December, 2013

No. 85

NMKL Newsletter

NORDIC COMMITTEE ON FOOD ANALYSIS

CONTENTS

The following methods have been made available in Finnish:

NMKL 99 Histamine

NMKL 194 Nitrate and nitrite

NMKL 195 Pesticide residues

NMKL 196 Biogenic amines

NMKL Wishes You All a Merry Christmas

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NMKL Secretary General: Hilde Skår Norli

Aerobic Microorganisms. Determination in foods at 37°C, 30°C, 25°C, 20°C, 17/7°C or 6.5°C by the colony count method.

New revised NMKL Method 86, 5th Ed., 2013.

Aerobic count is often used as a surveillance of hygiene in production of food. The number of microorganisms in a food product will be of importance in evaluating sanitary practices during processing and handling. When doing a surveillance it is important to use exactly the same conditions every time the analysis is performed. It might though be possible to use other incubation temperatures and incubation times than the ones described in this method as long as the temperature is kept the same in one surveillance, and especially if a business has an experience with another time/temperature combination.

Referee: Dr. Maijbritt Moos, Eurofins Denmark

NMKL 86 is suitable for determining the number of viable aerobic microorganisms in foods. The aerobic count can sometimes be used to indicate the quality and spoilage level of the product. The method can be applied to all kinds of foods. However, for aerobic count in fish and fish products, NMKL 184 could preferably be used.

This method is a defining method. Aerobic microorganisms are microorganisms, growing under aerobic conditions when the test is carried out according to the method described. Psychrotrophic microorganisms are aerobic microorganisms capable of relatively rapid growth at temperatures between 0 and 10°C. Psychrotrophic microorganisms usually have considerable enzymatic activity.

The aerobic plate count is determined by preparing a dilution series of the sample material according to general microbiological principles, followed by pour-plating into an agar medium in Petri dishes. The samples are incubated under aerobic conditions at either 37°C for 3 days, 30°C for 3 days, 25°C for 3 days, 20°C for 3 days, 17°C for 1 day followed by 7°C for 3 days or 6.5°C for 10 days. The number of viable aerobic microorganisms per millilitre or gram of sample is calculated from the number of colonies counted on selected plates.

This method replaces the 4th edition of NMKL 86 and NMKL 74 for Psychrotrophic microorganisms. Those methods will be withdrawn, and should no longer be referred to.

Majbritt Moos, Eurofins Stein, Denmark, has revised this method. Anu Surakka (Finland), Laufey Karlsdottir (Iceland), Kari Dommarsnes (Norway) and Jörgen Nilsson (Sweden) has been to her assistance in the revision.

The method is made available for the NMKL method subscribers. If you do not have a subscription, the method can be ordered at NMKL Web Shop.

CHALLENGES WITH FATS

BY PÄIVI TEIVAINEN-LÆDRE, SKRETTING AQUACULTURE RESEARCH CENTRE

DEFINITION OF FAT

Fats are generally considered to be esters of glycerol with various carboxylic acids, and hence what in most cases is meant by fat is triglyceride. As fat consists of a diverse range of organic compounds with varying physical properties makes it difficult to give fat "a water -tight"" definition that applies to all fat components. Thus, several definitions can be used. The US Nutrition Labelling and Education Act of 1990 defined total fat as: "*Total lipid fatty acids, that is the sum of fatty acids from mono, di and triglycerides, free fatty acids, phospholipid fatty acids and sterol fatty acids to be expressed as the amount of triglycerides that would provide the analytically measured amount of total lipid in the food*." In other words: **Total fat = sum of fatty acids from all sources, expressed as triglyceride.**
Hence only lipids that give fatty acids on hydrolysis plus free fatty acids are included in the definition.

METHODS FOR FAT ANALYSES

The two important major factors for fat analyses are as follows:
• Triglyceride is the major fat of fish and fish feed
• Triglycerides are non-polar hydrophobic ("water hating") compounds.

Historically, a number of methods that have been developed for the analysis of fats are based upon the ease or difficulty of removing fat from a given matrix. When extracting fat from different sources, the method and the solvent used are critical, in order to obtain complete extraction with minimum undesired impurities. It is a challenge for laboratories to measure exact quantity, "true value" of fat in different products.

The current reference method, Soxhlet, is efficient in extracting triglycerides and other non-polar fat compounds, but vary considerably in the efficiency of extraction of other more polar fat compounds (e.g. free fatty acids, mono-glycerides and phospholipids). This means that the fat tests are particularly efficient in measuring fat from fish consisting of 99 % triglyceride, but are not

Continued on page 4

NON-DESTRUCTIVE ANALYTICAL METHODS: CURRENT INSIGHT INTO NIR AND NMR TECHNOLOGIES

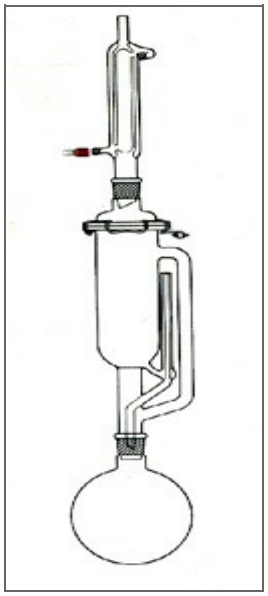
34 people from Belgium, Denmark, Finland, Iceland, Germany, the Netherlands, Norway, Sweden and United Kingdom participated at the seminar held 21 November at the University of Copenhagen. Invited speakers from industry, instrument suppliers and academia discussed current research, results and problems of both theoretical and practical nature.

The seminar was a success, and showed that there is a need to narrow the knowledge gap between current research and industry. Several examples on recent method and instrument development were given. For instance IR spectroscopy procedures such as on-line or in-line NIR and low- and high-field nuclear magnetic resonance spectrometry (NMR) that can increase the potential to substantially improve the accuracy, sensitivity, and speed of determination of quality parameters.

Members of organizing committee, Dr. Päivi Teivainen-Lædre from Skretting Aquaculture Research Centre, Dr. Erik Nordkvist from National Veterinary Institute, Sweden and NMKL Secretary General Hilde Skår Norli, acknowledge Professor Søren Balling Engelsen who gave us the opportunity to arrange the seminar at the University of Copenhagen.

CONTINUED FROM PAGE 3 : ## CHALLENGES WITH FATS

particularly efficient in measuring fat from products that have a high percentage of polar lipids such as phospholipids or free fatty acids. Plant based raw materials are increasingly used in fish feed. It is notable that plant oils have a fat composition that differs greatly from fish oil, traditionally the most common source of fat in fish feed. This will challenge current quality control laboratories to establish technics that are able to quantify fat from different sources and with diverse fat profile. Thus, it is important to evaluate available methods to analyse fat and to minimize uncertainties and potential economic consequences.



Soxhlet Extraction Glassware setup

New methods and technics have been developed to quantify fat in different samples. Recent method and/or instrument development, e.g. IR spectroscopy procedures, ionic-liquid GC columns, multidimensional chromatographic techniques and nuclear magnetic resonance spectrometry (NMR), have increased the potential to substantially improve the accuracy, sensitivity, and/or speed of fat determination. Comparative study to evaluate and to approve low-field (LF) NMR as reference method for fat analysis in fish and fish feed is currently on-going.

NMR VS. EXTRACTION TECHNICS SUCH AS SOXHLET

Data has indicated that NMR method systematically leads to higher values than those measured using the Soxhlet method, which might be due to the extraction efficiency of chemical methods, linked to the structure of the samples. Previous results have shown that the structure of the sample (particle size) have a significant impact on the chemical extraction, and, thus, recovery of fat.

The second source of error using Soxhlet might be losses that occur during transfer of the lipid phase with a pipette. During this step, the operator usually prefers to end a little early for not risking transfer of non-lipid phase. Although the boundary between the two phases is not easily detectable, the non-lipid phase contains visible solid elements. Thus, if the operator notices that he/she has drawn off from the non-lipid phase, she/he will discard this pipetted volume even if it certainly contains a small amount of the lipid phase.

Thirdly and as mentioned above, with a non-polar solvent, only non-polar molecules in the sample dissolve while polar ones do not. Problems arise in cases where lipids are bound in animal or plant cell membranes. Animal and plant cell membranes are made up of molecules that have both polar and non polar regions such as triglycerides (molecule with polar glyceride heads and non-polar fatty acid tails) and phospholipids (similar to triglycerides, but a phosphate group replaces the fatty acid tail). These molecules end up grouping together with their polar heads sticking outwards and non-polar tails inwards making it difficult for non-polar solvents to interact with the non-polar tails and extract them. As these molecules are part non-polar and part polar, we need a method, such as LF-NMR method, that is able to directly measure both phospholipids and triglycerides.

NMR Instrument

NMKL IS ELABORATING A NMR METHOD FOR FAT IN FISH AND FISH FEED

Department manager Päivi Teivainen-Lædre, Skretting ARC laboratory has elaborated and arranged interlaboratory validation studies for the determination of fat by NMR. At a project meeting held 22nd November 2013 in Copenhagen, data from a pre-validation study (2012) and the validation study (2013) were discussed. In the studies, there were participants from the Nordic countries, Germany and UK, with representatives from research laboratories, instrument suppliers and industry. The results were satisfactory and it is expected that NMR fat method for quantification of fat in feed and fish will be approved as NMKL method in 2014.

There has also been conducted a comparison studies between NMR spectroscopy and Soxhlet extraction for the determination of fat in fish feed with different fat contents. Current results show that NMR offers better precision than the Soxhlet extraction method (as discussed on page 4).

Finally, since NMR is faster, requires less sample handling and is more robust with regard to the structure of samples, it is considered as the most suitable of the two techniques for routine measurements of fat, according to the Referee Päivi Teivainen-Lædre.

The final conclusion of method-performance of NMR and proficiency testing and the method procedure will be published in 2014, and hopefully this will contribute to solve the problems and uncertainties concerning fat determination in fish, feed and raw materials.

Picture : NMKL NMR fat method project group. From left Anna Hesby Nessa (Skretting ARC), Cecilia Rumin (Eurofins), Päivi Teivainen-Lædre (Skretting ARC), Kevin Nott (Oxford Instruments), Hilde Skår Norli (NMKL), Harald Todt (Bruker BioSpin), Heiða Pálmadóttir (Matís ltd.), Astrid Nordbotten (Norwegian Food Safety Authority) and Frank Lundby (Nofima).

News from NordVal International

NordVal International has renewed the following certificates:

No. 022 RAPID'*L.MONO* from Bio-Rad Laboratories

The method is applicable for the detection and the enumeration of *Listeria monocytogenes* and for the detection of other *Listeria* species in food and environmental samples. The principle of the RAPID'*L. mono* medium relies on the chromogenic detection of the *Listeria monocytogenes* phosphatidylinositol-specific phospholipase C (PIPLC) and on the inability of these species to metabolise xylose. After 24 ± 2 hours of incubation, *Listeria monocytogoenes* forms characteristics blue (pale blue, grey blue to dark blue) colonies without a yellow halo. Colonies formed by other species of *Listeria* are white, with or without a yellow halo. The particularity of *Listeria ivanovii* species, infrequently found in food matrices, should be noted: it presents blue-green colonies with a yellow halo (xylose positive character). This halo can appear after 24 to 48 hours of incubation. The selective mixture in the medium allows the inhibition of most interfering flora (Gram- positive and Gram-negative bacteria, yeast and mould). Thus RAPID*' L mono* detects *Listeria monocytogenes* in 24 hours and other *Listeria* species in 24 and 48 hours.

The method has been compared against ISO 11290-1(1996/ amendment 2004): Food microbiology - Horizontal method for the detection and enumeration of *Listeria monocytogenes* -- Part 1: Detection method and Part 2: Enumeration method. NordVal International has concluded that it has been satisfactorily demonstrated that the requirements of the NordVal validation protocol are fulfilled for RAPID' *L. mono,* there are no statistical differences in the performances of *RAPID' L. mono* and the reference method for the detection and the enumeration of *Listeria monocytogenes* and the detection of *Listeria spp.* in foods and environmental samples.

No. 026 **food**proof® *E.coli* O157 Detection Kit in combination with **food**proof® ShortPrep II Kit from Biotecon Diagnostics GmbH

The **food**proof® *E.coli* O157 Detection Kit in combination with **food**proof® ShortPrep II Kit is intended for the detection of *E.coli* O157 DNA isolated from enrichment cultures prepared by various valid methods inoculated with food samples that are potentially contaminated with *E.coli* O157. The method is applicable for foods.

The method has been compared against EN ISO 16654:2001: Microbiology of food and animal feeding stuffs -- Horizontal method for the detection of *Escherichia coli* O157**.** The results document no statistical difference in the performances between the **food**proof® *E.coli* O157 Detection Kit in combination with **food**proof® ShortPrep II Kit and the reference method for the detection of *Escherichia coli* O157. NordVal International has concluded that it has been satisfactorily demonstrated that the requirements for the sensitivity and the agreement between the methods are fulfilled, further, that confirmation of obtained positives are not necessary.

Number	Available NMKL Procedures / Guides
1	Calibration and performance checking of laboratory balances
3	Control charts and control materials in internal quality control in food chemical laboratories
4	Validation of chemical analytical methods
5	Estimation and expression of measurement uncertainty in chemical analysis
6	Guide in quality assurance of sensory laboratories (Available in Danish and Finnish only)
7	Checking of UV/VIS spectrophotometers
8	Measurement of uncertainty in quantitative microbiological examination of foods
9	Evaluation of method bias using certified reference materials
10	Control of microbiological media
11	Procedure for sensory analysis of drinking water
12	Guide on sampling for analysis of foods
13	Volumetric control
14	SENSVAL: Guidelines for internal control in sensory analysis laboratories
16	Sensory quality control.
17	Guidelines for requirement specifications for food analyses
18	The use of reference materials, reference strains and control charts in a food microbiological laboratory
19	Guideline for sensorial Analysis of Food containers/packages
20	Evaluation of results from qualitative methods
21	Guide for sensory analysis of fish and shellfish
22	Considerations regarding evaluation of immunochemical test kits for food analysis
23	Guide on quality assurance in microbiological laboratories
24	Guidelines fro quality assurance for food chemical laboratories
25	Recovery information in analytical measurement
26	Control and internal calibration of thermometers and temperature control on microbiological laboratories
27	Measurement uncertainty in sensory analysis

Joint InterAgencyMeeting/MoniQA Workshop

SAMPLING: NEW APPROACHES IN VIEW OF CODEX STANDARDS

2 March 2014 **13:00-17:00**

Budapest, Hungary

Just prior to the CCMAS Meeting

DRAFT PROGRAMME:

13:00	Registration
13:30	Opening on behalf of IAM and MoniQA Richard Cantrill, USA and Roland Poms, Austria
13:40	Introduction to sampling issues including an outline of acceptance sampling Roger Wood, United Kingdom
14:10	Sampling commodity goods and interpretation of the compliance with maximum limits from the producer/exporter and the competent authority/importer sides Arpad Ambrus, Hungary
14:40	Sampling uncertainty Steve Ellison, United Kingdom
15:10	Sampling based on the NMKL Guide Astrid Nordbotten, Norway
15:40	Break
16:00	NZ joint industry-government programme project on sampling Roger Kissling, New Zealand
16:30	How does this meet Codex requirements? Future of sampling? Roger Wood, United Kingdom
17:00	Close

VENUE: The Aquincum Hotel Budapest • Árpád fejedelem útja 94. • Budapest 1036, Hungary
www.aquincumhotel.com

FEES:

Free:	IAM members, CCMAS delegates, speakers
Regular:	250 Euro
Discounts:	120 Euro for MoniQA Association Members and Students

Registration to: moniqa@moniqa.org

InterAgencyMeeting, IAM, is a forum for international organisations (such as AOAC, AOCS, IDF, ISO, NMKL) working in the area of analytical methods and quality assurance guides.

The organisations meet annually in connection with the meeting in the Codex Committee on Methods of Analysis and Sampling, CCMAS. For several years IAM has arranged a workshop in connection with the meetings.