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NMKL Newsletter

NORDIC COMMITTEE ON FOOD ANALYSIS

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NMKL Secretary General
Norwegian Veterinary Insitute
P.O.Box 750, Sentrum, N-0106 Oslo, Norway

Tel. +47 23 21 62 50 / +47 468 88 807
Org. No. 995 790 394

E-mail: nmkl@vetinst.no

www.nmkl.org

NMKL Secretary General: Hilde Skår Norli

Measurement uncertainty in sensory analyses

New NMKL Procedure:

Measurement uncertainty in sensory analyses

NMKL Procedure No. 27, 2013

Sensory analysis, or sensory evaluation, entails evaluating something using one or more of the human senses: taste, smell, sight, hearing and feeling (tactility).

The sensory characteristics of food can be complex. There are no "true" or strictly defined values to be measured. Sweetness can be perceived differently in different foods, even though the content of sucrose is the same. It can not be stressed often enough that a trained sensory panel is something completely different than a group of consumers, and this procedure describes exclusively the uncertainty of results obtained in sensory panels, leaving out validation and verification of consumer tests. A sensory panel consists of 3-15 trained participants depending on the type of test.

Sensory data can be divided into three main groups: profiling data, binomial data and quality control data. Guidelines for statistical processing and estimation of measurement uncertainty in different sensory analysis have been elaborated in an NMKL project under the leadership of Per Lea, Nofima, Norway,

Per Lea, Nofima

The guidelines provide examples on how to use classical statistical methods in the estimation of standard deviation and measurement uncertainty. In addition to Per Lea, the following project members participated in the elaboration of the procedure:
Gunnar Forsgren (Iggesund Paperboard, Sweden), Grethe Hyldig (DTU Food Institute, Denmark), Päivi Kähkönen (FINAS, Finland), Aðalheiður Ólafsdóttir and Kolbrún Sveinsdottir (Matis, Iceland) and Steffen Solem (Wine Monopoly, Norway).

The procedure is available in Norwegian and English versions from the general secretariat office of NMKL.

Course in measurement uncertainty in sensory analyses

21 May 2013, 2:00 - 5:00 PM, at
Helsinki Congress Paasitorni, Finland

Lecturer: **Per Lea, Nofima**

The deadline for registration has passed, but there may be some available places. The course will be held in conjunction with the Nordic Workshop in Sensory Science.

The course is based on NMKL Procedure No. 27, 2013, which will be distributed to the participants.

Course fee: NMKL subscribers and students: NOK 2000,
other participants: NOK 3000.
Registration to nmkl@vetinst.no
Language: **English**

Nitrate and Nitrite

Nitrate occurs naturally in plants (vegetables), and only a small fraction of the amount we consume is related to food additives. Nitrate (which is converted to nitrite) and nitrite inhibits the growth of bacteria. In addition, nitrite is used to preserve a fresh red colour in meat.

Nitrate and nitrite are among the most controversial preservatives as they are proven to be harmful in large doses. The ADI values (Acceptable Daily Intake) for nitrite and nitrate, are 0 - 0.2 and 0 - 0.5 mg/kg body weight, respectively.

Lenordo Merino

New NMKL Method:

Nitrate and nitrite. Determination of nitrate and/or nitrite in foodstuffs and water by spectrophotometry after zinc reduction and Griess reaction. (NMKL Method No. 194, 2013)

The method describes a spectrophotometric method for the determination of nitrate/nitrite content in foodstuffs and water.

Nitrate (NO_3^-) is reduced quantitatively to nitrite (NO_2^-) in the presence of zinc powder (Zn). The nitrite (originally present plus reduced nitrate) is determined by diazotizing with sulfanilamide and coupling with N-(1-naphtyl)-ethylenediamine dihydrochloride to form a highly coloured azo dye that is measured at 540 nm. The nitrite present in the sample is determined by analyzing without the reduction step. The nitrate is calculated as the difference between the total nitrite content after reduction, and the initial nitrite concentration.

The method has been validated in extensive internal validations over a period of six months testing salad, meat, baby food, dairy products and water. The nitrate levels tested ranged from 21 to 203 mg/kg, with satisfactory precision (HorRat ≤ 2). The recovery of nitrate ranged from 70-109%. For nitrite levels ranged from 27 to 1579 mg/kg. The precision was satisfactory. The recovery of nitrite ranged from 73-105%.

This method has been elaborated by Leonoardo Merino in cooperation with Mailani Åström. Both are employees of the National Food Administration in Sweden. Udo Jensen (Denmark), Christina Bäckman (Finland), Jón Baldur Vigfusson (Iceland) and Sharon M. Løver (Norway) have been contact persons.

Remember to renew your NMKL subscription!

NMKL offers online access to NMKL Methods and NMKL Procedures.

Histamine - Biogenic amines

Biogenic amines are common metabolic constituents of many foods. The formation of large amounts of biogenic amines are mainly reported in expired, fermented or rotten products, especially cheeses, fish and meat, wine and some vegetables. Biogenic amines are formed by microbial decarb-oxylation of amino acids.

Low levels do not normally present any risk to consumers. However, if large amounts of biogenic amines are ingested and/or the natural mechanism for the catabolism of amines is inhibited or deficient, toxic symptoms may occur.

Maximum Limits and Method Criteria for Histamine:

According to EC 2073/2005 on microbiological criteria for foodstuffs, the maximum limit of histamine in some fish products is 200 mg/kg. In Codex, the maximum limit of histamine in fish is 100 mg/kg and 200 mg/kg. The Codex Committee on Methods of Analysis and Sampling suggested at their meeting in March 2013 the following criteria for a suitable method for the determination of histamine in fish:

Minimum applicable range: 79-243 mg/kg

Limit of detection: 10 mg/kg

Limit of quantification: 20 mg/kg

HorRat value: ≤ 2

Recovery: 90-107%.

New NMKL Method: Biogenic amines. HPLC determination in foods. (NMKL Method No. 196, 2013)

This method meets the method criteria proposed by the Codex Committee on Methods of Analysis and Sampling (CCMAS). CCMAS has recommended this method for the analysis of histamine in fish and fish products.

This method is elaborated for the quantitative determination of different biogenic amines: tryptamine (Tryp), phenylethyl-amine (Phe), putrescine (Put), cadaverine (Cad), histamine (His), serotonin (Ser), tyramine (Tyr), spermidine (Spd) and spermine (Spr). It is applicable to the analysis of fish and fish products, meat products, cheese and fermented vegetables.

Biogenic amines are extracted from a homogenised sample with diluted perchloric acid. An aliquot of the extract is derivatised with dansyl chloride. Separation and quantification of dansylated amines are performed by reversed phase liquid chromatography with UV detection at 254 nm.

This method was collaboratively validated in 2004. Nine laboratories participated. The results of the validation were not considered satisfactory, and hence the method was not published. This was due to the fact that outliers had not been excluded and that the analyses had been carried out on very low levels (levels at the limit of detection) which yielded an unsatisfactory high standard deviation. In 2012, the results were reviewed again, this time omitting outliers and including results from the expert laboratory.

To be continued on page 5

Histamine - Biogenic amines (cont.)

Cont. from page 4. NMKL 196, 2013

After the study was performed in 2004, the expert laboratory has used two other dimensions of the LC column than the one used in the collaborative validation. The results of internal validations of these columns have also been satisfactory. The following results have been obtained for the current LC column in use, 75 x 3.0 mm id.:

- Limit of Detection: 0.5 mg/kg
- Limit of Quantification: 1.0 mg/kg
- Tested levels with satisfactory precision: 2.4 - 100 mg/kg
- Recovery: 96-101%

The method was elaborated and validated by Susanna Eerola, Tiina Ritvanen, Kristiina Kuitunen and Mervi Rokka, Evira, Finland.

Establishing numeric values for the method criteria according to Codex

In order to give laboratories a freedom of choice in the use of appropriate analytical methods, Codex has developed a set of criteria based on maximum levels (ML). A method shall be considered appropriate if the following criteria are met:

Minimum applicable range: For ML ≥ 0.1 mg/kg: [ML - 3sR, ML+ 3sR]
For ML < 0.1 mg/kg: [ML - 2sR, ML+ 2sR]

Limit of Detection, LOD: For ML ≥ 0.1 mg/kg: LOD ≤ ML·1/10
For ML < 0.1 mg/kg: LOD ≤ ML·1/5

Limit of Quantification, LOQ: For ML ≥ 0.1 mg/kg: LOQ ≤ ML·1/5
For ML < 0.1 mg/kg: LOQ ≤ ML·2/5

Precision: For ML ≥ 0.1 mg/kg: HorRat ≤ 2
For ML < 0.1 mg/kg: RSDR ≤ 44%

Recovery: 100 g/100g– 10g/100g: Recovery (%): 98-102
1 g/100g: Recovery (%): 97-103
1 mg/g: Recovery (%): 95-105
100 mg/kg: Recovery (%): 90-107
10 mg/kg - 100 µg/kg: Recovery (%): 80-110

NMKL has been involved in the elaboration of the method criteria in Codex, and has prepared a simple spreadsheet that calculates the values of the method characteristics: application range, LOD, LOQ and precision, when the limit (ML) is inserted. **The spreadsheet is available free of charge at NMKL's website under "Download Excel spreadsheet" and "How to get method criteria based on ML".**

NMKL Method No. 99: Histamine. Fluorimetric determination in fish

This method is endorsed by the Codex Committee on Food Analysis and Sampling for the determination of histamine in fish products. The method harmonises with AOAC 977.13.

NMKL Method No. 99 is under revision. In the new edition, which will be released in 2013, the extraction solution is changed from 100% to 75% methanol. Further, data from a collaborative validation is included in the method. The validation shows that the method satisfies the criteria for histamine (given on page 4), except when it comes to recovery. In the validation, the obtained recovery was 85-120%, not 90-107% as required. The method is validated for histamine in fish at levels from 5.6 mg/kg to 158 mg/kg. The sample is extracted with 75% methanol, and purified on an anion exchange column in order to remove interfering substances. O-phthaldialdehyde solution is added to the eluate to form fluorescent histamine derivatives. The fluorescent intensity of the derivatives is measured spectrophotometrically, and histamine is quantified using external standards.

Sensory Quality control of drinking water (NMKL 183)

- safety assessment of chemicals

NMKL 183 describes how to test the quality of water samples by evaluate smell and taste against reference water. Prior to the tests, the judges are "calibrated" in order to assess their suitability. The calibration solutions contain a variety of chemicals, and for the safety of the judges a safety assessment of these chemicals has been carried out. A list of chemicals and their respective smells and tastes in drinking water is given in the table on the right hand side.

Smell/taste	Chemicals
Sour/acidic	Citric acid in water
Sweet	Sucrose in water
Salt	Sodium chloride in water
Bitter	Caffeine in water
Earthy	Geosmine
Mouldy	2-Metylisoborneol
Gras	Cis-3-hexen-1-ol
Vegetable (cucumber)	Trans-2-cis-6-nonadienal
Rotten fish	Trimethyl amine (25%)
Medicine	Iodoform (tri-odo-methane)
Plastic	Methyl metacrylate
Mouth/nose feeling	
Astringent	Lemon juice

A sensory test is performed by taking a large mouthful of the solution, rolling it around in all parts of the mouth and then spitting it out. Accidental swallowing of samples increase exposure. The concentrations of the chemicals are so low that immediate effects will not occur, but what about any long-term effects?

A safety assessment has been conducted by Pertti Kovisto, Evira, Finland and Kettil Svensson, National Food Administration, Sweden. Exposure calculations for each of the chemicals are given as an appendix to the method. Based on the calculations, the conclusion is that there are no health risks associated with the applied substances, if used as specified in the method.

Method requirements for chemical NMKL Methods, that are not collaboratively validated (NMKL Protocol No. 6)

Collaborative method studies involving 10-12 laboratories are extremely resource demanding. Given that there are fewer labs that can undertake to arrange collaborative studies, and to participate in collaborative studies without getting costs covered, while at the same time, the requirements for laboratory quality is more stringent, compelling the laboratories to be accredited and participate in PT schemes, the organisations are looking at alternative ways to evaluate new analytical methods.

NMKL will continue to strive to validate the methods collaboratively. If a chemical method is not validated in a collaborative study, the method has to be validated according to NMKL Procedure No. 4: *Validation of chemical analytical methods*. Further, the method must meet Codex's method criteria, as specified on page 5. NMKL Protocol No. 6, which describes this procedure, is available for free at www.nmkl.org.

Working instructions

- The referee and the contact persons prepare a draft of the method.
- The laboratory of the referee performs an internal validation of the method. Results of PT schemes and results obtained from internal validations for establishing estimates of measurement uncertainty can be used.
- The results of the internal validations are reviewed by the referee and the contact persons.
- The results are included in the method text.
- The draft method, including the results of the validations, is reviewed by the national committees for approval, firstly by the national committee of the referee.
- The approved method is finally reviewed by the Chair of the Chemical Committee and the Secretary General.
- The method is considered for revision after 5 years.

Seminar: Preparedness for emergencies in the food chain!

30 August 2013, at Kalmarsund Hotel, Kalmar, Sweden

This very relevant seminar will be held in Swedish language. For program and registration please see www.nmkl.org.

Available NMKL Procedures

No 1, 2. Ed. 2005	Calibration and performance checking of laboratory balances
No 3, 1996	Control charts and control materials in internal quality control in food chemical laboratories
No 4, 3. Ed. 2009	Validation of chemical analytical methods
No 5, 2. Ed. 2003	Estimation and expression of measurement uncertainty in chemical analysis
No 6, 1998	Yleiset ohjeet aistinvaraisten laboratorioiden laadunvarmistukseen (avail. Danish/Finnish)
No 7, 1998	Checking of UV/VIS spectrophotometers
No 8, 4. Ed. 2008	Measurement of uncertainty in quantitative microbiological examination of foods
No 9, 2. Ed. 2007	Evaluation of method bias using certified reference materials.
No 10, 2001	Control of microbiological media
No 11, 2.Ed. 2010	Procedure for sensory analysis of drinking water
No 12, 2002	Guide on sampling for analysis of foods
No 13, 2003	Volumetric control
No 14, 2004	SENSVAL: Guidelines for internal control in sensory analysis laboratories
No 16, 2005	Sensory quality control
No 17, 2006	Guidelines for requirement specifications for food analyses
No 18, 2006	The use of reference materials, reference strains and control charts in a food microbiological laboratory
No 19, 2007	Guideline for sensorial analysis of food containers/packages
No 20, 2007	Evaluation of results from qualitative methods
No 21, 2008	Guide for sensory analysis of fish and shellfish
No 22, 2008	Considerations regarding evaluation of immunochemical test kits for food analysis
No 23, 2008	Guide on quality assurance in microbiological laboratories (replaces NMKL Report No. 5)
No 24, 2010	Guidelines for quality assurance for food chemical laboratories
No 25, 2012	Recovery information in analytical measurement
No 26, 2012	Control and internal calibration of thermometers and temperature control on microbiological laboratories
No 27, 2013	Measurement uncertainty in sensory analysis

NordVal has become NordVal International

NordVal has changed its name to NordVal International. The reason for the name change is to underline that NordVal evaluations are recognised world wide, not only within the Nordic countries.

NordVal International is still a committee under NMKL, with its own Steering Group. The Chairman is Dr. Sven Qvist, Denmark.
NordVal validates both chemical and microbiological proprietary and other

Hygicult® TPC - Aerobic microorganisms - NordVal Certificate 018

Hygicult®TPC is a culture slide for rapid monitoring of microbiological hygiene in different types of materials, both solid and liquid. The slide is covered on both sides with Total Plate Count (TPC) agar which supports rapid growth of the most common bacteria and fungi.

Hygicult®TPC has been tested in a collaborative study, and was compared against NMKL Method No. 5. The incubation temperatures tested were 25 and 30°C, and the incubation times were 48 and 72 hours. There were no significant statistical differences in the results of the two methods. The certificate is renewed until 2015.

Manufacturer and supplier of Hygicult®TPC is Orion Diagnostica Oy.

Campylobacter real-time PCR - NordVal Certificate 017

Eurofins has renewed the NordVal certificate for a method for the determination of human pathogenic thermo-tolerant Campylobacter (*C. jejuni*, *C. coli* and *C.lari*) using real-time PCR in raw chicken meat, faeces, sewage swabs and shoe covers. The sensitivity of the method is 1-10 cfu/25g in raw chicken meat, and 100-1000 cfu/ml in diluted swabs and faeces samples collected on disposable shoe covers. The method is open, i.e. all reagents are described. The method is tested against ISO 10272-4 and NMKL 119. There were no statistically significant differences in the results obtained by the different methods.

Salmonella ELISA Test OPTIMA - NordVal Certificate 010

Salmonella ELISA Test OPTIMA is an immuno-enzymatic test using a microtiter plate coated with specific antibodies directed against *Salmonella, and* ready-to-use reagents. The test allows the detection of *Salmonella* after enrichment (for approx. 40 hours) and a heat shock releasing any *Salmonella* antigens present in the sample. The antigens are detected by a sandwich ELISA (Enzyme Linked Immuno Sorbent Assay). The method is tested according to ISO 16140 and the NordVal Protocol. The reference method was ISO 6579.

The manufacturer and supplier of *Salmonella* ELISA Test OPTIMA is Diaktek AG, Switzerland.

NordVal Certificates are available for downloading at www.nmkl.org