under 3.0 log CFU/g in samples from plant B. Five samples from plant A and one sample from plant C exceeded an APC value of 7.0 log CFU/g after 6 weeks. In all samples from plant B, the APC was under 7.0 log CFU/g. By week 8, APC in samples from the plant A was significantly higher than in samples from both plant B ($p<0.01$) and plant C ($p<0.05$).

The mean and range of LAB levels and the mean taste scores are presented in Table 2.

After 8 weeks' storage, the lampreys still received good sensory scores from the panel. The

scores decreased slightly only in samples from plant C. No association was observed between taste scores and aerobic plate or LAB counts. The salt concentration obtained from vacuum-packaged samples was 2.5% (1.5-3.4%). The a_w values tested varied between 0.93 and 0.98.

After 2 weeks' storage, LAB counts of all samples incubated in anaerobic conditions were under 3.0 log CFU/g (Table 2), and no significant difference ($p>0.05$) was present between plants in any week. The level of bacteria grown on MRS under aerobic conditions was higher in samples from plant A than from plants B and C ($p<0.05$) after 7 weeks' storage. Overall, the counts of bacteria grown under aerobic conditions were higher than those grown under anaerobic conditions. After 3 weeks' storage, the proportion of LAB compared with APC from samples of plant A was 53 %, but this diminished after 6 and 8 weeks to 25% and 7%, respectively. The mean APC was higher after 4-8 weeks' storage than the mean LAB levels; thus microbes in addition to LAB were present in the spoilage population.

Based on the isolates obtained from samples of plants A and C, we found five different ribotypes (Fig. 1). Ribotypes I (1 isolate) and IV-V (2 and 12 isolates, respectively) were isolated from samples originating from only plant A. Twelve isolates (ribotype V) from plant A possessed identical patterns to *Lactobacillus curvatus* subsp. *curvatus* (type strain DSM 20019) and two (ribotype IV) to *Leuconostoc mesenteroides* (type strains DSM 20484, DSM 20343). One isolate (ribotype I) from plant A showed a similar ribotype to that of *Weissella halotolerans* (type strain ATCC 35410).

Eleven isolates (two strains) were not identified to species level by the RFLP LAB database. When their 16S ribosomal encoding gene sequence similarities were compared with the sequences in GenBank, both strains showed the highest similarities to *Staphylococcus* strains. The following sequences were deposited in the GenBank (<http://www.ncbi.nlm.nih.gov>): lamprey strains 24/1 (ribotype II) and 34/1 (ribotype III), with accession numbers AY578091 and AY578092, respectively.

Nine isolates (ribotype III) from plant C showed 99.9% similarity to *Staphylococcus warneri* type strain ATCC 27836 (GenBank accession number L37603). Two isolates from plant A and one from plant C possessed ribotype II and showed 99.9% sequence similarity to *Staphylococcus pasteurii* type strain ATCC 51129 (GenBank accession number AB009944).

4. Discussion

Our results indicate that microbial growth in lampreys is relatively slow. After 3 weeks' storage, the mean APC was 3.48 log CFU/g or less in the products of two plants and under the detection limit in the product of third plant. After 8 weeks, the highest mean APC reached was 6.01 log CFU/g. Magnússon and Traustadóttir (1982) reported an APC in vacuum-packaged smoked herring fillets of 6.0 log CFU/g in less than 3 weeks' and >7.5 log CFU/g after 6 weeks' storage. Thus, the lamprey product appears to remain edible for at least 5 weeks provided that the quality of the initial product is similar to ours. The sensory scores remained almost at baseline levels after 8 weeks' storage. This is probably due to slow development of microbial population. The product reached an APC of 7.0 log CFU/g but after 6 weeks or even longer storage. According to one sensory panel, vacuum-

packaged smoked herring fillets (Magnússon and Traustadóttir, 1982) were still of high quality after 12 weeks' storage at 10°C, although a rapid increase in microbes occurred during the first few weeks. The authors speculated that the high numbers of LAB found might play a role in the long shelf-life of the product. With regard to charcoal-broiled river lamprey, this does not appear likely.

The predominant LAB species found here was *L. curvatus* subsp. *curvatus*. Only two out of 27 isolates were identified as *Leuconostoc* spp. Jeppesen and Huss (1993) reported that all isolated LAB from samples of vacuum-packaged minced herring were *Leuconostoc* spp. Lyhs et al. (1999) found *Leuconostoc mesenteroides* subsp. *mesenteroides*, *Leuconostoc citreum*, *Lactobacillus sakei* and *Lactobacillus curvatus* to be the main species associated with spoilage of vacuum-packaged cold-smoked rainbow trout. In our study, ribotyping revealed that only the products from plant A possessed LAB species, three different ribotypes were detected, *Lactobacillus curvatus* subsp. *curvatus* most often. Surprisingly, the strains in samples from plant C were identified as *Staphylococcus* spp. This shows that the MRS medium is not entirely selective for lactic acid bacteria. One ribotype (II) was common to both plants A and C.

Our results are different from findings obtained in studies investigating other fish products. Magnússon and Traustadóttir (1982) stated that LAB increased in vacuum-packaged smoked herring at the expense of other genera, reaching a level of 76% after 9 weeks' storage. Lyhs et al. (1998) found LAB to predominate in the spoilage population of all samples from vacuum-packaged cold-smoked rainbow trout fillets cured with NaCl. In lampreys, the mean APC values being higher than the mean LAB values indicates that microbial groups other than LAB are also present. This may be due to the different

processes involved, i.e. smoking versus charring, in the latter of which relatively high temperatures (300°C in average) burn the surface of the product, resulting in a dry crispy coating. The values of water activity between 0.93 and 0.98 don't explain the slow microbial development. Although bacteria recovered in the product such as *Lactobacillus*, *Leuconostoc*, *Weissella* and *Staphylococcus* have growth potential at that range. Water activity as low as 0.91 allows growth of microorganisms similar to those isolated, although the measurement is complicated by the methodology (Doe, 2002). Because the lampreys stop feeding at the beginning of spawning season and their energy is deposited to fat, they may also have a different nutritional value for microbes, especially for LAB, which usually rely on fermentative carbohydrate metabolism. Since the LAB species detected have been associated with spoilage in other types of fish products, this warrants further study. The different handling of lampreys might also affect the product's initial microbial population; in lamprey processing no raw materials or products other than lampreys are present, and thus, cross-contamination is minimized.

5. Conclusions

Our study and the findings of Merivirta et al. (2003) indicate that microbial development in charred river lampreys is slow, and therefore the shelf-life of the product is longer than that of some other fishery products. LAB was not the main bacterial group present. We found the shelf-life of vacuum-packaged-charcoal broiled river lampreys stored at 8°C to vary between 5 and 8 weeks depending on the manufacturer. Further experiments are required before conclusions can be drawn of the reasons resulting in low LAB values in lampreys.

Acknowledgements

The Regional Council of Satakunta partially funded this study. The authors thank the City of Pori (Porilab) for use of their facilities and Ms. Henna Niinivirta for excellent technical assistance.

References

- Amerine, M.A., Pangborn, R.M., Roessler, E.B., 1965. Principles of sensory evaluation of food. Academic Press, New York and London, pp.354-366.
- Altschul, S.F., Madden, T.L., Schäffer, A.A., Jinghui Zhang, Zheng Zhang, Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* 25, 3389-3402.
- Björkroth, J., Korkeala, H., 1996a. Evaluation of *Lactobacillus sake* contamination in vacuum-packaged sliced cooked meat products by ribotyping. *Journal of Food Protection* 59, 398-401.
- Björkroth, J., Korkeala, H., 1996b. rRNA gene restriction patterns as a characterization tool for *Lactobacillus sake* strains producing ropy slime. *International Journal of Food Microbiology* 30, 293-302.
- Björkroth, J., Korkeala, H., 1997. Characterization of *Lactobacillus fructivorans* spoilage in ketchup. *Journal of Food Protection* 60, 505-509.
- Björkroth, J., Vandamme, P., Korkeala, H., 1998. Identification and characterization of *Leuconostoc carnosum*, associated with production and spoilage of vacuum-packaged, sliced, cooked ham. *Applied and Environmental Microbiology* 64, 3313-3319.

346 Björkroth, K.J., Geisen, R., Schillinger, U., Weiss, N., De Vos, P., Holzapfel, W.H.,
 347 Korkeala, H.J., Vandamme, P., 2000. Characterization of *Leuconostoc gasicomitatum* sp.
 348 nov. associated with spoiled raw tomato marinated broiler meat strips packaged under
 349 modified atmosphere conditions. Applied Environmental Microbiology 66, 3764-3772.
 350 Blumberg, H.M., Kielbauch, J.A., Wachsmuth, K., 1991. Molecular epidemiology of
 351 *Yersinia enterocolitica* O:3 infection: use of chromosomal DNA restriction fragment
 352 length polymorphism of rRNA gene. Journal of Clinical Microbiology 20, 2368-2374.
 353 Civera, T., Parisi, E., Amerio, G.P., Giaccone, V., 1995. Shelf-life of vacuum-packed
 354 smoked salmon; microbiological and chemical changes during storage. Archiv für
 355 Lebensmittelhygiene 46, 1-24.
 356 Doe, P.E., 2002. Fish drying. In: Bremner, H.A. (Ed.), Safety and quality issues in fish
 357 processing. Woodhead Publishing Limited, Cambridge, pp.350-358.
 358 Finnish Game and Fisheries Research Institute, 1999. Fish processing in Finland 1997,
 359 Helsinki, Finland, pp.16-17.
 360 Ikonen, E., Kokko, U., Tuunainen, P., Auvinen, H., 1983. Nahkiaisen pyynti Suomessa
 361 vuonna 1982. Suomen Kalatalous 50, 90-98.
 362 International Organization for Standardization, 1991. International standard, ISO 4833.
 363 Microbiology – General guidance for the enumeration of micro-organisms - Colony count
 364 technique at 30° C. 2nd ed. Geneva.
 365 Jeppesen, V.T., Huss, H.H., 1993. Characteristic and antagonistic activity of lactic acid
 366 bacteria isolated from chilled fish products. International Journal of Food Microbiology 18,
 367 305-320.
 368 Leroi, F., Joffraud, J., Chevalier, F., Cardinal, M., 1998. Study of the microbiological
 369 ecology of cold-smoked salmon during storage at 8°C. International Journal of Food
 370 Microbiology 39, 111-121.

371 Lyhs, U., Björkroth, J., Hyytiä, E., Korkeala, H., 1998. The spoilage flora of vacuum-
 372 packaged, sodium nitrite or potassium nitrite treated, cold-smoked rainbow trout stored at
 373 4° C or 8° C. *International Journal of Food Microbiology* 45, 135-142.
 374 Lyhs, U., Björkroth, J., Korkeala, H., 1999. Characterisation of lactic acid bacteria from
 375 spoiled, vacuum-packaged, cold-smoked rainbow trout using ribotyping. *International*
 376 *Journal of Food Microbiology* 52, 77-84.
 377 Magnússon, H., Traustadóttir, K., 1982. The microbial flora of vacuum-packed smoked
 378 herring fillets. *Journal of Food Technology* 17, 695-702.
 379 Merivirta, L., Korkeala, H., Björkroth, J., 2003. Microbiology of Charcoal-Broiled
 380 European River Lampreys (*Lampetra fluviatilis*) Stored at 3 and 22°C. *Journal of Food*
 381 *Protection* 66, 2332-2335.
 382 Pitcher, D.G., Saunders, N.A., Owen, R.J., 1989. Rapid extraction of bacteria genomic
 383 DNA with guanidium thiocyanate. *Letters in Applied Microbiology* 8, 151-156.
 384 Shimasaki, T., Miake, K., Tsukamasa, Y., Sugiyama, M., Minegishi, Y., Shinano, H.,
 385 1994. Effect of water activity and storage temperature on the quality and microflora of
 386 smoked salmon. *Nippon Suisan Gakkaishi* 60, 569-576.
 387 Truelstrup Hansen, L., 1995. Quality of chilled, vacuum-packed cold-smoked salmon.
 388 Thesis, Danish Institute of Fisheries Research, Department of Seafood Research, Technical
 389 University, Denmark.
 390 Tuunainen, P., Ikonen, E., Auvinen, H., 1980. Lamprey and lamprey fisheries in Finland.
 391 *Canadian Journal of Fisheries and Aquatic Sciences* 37, 1953-1959.
 392
 393
 394
 395

Legend to the figure

Fig. 1. Ribopatterns generated by HindIII restriction enzyme and the numerical analysis of the patterns presented as a dendrogram. Left side of the banding patterns, high molecular masses (<23 kbp); right side, low molecular masses (>500 bp).

Fig. 1. Merivirta et al.

Table 1
Mean and range of aerobic bacterial counts ($\log_{10}$ CFU/g) and mean taste scores of vacuum-packaged charcoal-broiled river lampreys stored at 8°C.

Storage time (weeks)	n	Plant								
		A			B			C		
		Mean	Range	Taste ^a	Mean	Range	Taste	Mean	Range	Taste
1	5	ND ^b		7.6	ND		7.7	ND		7.5
2	5	3.20A ^c	ND-4.41	7.5	ND		6.4	3.07A	ND-3.78	7.5
3	5	3.35A	ND-5.94	7.6	ND		7.3	3.48A	ND-4.85	7.1
4	5	4.04A	ND-6.59	7.6	ND		7.6	3.49A	ND-4.88	7.5
5	5	3.00A	ND-3.85	7.2	ND		7.1	4.01B	ND-5.15	6.7
6	5	4.82A	ND-7.11	7.6	2.86A	ND-3.48	6.7	4.87A	ND-8.32	6.9
7	5	5.04A	ND-7.98	7.8	ND		7.3	3.53A	ND-4.89	7.1
8	5	6.01A	4.74-8.11	7.7	ND		8.0	3.85B	ND-5.30	6.6

^aScore range 0-10.

^bND=not detected; assigned 2.70 log CFU/g for calculation of mean.

^cMeans within a row with same letter are not significantly different at $p>0.05$.

425

426

427

Table 2

Mean and range of lactic acid bacteria counts ($\log_{10}$ CFU/g) after anaerobic incubation and mean taste scores of vacuum-packaged charcoal-broiled river lampreys stored at 8°C.

Storage time (weeks)	n	Plant								
		A			B			C		
		Mean	Range	Taste ^a	Mean	Range	Taste	Mean	Range	Taste
1	5	ND ^b		7.6	ND		7.7	ND		7.5
2	5	ND		7.5	ND		6.4	ND		7.5
3	5	3.40	ND-6.20	7.6	ND		7.3	ND		7.1
4	5	3.46	ND-6.49	7.6	ND		7.6	ND		7.5
5	5	ND		7.2	ND		7.1	3.10	ND-4.72	6.7
6	5	4.34	ND-7.08	7.6	ND		6.7	3.07	ND-4.56	6.9
7	5	4.07	ND-7.97	7.8	ND		7.3	3.26	ND-5.48	7.1
8	5	4.86	ND-8.04	7.7	ND		8.0	ND		6.6

428 ^aScore range 0-10.

429 ^bND=not detected; assigned 2.70 log CFU/g for calculation of mean.