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New NMKL method

NMKL method No 184, 2006: Aerobic count and specific spoilage organisms in fish and fish products.

This method is applicable to the determination of aerobic count and hydrogen sulphide producing bacteria in fresh and lightly preserved (e.g. cold-smoked and brine-cured) fish and fish products.

Enumeration of hydrogen sulphide producing and other non-heat labile bacteria is performed by pour plating in Iron Agar and incubating the plates at 20 – 25 °C for 72 ± 6 hours. Hydrogen sulphide producing bacteria, e.g. *Shewanella* species are frequently found as specific spoilage organisms in chilled and aerobically stored fresh fish. Hydrogen sulphide producing bacteria also include some types of *Aeromonaceae, Enterobacteriaceae, Vibrionaceae* and lactic acid bacteria that occur in fresh and lightly preserved seafood. Hydrogen sulphide producing bacteria form black colonies by decomposing thiosulphate and/or L-cysteine. The black colour is caused by precipitation of iron sulphide (FeS). A thin overlay is poured on top of the Iron Agar to avoid fading of the black colonies due to oxidation of FeS.

Quantitative determination of the aerobic viable count at 15 °C is performed on the surface of Long & Hammer Agar, incubated aerobically at 15.0 ± 1.0 °C for 5-7 days. Luminous colonies can be counted after 4 days in a dark room. The aerobic count of Long & Hammer Agar is used for determination of aerobic plate counts in fresh and lightly preserved seafoods. Spread plating on Long & Hammer Agar allows detection of psychrotolerant and heat labile micro-organisms. These micro-organisms include *Photobacterium phosphoreum* which is CO_2-resistant and often dominates the spoilage microflora of fresh marine fish, particularly for products in vacuum and modified atmosphere packaging. Psychrotolerant and heat labile micro-organisms may also dominate the microflora in fresh minced fish and lightly preserved seafood.

This NMKL procedure has been elaborated by Lis Nielsen, Danish Food and Veterinary Research, Århus, Denmark, and Paw Dalgaard, Danish Institute for Fisheries Research, Lyngby, Denmark. Contact persons have been Ulrike Lyhs, University of Helsinki, Finland, Hélene L. Lauszon, The Icelandic Fisheries Laboratories, Iceland and Olaug Taran Skjerdal, Det Norske Veritas (DNV), Norway.

All the NMKL methods are made available on Internet

To access the NMKL methods on Internet, sign up for an online method subscription. Then you will have access to a complete and updated collection of methods at all times. The general secretariat of NMKL is responsible for keeping the collection up to date, and for notifying subscribers when new methods are published. The methods are available as pdf files. User name and password are requested to log on with.

Prices for online method collection/continuous updates for 1-3 users:
For subscribers: NOK 1500
For new subscriptions: NOK 2500
The password will be forwarded when payment has been received.

NMKL's homepage:
www.nmkl.org

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NMKL method No. 155, 2nd. Ed., 2006: Lactose and galactose. Enzymatic determination in foods.

This is an enzymatic method for the determination of lactose and galactose in foods. The method is applicable to various types of foods, such as liquid and solid dairy products, meat products, bakery products, cakes and pastries, baby foods and chocolate. The method is also applicable to lactose-free foods, but not to products in which the lactose has been hydrolysed (so called HYLA products).Under optimal conditions it is possible to determine concentrations down to lower than 100 mg/kg. This method is not applicable to foods containing lactose which has been hydrolyzed to glucose and galactose using enzymes. In general, difficulties may be expected in the case of samples containing more than five times more galactose than lactose.

In the presence of the enzyme β-galactosidase and water, lactose is hydrolyzed to D-glucose and D-galactose (1). D-galactose is oxidized to galactonic acid by nicotinamide adenine-dinucleotide (NAD) in the presence of the enzyme galactose dehydrogenase (Gal-DH) (2).

(1) Lactose + H_2O β-galactosidase D-glucose + D-galactose

(2) D-Galactose + NAD^+ Gal-DH Galactonic acid + NADH + H^+

The NADH thus formed (reaction 2) is stoichiometrically dependent on the amount of lactose and galactose. The NADH formed is quantitatively determined by measuring the absorbance of the solution spectrophotometrically at 340 nm.

The method was studied in an interlaboratory methods performance study with 12 participating laboratories from four countries. Six materials of commonly used foodstuffs with lactose concentrations varying between about 0.4 g/100 g and about 40 g/100 g and galactose concentrations up to 0.7 g/100 g were included in the study. The materials were crisp rye bread, milk chocolate, sausage, cheese, margarine and baby food containing milk powder. They were distributed to the 12 participants as 12 randomly numbered test samples, which were blind duplicates of the six materials. The relative reproducibility standard deviations (RSD_R) for the determination of lactose were estimated to vary between 2.3 and 11 %, and for galactose between 6.8 and 50 %.

The changes from the previous version consist of inclusion of the results from the study as well as references. The method-performance study was arranged in the mid 1990ies by Dr. Antti Mustranta, VTT Biotechnology and Food Research in Espoo, Finland.

NMKL method No. 154, 2nd Ed., 2006: *Fusarium*. Determination in foods and feedstuffs.

Most *Fusarium* species occurring in foods and feeds are known to produce mycotoxins, e.g. trichothecenes, fumonisins and zearalenone. A selective medium for *Fusarium* species would allow microbiological monitoring of the occurrence of these fungi indicating the possibility of toxin production. Several PCNB [pentachloronitrobenzene]-based media selective for *Fusarium* are known. However, as PCNB is suspected to be carcinogenic, there has been considerable effort to find a substitute. In 1987 a *Fusarium* selective medium CZID [Czapek-Dox iprodione dichloran agar] was developed, and it has since been used in the examination of several hundred food and feed samples. Comparison with two other proposed *Fusarium* selective media DCPA [dichloran chloramphenicol peptone agar] and PPA [pentachloronitrobenzene agar] showed that the *Fusarium* counts on CZID were not significantly different. Furthermore, it is possible to discriminate between different *Fusarium* species on CZID, which is impossible on DCPA and PPA.

By subsequent isolation of cultures, representing the different *Fusarium* types, it will be possible to identify the observed fungi to species level.

Using CZID and either dilution or direct plating (or both) it is possible to count the number of colony forming units (cfu) per gram of sample or the percentage infection of *Fusarium* in particles. The dilution plate count of *Fusarium* is obtained by a surface plating technique. After homogenizing of a specified quantity (preferably 40 g), a serial dilution is carried out. Of each dilution 0.1 ml is spread plated onto the medium in duplicate. After incubation at 25.0 ± 1.0 °C for 5-7 days under alternating light-dark conditions (12 h light/12 h dark), the colonies with a characteristic appearance are counted. Each colony type is counted separately. Representative colonies of each type may be purified on SNA [Spezieller Nährstoffarmer agar] and identified to species level. The number of cfu of each *Fusarium* species per gram of sample is calculated.

The direct plating count of *Fusarium* is performed by placing 100 particles (often kernels or seeds) onto the medium, 5-10 on each 9 cm Petri dish. If only the amount of *Fusarium* inside the sample is of interest the sample should be surface disinfected before plating. The number of particles, from which at least one *Fusarium* colony grows out, is recorded. Each colony type is counted separately. Representative colonies of each type may be purified on SNA and identified to species level. The percentage infection of each *Fusarium* species is calculated.

This method is not validated in a collaborative study.

This NMKL method has been elaborated and revised by Ulf Thrane, BioCentrum-DTU, Technical University of Denmark. Contact persons have been Sinikka Marmo, Plant Production Inspection Centre, Finland, Monica Olsen, former employed at the National Food Administration, Sweden, Mona Torp, former employed at the National Veterinary Institute, Norway.

Comments to the NMKL method No 145, 2nd Ed. 1997: Starch and glucose. Enzymatic determination in foods.

The method uses the enzyme Termamyl to break down starch to amyloglucosidase. Termamyl is available from Foss Tecator, and is produced by Novo industri A/S. At present, only 300 L is available for sale. According to Tecator, the solution can be diluted into 120 L. The concentration in 300 L is 300 KNU/g, and in 120 L, it is 120 KNU/g.

By using Termamyl for the analysis of a starch reference material from a Boehringer MannHeim/R-Biofarm kit, and analysing according to NMKL Method No. 145, satisfactory results can be obtained for starch when only total glucose is analysed. The starch reference material is said to contain 86.5% starch. By analysis (n=7), a mean of 85.4% is obtained, which yields a recovery rate of 98.7%. If only glucose is analysed, a content of 2-5% glucose is obtained in the sample. In a reference sample from Fapas containing 46% starch and 0.3% glucose, 7 analyses gave a mean value of 45.6% for total glucose and 2-4% for glucose alone. The mean value (n=7) is 3.27%. This indicates that the Termamyl hydrolyses the starch to glucose, or that it is contaminated by enzymes that break down amyloglucosidase to glucose.

To simplify the method's boiling procedure, analyses of samples kept in the oven at 90°C for 30 minutes were carried out parallel with the analyses of samples with the procedure in Method No. 145. The results of these comparisons show no significant differences. Starch samples left to stand overnight at 90°C, give a result of 12% glucose.

To test the concentration dependency of Termamyl, a dilution curve was made. Samples were left in the oven at 90°C for 30 min. The analyses were carried out on starch reference materials from Boehringer MannHeim/R-Biofarms kit with a given starch content of 86.7%.

Termamyl KNU/g	Starch tot. glucose %	Glucose %
300	87.6	4.9
150	85.3	2.7
75	81.7	0.6
36.5	81.5	1.0
18.25	72.9	0

Conclusion:
The method works well for analysing total glucose on hydrolysed and free glucose. For analysis of free glucose, the samples should not be treated with Termamyl.

The boiling procedure, in which Termamyl is added, can be replaced by keeping the samples in an oven at 90°C, with some shaking or stirring during this period. Termamyl breaks down maltose, but not sackarose.

The comments have been given by Anders Eriksson, from the National Food Administration of Sweden. The chemical committee of NMKL has decided that the comments should be enclosed with the method.

Workshop in Brominated flame retardants (BFR)

Time / location: 25 – 26 April 2006, Hotel Linné (First Hotel) Uppsala
Language: English
Organiser: NMKL / EK-Livs (the Nordic Committee of Senior Officials for Food Issues) / National Food Administration, Sweden

Programme
Day 1: 25 April
10.00-10.30 Registration, Coffee
10.30 10.50 Introduction, *Dr Leif Busk, Dep Dir. National Food Administration (NFA), Sweden*
10.50-11.10 Presentation of NMKL, *Hilde Skår Norli, Sec. Gen. NMKL*
11.10-11.30 Aim, schedule and practical details, *Dr Håkan Johnsson, NFA, Sweden*
11.30-12.30 BFR-overview: old and new issues, scope of application etc. *Prof. Jakob de Boer, the Netherlands.*
12.30-13.30 Lunch
13.30-15.00 BFR analysis/projects in the Nordic countries. Presentations by appointed delegates from the Nordic countries (about 20 minutes each country)
15.00-15.30 BFR toxicology, *Dr. PO Darnerud, NFA*
15.30-16.00 Coffee
16.00-16.30 Neurotoxicity of PBDE and HBCD in rodents, *Prof. Per Eriksson, University of Uppsala*
16.30-17.00 Endocrine-mediated developmental effects of BFRs. *Dr Helmut Lilienthal, BGFA University Bochum, Germany*
17.00 Discussions. The toxicologists view on the future with respect of BFRs. New substances, new analytical methods.

Day 2: 26 April
9.00-9.45 Procedures and challenges in BFR analysis. *Prof. Åke Bergman, University of Stockholm*
9.45-10.15 BFR methods in the Nordic countries. Presentation by the delegates (about 20-30 min per country) – to be cont.
10.15-10.45 Coffee
10.45-12.00 (cont.) BFR methods used in the Nordic countries
12.00-13.00 Lunch
13.00-13.15 Isomer specific analysis of HBCD with LC/MS
13.15-13.45 Proficiency testing schemes
13.45-15.15 Discussions regarding analysis and possible interest in standardising a method.
15.15-15.45 Conclusion and closing
Coffee.

Registration fee: NOK 500,- (coffees + 2 lunches) – No fee for the workshop.
Registration to: NMKL on nmkl@vetinst.no **Deadline for the registration:** 5. April 2006
Leader and contact person for the workshop is Dr. Håkan Johnsson, (e-mail: hajo@slv.se).

Nordic Workshop in Sensory Science
FOCUS ON THE NORDIC CONSUMER

Time / location: Thon Hotel, Ski, Norway, 3 - 5 May 2006
Language: English
Organiser: Matforsk

What kind of food does the Nordic consumer like or dislike? Without sensory analysis, the answer to this question could easily remain unknown. Nordic scientists and industry know a lot about the Nordic consumer's preferences, and are happy to share this knowledge with you.

The workshop will cover the following themes: The meal, trends, tomorrow's food, health and diet, traditional food as well as non-food.

For further information, programme and registration please visit Matforsk's homepage: **www.matforsk.no**
Please note that the deadline for registration is **1 April 2006.**

Seminar: Multidimensional food in all directions
Chemistry – Microbiology – Sensory topics

Time/ location: 24 August 2006 at Thon Hotel Opera, Oslo
Language: Scandinavian languages
Organiser: The Norwegian National Committee of NMKL

In connection with the 60th NMKL Annual Meeting, the Norwegian National Committee of NMKL will arrange a seminar on multivariable analysis. The purpose of the seminar is to show how multivariable techniques are used, and what they can be used for when processing results and developing new methods.

Multivariable methods are used for comparing and finding correlations between large amounts of data. They may be used for large, complex problems, or seemingly simpler tasks, for example describing which properties are important for different varieties of apples or different types of brown bread. They also make it possible to see whether there are any probable correlations between different analysis parameters obtained by chemical, physical, microbiological and sensory measurings.

The programme for the seminar is available at NMKL's homepage in Scandinavian version.

Registration to NMKL at nmkl@vetinst.no **before 1 June 2006**.
Registration fee: NOK 2000,-

NMKL procedure No 11: Procedure for sensory analysis of drinking water, is now also made available in Finnish as

NMKL-Menettelyohje Nro 11 (2006) : **JUOMAVEDEN AISTINVARAINEN ARVIOINTI**

Tämän menettelyohjeen tarkoitus on neuvoa ja opastaa juomaveden aistinvaraisen arvioinnin suorittamisessa. Menettelyohjeen avulla annetaan ohjeita arvioijien valinnasta, koulutuksesta, arviointiin valmistautumisesta, arvioijille asetettavista vaatimuksista, arviointitiloista ja -olosuhteista, näytteiden esikäsittelystä, tarvittavien välineiden valinnasta sekä näytteiden esittämisestä. Tämä on ts. tarkoitettu ohjeeksi arviointia tekeville laboratorioille tai muille juomaveden arviointia suorittaville tahoille. Vaikka menettelyohje on ennen kaikkea tarkoitettu avuksi aistinvaraisia analyysejä tekeville laboratorioille, sitä voidaan käyttää myös ohjeena kenttätutkimuksissa esim. eri vedenjakelupisteissä.

Menettelyohje perustuu olemassa oleviin kansallisiin ja kansainvälisiin juomaveden laatumääräyksiin ja standardeihin sekä juomaveden laatua tutkivien laboratorioiden ja muiden aistinvaraisten asiantuntijoiden käytännön kokemuksiin. NMKL:n menettelyohje nro 6 vuodelta 1998 antaa yleisohjeita aistinvaraista arviointia tekeville laboratorioille.

Tämä menettelyohje on toteutettu NMKL-projektina. Urd Bente Andersen, Norsk Matanalysestä toimi referenttinä ja menettelyohjeen kirjoittajana. Muista pohjoismaista kontaktihenkilöinä olivat Ulf Eriksson, VA-prosjekt, Uppsala (Ruotsi), Robert G. Nielsen, Bioteknologisk Institut (Tanska), Juhani Airo, Helsingin kaupungin ympäristökeskus (Suomi) ja Tyri Valdimarsdóttir, Fiskeriinsindustriens Forskningslaboratorium, Reykjavik, (Islanti). He kaikki avustivat menettelyohjeen laatimisessa arvokkain kommentein ja palauttein.