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# Newsletter for the Nordic Committee on Food Analysis

Contents:



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NB! New postal address for the NMKL Secretary General:
NMKL c/o National Veterinary Institute,
**P.O.Box 750, Sentrum, N-0106 Oslo**, Norway.

NMKL Secretary General:
Hilde Skaar Norli,
E-mail: nmkl@vetinst.no

## New NMKL Procedure:

### Guide for sensory analysis of fish and shellfish.
NMKL Procedure No. 21, 2008

A working group established by NMKL has elaborated this guide. The members of the group come from Seafood Research Institutes, and have been working with sensory analysis of seafood for several years.

The following persons participated in the project group:
Denmark: **Grethe Hyldig**, The Technical University of Denmark, National Institute of Aquatic Resources (Project leader, photo)
Norway: Mats Carlehög, Fiskeriforskning (now Nofima)
Iceland: Emilia Martinsdóttir, Kolbrún Sveinsdóttir, Matís
Finland: Leena Lilleberg, Finnish Food Safety Authority Evira

The procedure is elaborated on the basis of Nordic and international guidelines and standards. The procedure focuses primarily on aspects which are specific to sensorial analysis of fish and shellfish.

Fish and shellfish are highly perishable foods with limited shelf life. The sensory changes which occur during the rather short shelf life of fish, are very rapid. The early sensory changes of fish after it has been caught/slaughtered and during storage, are most prominent in appearance and texture. Thus, several aspects have to be considered when performing sensory analyses on fish and shellfish.

There are some sensorial characteristics that are specific to fish and shellfish, and some people may be ”blind” to them, i.e. they cannot taste or smell them. Examples of such sensorial characteristics are rancid flavour, iodine and geosmin. Also, some people have a very low response to cold-storage flavour and rancidity. This has to be considered when selecting and training assessors for sensory analysis of fish and shellfish.

Such conditions are described in the procedure along with handling and sample preparation. Further, the procedure includes specifications for sensorial methods elaborated specifically for fish.

www.nmkl.org

# Revised NMKL Method No. 171 for spoilage microbes in salt-cured fish.

By: Taran Skjerdal, National Veterinary Institute, Norway (photo)

Before refrigerators were introduced, halophilic and osmophilic microbes caused huge spoilage problems in salt-cured fish products in Scandinavia. During recent years, the problems have recurred, partly due to warmer weather conditions in the production season. NMKL has revised the method for detection of these microbes.

Salt-cured fish products such as klippfish and bacalao, may be unknown to many Scandinavians, but are produced and exported in large amounts from Norway, Iceland and the Faeroe Islands to Latin countries. In 2006, the export value of the Norwegian export alone was NOK 3 billion.

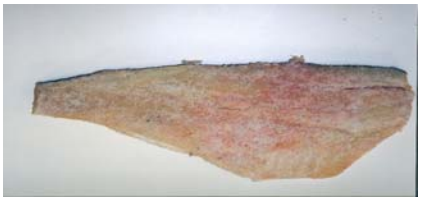
Salt-cured fish products contain 15-22% NaCl, and are perfect growth media for red halophilic bacteria (also called salt-loving bacteria). They require temperatures above 10-12°C to grow, and this is the reason why red spots are formed on salt-cured fish only at abuse storage temperatures. Salt-cured fish products are produced from 2 parts fish and 1 part sea salt, also called solar salt, which is produced in salt ponds from evaporated sea water. Many salt ponds contain the red halophilic bacteria *Halobacterium* spp. and *Halococcus* spp. The red colour is visible in the most salt-saturated chambers. Even though salt-cured cod have been produced without chilling facilities and with the bacteria present in the salt-ponds and salt-cured fish products for centuries, the formation of red spots on the products has been a limited problem for the producers in the Nordic countries due to the cold weather in the production season. Spoilage by red halophilic bacteria has therefore mainly been a problem during transport and distribution in the market countries. After year 2000, the winters have become warmer in the Nordic countries, and spoilage by halophilic bacteria is more common. In fact, there have been large losses and even lawsuits about spoiled salt-cured products in the last few years.

When the first version of NMKL Method No. 171 was published in 2002, the objective was to give the stakeholders in the trade of salt-cured fish a tool to quantitatively analyse low levels of the microbes, i.e. levels not sufficiently high to form visible red and brown spots, and thereby a tool to avoid complaints and incorrect information. So far, the method has only been used to a limited extent, but an increased interest for halophilic microbes has been observed among insurance companies, food authorities, laboratories and some producers. There is hope that the method will be used so that further losses and lawsuits can be avoided.

Red halophilic bacteria usually require more than 15% NaCl to grow. In addition, they need high amounts of KCl, magnesium salts and/or amino acids. They are able to carry out photosynthesis via the red pigments, and light can partly replace the need for complex organic compounds. However, as long as the growth medium contains organic compounds, light does not further stimulate growth. The growth medium can be made selective for some genera by lowering the content of NaCl and other compounds, but as red halophilic bacteria may be found in several genera, a rich growth medium containing 250 g NaCl/l and dark incubation has been chosen for NMKL Method No. 171. Recent studies have revealed that most halophilic bacteria are strictly aerobic. The revised method therefore specifies that surface sampling is essential. The bacteria may be unevenly distributed on the fish surface, but it is not likely that some areas are heavily contaminated and other areas do not contain any bacteria.

NMKL Method No. 171 also covers detection of microbes that cause brown spots on salt-cured fish. These microbes are osmotolerant fungi like *Wallemia* spp. They, too, are found in salt ponds, but may also have other origins. The method for these microbes has not been changed during the revision.

For further information:
taran.skjerdal@vetinst.no.



Picture 1: A salt-cured cod fillet with 'pink' and 'dun'. The red areas are caused by red halophilic bacteria, most likely a *Halobacterium salinarium*, and the brown spots by osmophilic moulds, most likely *Wallemia sebi*. Photo: Grete Lorentzen

Picture 2: From a salt-pond in Slovenia. The wooden wall separates two chambers with different salt concentrations. The chamber with the strongest colour has the highest salt concentration. Photo: Taran Skjerdal, 2004

Picture 3: A commercial sample of salt-cured fish naturally contaminated with red halophilic bacteria. The fish has been stored at 37°C for approximately a week, in order to stimulate the growth of the bacteria. The fish was torn in two pieces by hand immediately before the photo was taken. Photo:Taran Skjerdal.

## New NMKL Method:

## NMKL Method No. 56, 4th Ed., 2008: Anaerobic sulphite reducing bacteria. Determination in foods.

The new edition of this NMKL method is a technical revision. The main change is that this method now includes anaerobic sulphite reducing **bacteria**, whilst the previous editions have only dealt with anaerobic sulphite reducing ***Clostridia***. In addition, the method describes the determination of spores of anaerobic, sulphite reducing bacteria. The method also makes it possible to determine sulphite reducing *Clostridia* and *C. perfringens* after confirmation.

Sulphite reducing bacteria are bacteria capable of anaerobic growth, e.g. *Clostridia,* and some Gram-negative rods (e.g. *Salmonella*). The genus *Clostridium* comprises spore-forming, mainly motile, Gram-positive, anaerobic rods. They appear in soil and in the gastrointestinal tract of animals and humans. In vacuum-packed raw and cooked meat, psychrotrophic *Clostridia* can cause spoilage problems (bombage and/or foul smell). The psychrotrophic *Clostridia* will not necessarily be measured using this method, as they might need a lower growth temperature and addition of further nutrition factors (e.g. carbohydrates and vitamin $K_1$).

The number of sulphite reducing bacteria is determined by pouring a known amount of sample into a suitable medium containing ferri(III)ammonium citrate (ammonium iron(III) citrate) and disodium disulphite (sodium metabisulphite). After incubation for 1-2 days at 37ºC (final reading after 2 days), possibly at 50ºC if thermophilic bacteria are present, typical black-coloured colonies are counted. If psychrotrophic bacteria are suspected, a lower temperature should be used, e.g. 20.0 ± 1.0°C.

The black colour of the colonies and the surrounding zone is caused by the formation of iron sulfide as a result of the reaction between sulfide ions and trivalent iron ($Fe^{3+}$; ferri-ions) present in the medium. If the number of sulfite-reducing *Clostridia* is to be determined, further verification should be carried out.

If the number of spores is to be determined, the primary dilution should be heat-treated at 80°C for 10 minutes, in order to eliminate vegetative cells.

This method has been technically updated by Lisbeth Etzerodt (photo), Regional Veterinary and Food Administration, Herning, Denmark. Niels Skovgaard, Denmark, Seija Kalso, City of Helsinki Environment Centre, Finland, Laufey Karlsdóttir, SYNI Laboratory Service, Iceland, Per Einar Granum, Norwegian School of Veterinary Science, and Christer Wiberg, National Food Administration, Sweden, have assisted Etzeroth in her work.

### Clarification to NMKL Method No. 136, 4th Ed., 2007: *Listeria Monocytogenes.* Detection in foods and feeding stuffs and enumeration in foods.

An annex to the method has been elaborated in order to clarify that the detection and enumeration procedure can be performed either simultaneously, or as separate analyses, and that it is sufficient to streak 5 colonies from 1 countable dish onto blood agar for confirmation by haemolysis.

### Erratum to NMKL Method No. 107. 2nd Ed., 2006: Titratable acidity. Determination in Milk and Cream.

There is a divergence between the Danish and the English text regarding the concentration of the sodium hydroxide solution in chapter 5.2. The English text, stating a sodium hydroxide concentration of 0.1 M ± 0.002, is correct.

### NMKL's prices for 2008 (same as for 2007):

- Online subscription, a complete method collection with continuous updates, for existing subscribers: NOK 3 000 for 1-3 users.
- New subscription for online method collection: NOK 5 000
- Annual hard copy subscription: NOK 2 000
- Annual electronic subscription via e-mail: NOK 1 500
- Single NMKL methods: NOK 400
- Single NMKL procedures (guidelines): NOK 300

New NMKL Procedure:

## Measurement uncertainty in quantitative microbiological examination of foods.

NMKL Procedure No 8, 3rd Ed., 2008.

In connection with the quality assurance and accreditation of microbiological methods, according to EN ISO/IEC 17025, a calculated value for the uncertainty shall be given in connection with an analytical result. In cases where it is not possible to estimate a value for the uncertainty, the laboratories shall be able to identify sources of uncertainty, and provide a reasonable estimate of the proportion of the various contributions.

The first NMKL procedure on estimation of uncertainty for microbiological analyses, was published already in 1999. Professor Eystein Skjerve at the Norwegian School of Veterinary Science was project leader and arranged courses in the use of the procedure. Skjerve also revised the procedure, and the second edition was published in 2002. In the previous editions, the measurement uncertainty (MU) was estimated from the extra variance in addition to the Poisson variance. Comments received on the previous editions, indicate that the estimates were too narrow and that the procedure was somewhat complicated.

**The procedure shows how to estimate MU based on internal reproducibility data, and how to check that your estimate is adequate by participation in proficiency testing schemes.**

The aim was to make a simple and user-friendly guideline by using real examples, in order to get laboratories started on conducting estimations of MU. The procedure also shows how to use data from collaborative studies for estimation of the MU, which presupposes that the laboratory verifies the obtained precision of the study. Further, the procedure describes the relation between standard deviations and concentration levels based on a number of parallels from collaborative studies, which in turn indicates the precision expected of the laboratory. Read the procedure and find out.

The revised procedure has been elaborated by the following individuals:

Denmark: Erik Dahm, Danish Veterinary and Food Administration, Region North
Iceland: Snorre Þórisson /SYNY Laboratory service
Norway: Marianne Økland, National Veterinary Institute
Sweden: Lennart Larsson, LaVet
Project leader, NMKL Secretary General: Hilde Skaar Norli (photo), National Veterinary Institute

## Call for collaborators for collaborative studies on:

### "Determination of vitamin $K_1$ and $K_2$ (MQ-4 and MQ-7) in foodstuffs by HPLC" NMKL file reg. no. 8.14.4

**Instructions to participants**
The method describes determination by HPLC using fluorescence detection. The fat is removed by lipase, and the vitamin is extracted. The chlorophyll bound vitamin K is removed from the matrices by crushing the material with sand followed by extraction. After the HPLC separation, vitamin K is reduced to vitamin K hydroquinone by a post-column reaction with zinc. Vitamin K is quantified by an external calibration curve. The collaborative study aims to test the method, thus the collaborators are required to follow the method carefully, even though there are steps that could be optimised.

**Test samples and time table**
The number of test samples (food samples) to be analysed will be between 12 and 16. All test samples are to be analysed as single determinations. Two reagent blanks are to be analysed together with the test samples.

The collaborative study will be carried out in May 2008.

**Contact person**
For joining the study or for further information, please contact Kaare Julshamn (e-mail: kju@nifes.no)

### PAH. Determination in foods. NMKL file reg. no. 17.1

**Instructions to participants**
The method, which has to be followed carefully, describes hydrolysis of the sample with potassium hydroxide in methanol/water. The sample is extracted with cyclohexane, washed with methanol/water, cleaned up on SPE-columns, and quantified by GCMS analysis in SIM-mode with impact ionisation. Internal standards are used for quantification.

Information about test samples and time table will be available soon.

**Contact person**
For joining the study or for further information, please contact Sören Wretling, National Food Administration (e-mail: sowr@slv.se)

## News from NordVal:

A list of NordVal approved methods is available at www.nmkl.org under NordVal. There you will also find pdf files of the issued certificates, which you might like to print out for accreditation purposes.


## Bio-Rad has received NordVal Certificate No. 22 for RAPID'*L.mono*

RAPID'*L.mono* is applicable to the detection and enumeration of *Listeria monocytogenes,* and the detection of other species of *Listeria* spp. in all foods and in environment samples.

The new version of RAPID'*L.mono* has been validated by Institute Pasteur de Lille. The validation was conducted according to EN ISO 16140:2003. RAPID'*L.mono* was compared with EN ISO 11290-1/A1:2004 (the detection part) and EN ISO 11290-2/A1:2004 (the enumeration part). The results have been evaluated against the criteria given in the NordVal validation protocol, and are found to be satisfactory for:

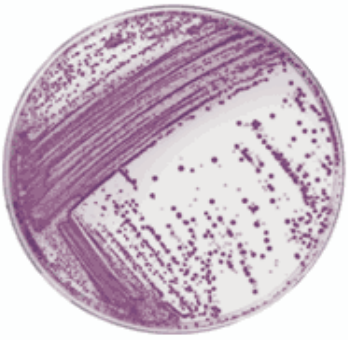
- Detection of *Listeria monocytogenes* within 24 hours after 24 hours of enrichment
- Enumeration of *Listeria monocytogenes* within 24 hours after 48 hours of enrichment
- Detection of other *Listeria* spp. within 48 hours after 48 hours of enrichment

The reading and enumeration of *Listeria* is easy to perform, due to RAPID'*L.mono*'s chromogenic principle. *L.monocytogenes* forms blue colonies without a halo, while *Listeria* spp. forms white or yellow colonies. Confirmation is not necessary.

*The information above was given in the NMKL Newsletter No. 67/2007, but as it contained a number of mistakes, it has been corrected and reprinted.*

## Bio-Rad has received NordVal Certificate No. 32 for RAPID'*Salmonella* - 24 hours test method

NordVal has studied the method description and the validation reports. The validation was carried out according to EN ISO 16140:2003. The RAPID'*Salmonella* 24 hours test method was compared with EN ISO 6579 Microbiology of food and animal feeding stuffs -- Horizontal method for the detection of *Salmonella* spp. The results were evaluated against the criteria given in the NordVal validation protocol, and were found to be satisfactory.



The RAPID'*Salmonella* 24 hours test method is based on the following steps:

- Resuscitation in buffered peptone water
- Enrichment in selective RVS broth for 24 h ± 2 h
- 10 µl plating on RAPID'*Salmonella* chromogenic medium

The principle of the medium relies on the demonstration of two enzymatic activities. *Salmonella* spp take the form of easily identifiable typical magenta colonies (detection of C8 esterase). Counter selection is used to reveal other bacteria with a different colour. RAPID'*Salmonella* permits detection of motile and non-motile salmonella, as well as lactose-positive *Salmonella,* including *Salmonella Typhi* and *Salmonella Paratyphi*.

The RAPID'*Salmonella* 24 hours test method is found to be applicable to food and feed analysis.

For information about NordVal certificates, the NordVal protocol, application forms, price list etc., please visit the NordVal pages under www.nmk.org, or contact the Chair of NordVal, Sven Qvist (sven@qvist.com).

# New NordVal certificates for 3M products:

## 3M™ Petrifilm™ *Enterobacteriaceae* Count Plate, NordVal Certificate No. 28

*Enterobacteriaceae* are of great importance since these organisms are indicators of fecal contamination in food products, and are involved in food spoilage, some of which are food borne pathogens. The 3M™ Petrifilm™ Enterobacteriaceae Count Plate enumerates all *Enterobacteriaceae* organisms, providing a more inclusive picture of potential contamination.


The Petrifilm *Entrobacteriaceae* Count Plate is a sample-ready culture medium system which contains modified Violet Red Bile (VRBG) nutrients, a cold-water-soluble gelling agent, and a tetrazolium indicator dye that facilitates colony enumeration.

The method consists of the following three steps:

- Inoculation of 3M Petrifilm Count Plate with 1ml of sample
- Incubation for 24h ± 2h at 30°C or 37°C ± 1°C .
- Interpretation: After incubation *Enterobacteriaceae* will appear as red colonies with yellow zones and/or red colonies with gas bubbles with or without yellow zones.

The method is applicable to all food products. It has been tested against ISO 21528:2004: Microbiology of food and animal feeding stuffs – Horizontal methods for the detection and enumeration of *Enterobacteriaceae.* Part 2: Colony-count method. The results were evaluated against the criteria given in the NordVal validation protocol, and were found to be satisfactory. The method has been collaboratively tested according to IUPAC harmonisation protocol, and is included in the Official Methods of Analysis (AOAC 2003.01 – Enumeration of *Enterobacteriace* in selected foods).

## 3M™ Petrifilm™ Select *E. coli* Count Plate, NordVal Certificate No. 27


The Petrifilm Select *E. coli* Count Plate is a sample-ready culture medium which allows selective enumeration of ß-glucuronidase producing *E. coli*. This enzyme reacts with BCIG (5-bromo-4-chloro-3-indolyl-ß-D-glucuronide) in the Petrifilm Select E. coli Count Plates, producing dark green to blue-green colonies.

The method consists of the following three steps:

- Inoculation of 3M Petrifilm Count Plate with 1 ml of sample
- Incubation 24h ± 2h at 42°C ± 1°C or 44°C ± 1°C
- Interpretation: After 24h ± 2h at 42°C ± 1°C or 44°C ± 1°C , *E.coli* colonies appear as dark green to light blue-green colonies, regardless of gas production. Colonies other than *E.coli* are difficult to see as they are colourless to light grey-beige.

The method is applicable to all food products. It has been tested against EN ISO 16649 part-2: 2001. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of β-glucuronidase positive *E.coli* – Part 2 : Colony count technique at 44°C using 5-bromo-4-chloro-3-indolyl β-D-glucuronide. The results were evaluated against the criteria given in the NordVal validation protocol, and were found to be satisfactory.

NordVal will be arranging a session at
Food Micro 2008 in Aberdeen, Tuesday 2 September, 14.15-17.15, entitled

**Validation of alternative methods - why, how and who?**

Why it is worth going to the trouble of validating alternative methods will be discussed from a legislator, industry and a test-kit producer's point of view. How these methods should be validated will be presented by test-kit producers, expert laboratories and the certification bodies Afnor, Microval and NordVal.

Chair of the session will be Chair of NordVal: Sven Qvist, (sven@qvist.com), (photo).

## Courses in: Requirement specifications for food analysis - Checklist for considerations in connection with tendering procedures

Whether you are calling for tenders or providing analytical services, it is important to learn about factors that should be considered in connection with tendering processes. NMKL has compiled guidelines (NMKL Procedure No. 17) to help the authorities, laboratories and food industry to specify their needs regarding this topic.

Target group:
- Food control authorities and other authorities – buyers of external analyses
- Laboratories buying internal/external analyses
- Food producers buying analyses for internal control
- Laboratory personnel – to learn about the requirements set by the authorities

Program content:
- General information about the guidelines
- General requirements for the purchase of services (incl. public requirements)
- Sampling, transportation and receipt of samples at laboratories
- Sample preparation, analytical methods, report & quality assurance
- Economical aspects
- Evaluation of possible laboratories with examples from microbiology, chemistry and sensorial analyses

For complete programme (in Scandinavian languages) and speakers, please visit **www.nmk.org.**

Date and place:

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|---|---|
| Norway: | **26 May 2008** at the National Veterinary Institute, Oslo |
| Sweden: | **2 June 2008** at the National Food Administration, Uppsala |
| Denmark: | October /November 2008 |
| Iceland: | October/ November 2008 |

The course will not be held in Finland. Participants from Finland are welcome to attend the courses in the other Nordic countries.

| | |
|---|---|
| Language: | Scandinavian in the Scandinavian countries. English in Iceland. |
| Fee: | NOK 2500, incl. course material (NMKL Procedure No. 17) + lunch and refreshments |
| Registration to: | **nmkl@vetinst.no**, see registration form at www.nmk.org |
| Registration deadline: | **28 April 2008** |

Course organisers:

| | |
|---|---|
| Denmark: | Lisbeth Lund, The Danish Accreditation and Metrology Fund – DANAK (llu@danak.dk ) |
| Finland: | Raymond Tuominen, The Finnish Meat Research Insitute (raymond.tuominen@ltk.inet.fi) |
| Iceland: | Franklin Georgsson, Matis ohf (franklin@matis.is) |
| Norway: | Astrid Nordbotten (project leader), Food Control Authority (asnor@mattilsynet.no) and Urd Bente Andersen, AS Vinmonopolet (urd.bente.andersen@vinmonopolet.no) |
| Sweden: | Håkan Johnsson, National food Administration (hajo@slv.se) |

## Do not miss this opportunity to learn about requirement specifications!

The Swedish National Committee of NMKL would like to welcome you to a Nordic seminar on

## Measurement uncertainty in analyses of foodstuffs - international guidelines, requirements and adaptation.

For those of you who understand Scandinavian languages, we recommend setting aside 22 August 2008 for this seminar, which will be held in Linköping, Sweden. The seminar will be held in connection with the 62nd Annual Meeting of NMKL.

Further information will be made available on www.nmkl.org in due time.

## Available Guidelines & NMKL Procedures:

| | |
|---|---|
| No. 1, 2nd Ed. 2005 | **Calibration and performance checking of laboratory balances.** (English and Swedish) |
| No. 2, 1995 | **Performance check and in-house calibration of thermometers.** (English and Swedish) |
| No. 3, 1996 | **Control charts and control materials in internal quality control in food chemical laboratories.** (English and Swedish) |
| No. 4, 2nd Ed., 2005 | **Validation of chemical analytical methods.** (English, Norwegian and Spanish) |
| No. 5, 2nd Ed. 2003 | **Estimation and expression of measurement uncertainty in chemical analysis.** (English, Spanish and Swedish) |
| No. 6, 1998 | **General guidelines in quality assurance for sensory analysis laboratories Yleiset ohjeet aistinvaraisten laboratorioiden laadunvarmistukseen.** (Danish and Finnish) |
| No. 7, 1998 | **Checking of UV/VIS spectrophotometers.** (Danish and English) |
| No. 8, 3rd Ed., 2008 | **Measurement uncertainty in microbiological examination of foods.** (English and Norwegian) |
| No. 9, 2001 | **Evaluation of results derived from the analysis of certified reference materials.** (English and Swedish) |
| No. 10, 2001 | **Control of microbiological media.** (English, Finnish and Norwegian) |
| No. 11, 2002 | **Procedure for sensory analysis of drinking water.** (English, Finnish and Norwegian) |
| No. 12, 2002 | **Guide on sampling for analysis of foods.** (English, Finnish, Norwegian and Polish). |
| No. 13, 2003 | **Volumetric control.** (Danish and English) |
| No. 14, 2004 | **SENSVAL: Guidelines for internal control in sensory analysis laboratories.** (English and Norwegian) |
| No. 15, 2004 | **Temperature control in microbiological laboratories.** (English and Swedish) |
| No. 16, 2005 | **Sensory quality control.** (English and Norwegian) |
| No. 17, 2006 | **Guidelines for requirement specifications for food analyses.** (English and Norwegian) |
| No. 18, 2006 | **The use of reference materials, reference strains and control charts in a food microbiological laboratory.** (English and Norwegian) |
| No. 19, 2007 | **Guideline for sensorial analysis of food containers/packages.** (English and Swedish) |
| No. 20, 2007 | **Evaluation of results from qualitative methods.** (English and Norwegian) |
| No. 21, 2008 | **Guide for sensory analysis of fish and shellfish.** (English) |