codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

JOINT OFFICE: Viale delle Terme di Caracalla 00100 ROME Tel: 39 06 57051 www.codexalimentarius.net Email: codex@fao.org Facsimile: 39 06 5705 4593

Agenda Item 9 **CX/MAS 01/10**

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON METHODS OF ANALYSIS AND SAMPLING
Twenty-Third Session
Budapest, Hungary, 26 February - 2 March 2001

ENDORSEMENT OF METHODS OF ANALYSIS PROVISIONS IN CODEX STANDARDS

Appendix I of this document contains Part I .Methods of analysis and Part II. Methods of Sampling proposed by the following Committees (adopted standards or standards under elaboration)

A. Codex Committee on Sugars

B. Codex Committee on Fish and Fishery Products

C. Codex Committee on Milk and Milk Products

D. Codex Committee on Cocoa Products and Chocolate

E. Codex Coordinating Committee for Asia

F. Codex Committee on Soups and Broths

G. Codex Committee on Vegetable Protein Products

Appendix II contains other methods of analysis and sampling temporarily endorsed by the last session or previous sessions of the CCMAS, and related matters requiring further consideration. They are submitted to the Committee for review of their status

A. General matters
- Method for the Determination of Copper
- Method for Determination of Lead, Cadmium, Zinc, Copper, and Iron

B. Codex Committee on Soups and broths
C. Codex Committee on Nutrition and Foods for Special Dietary Uses
D. Codex Committee on Processed Fruits and Vegetables

Appendix I

PART I . METHODS OF ANALYSIS FOR COMMODITY STANDARDS SUBMITTED FOR ENDORSEMENT

A. CODEX COMMITTEE ON SUGARS

COMMODITY	PROVISION	METHOD	PRINCIPLE	Note	Type	Status
Sugars (white sugar, plantation or mill white sugar, soft white sugar, soft brown sugar, powdered sugar, powdered dextrose, raw cane sugar)	Sulphur dioxide	ICUMSA GS 2-33 (1994)	Rosaniline procedure	Proposed as Type III, on addition to the Type II and Type IV methods already endorsed by CCMAS		
Sugars (powdered sugar)	Polarisation	ICUMSA (1994) GS 3-21	Polarimetry	Not endorsed by the last CCMAS[1]		
Sugars (fructose)	Conductivity ash	ICUMSA GS 2/3-17 (1994)	Conductimetry	As recommended by the last CCMAS.		
Sugars (soft sugars, brown sugar)	Invert sugar	ICUMSA GS 2/3-5 (1997)	Titrimetry (Lane & Eynon)	Replaces ICUMSA GS 4/3-3 (1994) (endorsed as Type I)		
Sugars (plantation or mill white sugar)	Invert sugar	ICUMSA GS 2-6 (1998)	Titrimetry	Replaces ICUMSA GS 1/3/7 (1994) (endorsed as Type I)		
Sugars (soft white sugar, powdered sugar, plantation or mill white sugar)	Colour	ICUMSA GS 2-10 (1998)	Photometry	Replaces ICUMSA GS 2/3-9 (1994) (endorsed as Type I)		
Honey[2]	Sample preparation	AOAC 920.180				
Honey	Moisture content	AOAC 969.38B MAFF validated method V21	Refractometry			
Honey	Fructose and Glucose (sum of both)	Harmonised method of the EHC	HPLC	Apidologie, Special Issue 28, 1997, Chapter 1.7.2		
Honey	Sucrose content	Harmonised method of the EHC	HPLC	Apidologie, Special Issue 28, 1997, Chapter 1.7.2		
Honey	Sugars added: for sugar profile	AOAC 991.41	Carbon isotope ratio mass spectrometry			
Honey	Water-insoluble solids content	MAFF validated method V22	Gravimetry			

[1] The CCS agreed to maintain the method, after filtration if necessary to remove any anti-caking agent, with a footnote “Care needs to be taken if anti-caking agents such as starch are present as the method may then not be appropriate”

[2] Literature references concerning methods are included in Annex 1, following the request of the CCS that they should be considered by CCMAS

COMMODITY	PROVISION	METHOD	PRINCIPLE	Note	Type	Status
Honey	Electrical conductivity	Harmonised method of the EHC		Apidologie, Special Issue 28, 1997, Chapter 1.2		
Honey	Acidity	MAFF Validated method V19	Titrimetry			
Honey	Diastase activity	Phadebas – Harmonised method of the EHC[3]		In addition to AOAC 958.09 endorsed as Type II		
Honey	Hydroxymethylfurfural	Harmonised method of the EHC	HPLC			

B. CODEX COMMITTEE ON FISH AND FISHERY PRODUCTS

1. Methods referred back to CCCFFP

Quick Frozen Fish Sticks (fish fingers) and Fish Portions-Breaded and in Batter(except for certain fish species with soft flesh)	Proportion of fish fillet and minced fish	WEFTA Method	Gravimetry	The CCFFP confirmed its decision to use the WEFTA method with the amendment concerning soft flesh fish (amendment endorsed by CCMAS)		NE
Quick Frozen Fish Sticks (fish fingers) and Fish Portions-Breaded and in Batter	Proportion of fish flesh in fish sticks (fish core)	AOAC 996.15 (with an adjustment factor of 2% for raw breaded and batter-dipped products; 4% for precooked products)	Gravimetry	AOAC 996.15 is a modified method of AOAC 971.13 which was endorsed previously. The CCFFP confirmed the applicability of the adjustment factors.	I	
Salted Fish of the *Gadidae* Family	Salt	See Annex II	Titrimetry (Mohr)	The CCFFP confirmed the applicability of the method to salted fish		

2. Methods proposed in standards under elaboration

Salted Atlantic Herring	Histamine	AOAC 977.13	Fluorimetry	Method endorsed for Quick Frozen Fish Sticks in 1995		

Note: Determination of histamine with AOAC 977.13 appears in the standards adopted in 1995 for the following products:
- Quick Frozen Fish Fillets
- Quick Frozen Blocks of Fish Fillets, Minced Fish Flesh and Mixtures of Fish Fillets and Minced Fish Flesh
- Quick Frozen Finfish, Uneviscerated and Eviscerated
- Canned Finfish
- Canned Sardines and Sardine Type Products

[3] In the CCS, the Delegation of Italy indicated that Phadebas method was only suitable for honeys with a diastase activity between 6 and 40 Shade Unit.

- Quick Frozen Fish Sticks

However, there is a record of endorsement only for the Standard the Quick Frozen Fish Sticks in 1995 (ALINORM 97/23, Appendix IV).

Since this method applies in all standards for fish and fishery products where histamine is an essential provision, the Committee is invited to consider the possibility of endorsing the method for "fish and fishery products" in general.

C. CODEX COMMITTEE ON MILK AND MILK PRODUCTS

1. Methods of analysis referred back to CCMMP (adopted standards)

COMMODITY	PROVISION	METHOD	PRINCIPLE	NOTE
Cheese	Moisture Solids	IDF Standard 4A:1982 ISO 5534:1985	Gravimetry, drying at 102 °C	[4]
Cheeses in Brine	Dry matter	IDF Standard 4A:1982 ISO 5534:1985	Gravimetry, drying at 102 °C	[4]
Sweetened Condensed Milks	Solids	IDF Standard 15B:1991 ISO 6734:1989	Gravimetry, drying at 102 °C	[5]
Milkfat Products	Peroxide value (expressed as milliequivalents of oxygen/kg fat)	AOAC 965.33	Titrimetry	Endorsed for anhydrous milkfat.[6]
Evaporated Milks Sweetened and Condensed Milks	Protein	AOAC 945.48H AOAC 991.20 – IDF 20B:1993	Kjeldahl, titrimetry	AOAC 945.48H was endorsed by CCMAS[7]
Milkfat Products Butter	Copper	IDF Standard 76A:1980/ISO 5738:1980/AOAC 960.40	Photometry, diethyldithiocarbamate	Not endorsed. CCMP was requested to consider applicability to high fat products and consider the applicability of AOAC 999.05[8]

[4] The CCMMP recognized that only one Type I method could be endorsed and recommended IDF Standard 4A:1982/ ISO 5534:1985 which used drying at 102 °C

[5] The CCMMP recommended IDF Standard 15B:1991/ ISO 6734:1989 which also used drying at 102 °C (see above note)

[6] The CCMMP agreed that it was applicable also to milkfat, butteroil, ghee, and anhydrous butteroil

[7] The CCMMP agreed that AOAC 945.8H (endorsed) referred to AOAC 991.20 for Kjeldahl determination and this was identified with IDF 20B:1993, which is currently under revision and being validated for the determination of total nitrogen in cheese

[8] The CCMMP reported back that IDF Standard 76A:1980/ISO 5738:1980/AOAC 960.40 was capable of determining levels as low as 0.05mg/kg of copper in butter and milkfat. No comment was made on the applicability of AOAC 999.05.

COMMODITY	PROVISION	METHOD	PRINCIPLE	NOTE
Whey Cheese	Dry matter (for denomination)	IDF Standard 58:1970 (confirmed 1993) ISO 2920:1974	Gravimetry, drying at 88± 2 °C	CCMAS noted that "CCMMP may wish to consider if the provision can be handled by other methods." No other proposals were made by CCMMP.
Whey Powders	Lactose	IDF 79B:1991/ ISO/DIS 5765	Enzymatic method: glucose moiety (method A), galactose moiety (method B)	CCMMP was asked to indicate which method (A or B) it preferred. It agreed that methods A and B were complementary

2. Methods of analysis proposed for standards under elaboration (advanced to Step 5 or 8)

Cheeses, individual	Dry matter (specified in individual standards)	IDF Standard 4A:1982 ISO 5534:1985	Gravimetry, drying at 102 °C
Cheeses, individual	Milkfat in dry matter	IDF Standard 5B:1986 ISO 1735:1987 AOAC 933.05	Gravimetry (Schmid-Bonzynski-Ratzlaff)
Creams, Whipped Creams and Fermented Creams	Milk solids-not-fat	IDF Standard 80:1977 ISO 3727:1977 AOAC 920.116	Gravimetry
Creams, Whipped Creams and Fermented Creams	Milk solid-not-fat	IDF Standard 11A:1986	Gravimetry
Cream	Milkfat	IDF Standard 16C:1987 ISO 2450:1999 AOAC 995.19	Gravimetry
Creams Lowered in Milkfat Content	Milkfat	IDF Standard 16C:1987 ISO 2450:1999 AOAC 995.19	Gravimetry
Edible Casein Products	Casein in protein	IDF Standard 29:1964	Titrimetry, Kjeldahl
Fermented Milks	Lactic acid	IDF Standard 150:1991 ISO 11869:1997	Potentiometry
Fermented Milks	Lactic acid	AOAC 937.05	Spectrophotometric (for lactate acid in milk & milk products)

Fermented Milks	Protein	IDF Standard 20B:1993 ISO DIS 8968 AOAC 991.20-23	Titrimetry (Kjeldahl)
Fermented Milks	Dairy starter cultures of lactic acid bacteria (LAB)	IDF Standard 149A:1997 (Annex A)	Colony count at 25 °C, 30 °C, 37 °C & 45 °C according to the starter organism in question
Fermented Milks (Yoghurt)	*Streptococcus thermophilus* & *Lactobacillus delbrueckii* subsp. Bulgaricus $>= 10^7$ cfu/g	IDF Standard 117B:1997 ISO DIS 7889	Colony count at 37°C
Fermented Milks (Yoghurt)	*Streptococcus thermophilus & Lactobacillus delbrueckii* subsp.bulgaricus $>= 10^7$ cfu/g	IDF Standard 146:1991 ISO CD 9232	Test for identification
Milk Products obtained from Fermented Milks Heat-Treated after Fermentation	Protein	IDF Standard 20B:1993 ISO DIS 8968 AOAC 991.20-23	Titrimetry (Kjeldahl)
Unripened Cheese Including Fresh Cheese	Dry matter [not decided (unripened/fresh cheese)] >= 3.5 % (cream cheese)	IDF Standard 4A:1982 ISO 5534:1985	Gravimetry, drying at 102 °C
Unripened Cheese Including Fresh Cheese	Dry matter [not decided (unripened/fresh cheese)] >= 35 % (m/m), < Restricted by the MMFB	IDF Standard 4A:1982 ISO 5534:1985	Gravimetry, drying at 102 °C
Unripened Cheese Including Fresh Cheese	Dry matter [not decided (unripened/fresh cheese)] >= 3.5 % (cream cheese)	AOAC 926.08	Gravimetry, vacuum oven
Unripened Cheese Including Fresh Cheese	Protein	IDF Standard 20B:1993 ISO DIS 8968 AOAC 991.20/920.123	Titrimetry, Kjeldahl

D. CODEX COMMITTEE ON COCOA PRODUCTS AND CHOCOLATE

COMMODITY	PROVISION	METHOD	PRINCIPLE	Note
Cocoa (Cacao) Mass, Cocoa/Chocolate (liquor) and Cocoa Cake	Fat content	AOAC 963,15 or IOCCC 14 (1972)		
Cocoa powders (cocoas) and dry mixtures of cocoa and sugars	Cocoa butter	ICCC 37 (1990) and IOCCC 14 (1972)	Total fat and total sterol content by GLC	IOCCC 14 (1972) already endorsed
Chocolate and Chocolate Products	Copper	AOAC 971.20	Atomic absorption Spectrophotometry	See Appendix 2

E. CODEX COORDINATING COMMITTEE FOR ASIA

Aqueous Coconut Products	Total solids	AOAC 925.23A (IDF-ISO-AOAC method)	Gravimetry	
Aqueous Coconut Products	Total Fats	AOAC 945.48G	Gravimetry (Röse-Gotlieb method)	

F. CODEX COMMITTEE ON SOUPS AND BROTHS

Soups and Broths	Creatinine	AIIBP Method 2/5 (2000)	HPLC	See Appendix II
Soups and Broths	Creatinine	AOAC 920.115	Colorimetry	
Soups and Broths	Total Nitrogen	AOAC 928.08	Kjeldahl	
Soups and Broths	Sodium Chloride	AIIBP No. 2/4	Titrimetry	Temporarily endorsed in 1994 as Type III See Appendix II

G. CODEX COMMITTEE ON VEGETABLE PROTEINS

Wheat Protein Products	Vital wheat gluten and devitalized wheat gluten	AOAC 979.09 (Wheat protein in grain N x 5.7)	Kjeldahl
Wheat Protein Products	Solubilized wheat protein	AOAC 920.87 (wheat protein in flour N x5.7)	Kjeldahl

PART II. METHODS OF SAMPLING IN COMMODITY STANDARDS SUBMITTED FOR ENDORSEMENT

A. COMMITTEE ON MILK AND MILK PRODUCTS

- Creams, Whipped creams and Fermented Creams - Fermented Milks	Sampling	IDF Standard 50C:1995 ISO 707:1997 AOAC 968.12	General instructions

Sampling of Cheese in Brine
(Amendment at Step 3 of the Accelerated Procedure)

Amend Section 8.1 of the Codex Standard for Cheeses in Brine (CODEX STAN 208-1999) concerning sampling as follows (struck-out text to be deleted):

8.1 Sampling

According to IDF Standard 50C:1995/ISO 707:1997/AOAC 933.12.

Special requirements for cheese in brine: A representative piece of cheese is placed on a cloth or on a sheet of ~~non-~~absorbent paper for 5 to 10 min. A slice of 2-3 cm is cut off and sent to the laboratory in a sealed insulated box for analysis.

B. COMMITTEE ON VEGETABLE PROTEINS

Draft Standard for Wheat Protein Products
Sampling: ISO 2170: 1980

APPENDIX II

METHODS OF ANALYSIS CONSIDERED AT PREVIOUS SESSIONS OF THE COMMITTEE AND RELATED MATTERS

A. GENERAL QUESTIONS

1. Determination of Copper

As stated in the Report of the last session of CCMAS (ALINORM/23 para 61), the AOAC method for copper 971.20: Codex General Method for Copper, needs to be replaced since the method was only validated for tea.

AOAC recommends to replace this method by AOAC Official method 999.11, Lead, Cadmium, Copper, Iron and Zinc in Foods, AAS after dry ashing. This method was also adopted (originally) by NMKL.

The text of the method is attached as Annex VI (not available in electronic version).

2. Determination of Lead, Cadmium, Zinc, Copper, and Iron (proposed by the Committee on Additives and Contaminants)

The 31st Session of the Committee on Additives and Contaminants agreed to forward the NMKL method for the determination of lead, cadmium, zinc, copper and iron to the CCMAS for consideration (NMKL method). The method is attached as Appendix V.

B. METHODS OF ANALYSIS PROPOSED BY SPECIFIC COMMODITIES

1. CODEX COMMITTEE ON SOUPS AND BROTHS - STANDARD FOR SOUPS AND BROTHS

Determination of sodium chloride

The 19th Session of the CCMAS (1994) temporarily endorsed the AIIBP Method N0.2/4 for sodium chloride in Soups and Broths and the following note appears in Appendix IV, ALINORM 95/23
"The Working Group agreed to retain the status of the method for lack of additional information."

No additional information was presented to the Committee since the 19th Session.

The following justification and the revised AIIBP method for the determination of sodium chloride (1998) were provided by the Secretariat of the host country (Switzerland) since the Committee is working by correspondence.

8.5 Determination of Sodium Chloride: 8.5.1 Justification for the Method 2/4 of the AIIBP Official Collection of Methods of Analysis, Revision 1998 (Salt Concentration)

This method has specific provisions for sample preparations of bouillons, soups and sauces. Since the Proposed Draft Revised Codex Standard for Bouillons and Consommés deals with such products, it is, in our opinion, important to include this critical step in a method. The second step of determination of the analyte is similar to the more general AOAC method although certain steps are clarified in order to properly reflect the scope of the method. The Codex Standard method (AOAC 971.27) is tuned for canned vegetables and has to be adjusted in several aspects before it can be applied to dehydrated products.

AIIBP has constantly worked for the advancement of analytical procedures within its range of interest (i.e. for bouillons, soups, sauces). During the 1990's the method for the determination of salt, which is based on potentiometric titration of chloride, was reviewed, expanded and tested in a collaborative study. While the potentiometric titration is described in many standards (including AOAC 971.27), the sample preparation for the widely-used dehydrated products (bouillon cubes, dehydrated soups and gravies) is poorly described.

The method provided herein (AIIBP 2/4, Revision 1998) deals extensively with the sample preparation of dehydrated products. While the provisional method, as referred to in the current Codex Proposal ALINORM 01/29, requests the ashing of the material, the new method allows for direct preparation of the

analyte solution for potentiometric titration from the products sold to the consumer. The part on analyte determination includes provisions for manual evaluation of the measured data and also reflects advances in instrumentation where certain calculations are performed electronically by the instrument.

The Codex Standard method for the determination of Salt (AOAC 971.27) should also be retained. It is best applied to products presented to the consumer in liquid form (e.g. canned bouillons) since its main scope is canned vegetables. As such, many sections in the sample preparation scheme are not directly applicable to dehydrated products.

The complete text of the method is attached as Appendix III.

Determination of Creatinine

The following justification and new AIIBP method for the determination of creatinine were provided by the Secretariat of the host country (Switzerland).

(New) 8.2.1 According to the new Method 2/5, Revision 2000, HPLC of the *AIIBP Official Collection of Methods of Analysis.*

The old method should be replaced as it is no longer reliable. Furthermore, comments received from Mexico (Sections 2.1.2 y 2.1.3) also support the view that the currently referenced method is no longer suitable.

The currently referenced method (ALINORM 01/29) for the determination of Creatinine in meat extracts, bouillons and soups (AIIBP 2/5, Hadorn Method) uses a colorometric reaction between creatinine and picrate and subsequent measurement of absorption in the visible light range. This method is not used any more in most laboratories in the world. It is tedious to operate and, importantly, may lead to false positive results or elevated values due to interferences with constituents from the sample.

AIIBP has continuously worked on the advancement of analytical procedures within its range of interest (i.e. bouillons, soups, sauces). During the 1990s a method for the determination of creatinine based on HPLC principles was elaborated, pre-tested among selected laboratories and tested in a collaborative study. The results of these activities were good and were beyond expectations. This method, besides describing the full analytical procedure with sample preparation, focuses on measures for internal quality assurance and control procedures.

The result of this collaborative study was intended to be published in collaboration with AOAC. Unfortunately the administrative procedure within AOAC did not allow for a timely inclusion into the Method book of AOAC. AIIBP therefore decided in fall 2000 to publish this method in its own collection of methods as a replacement for the current Method 2/5.

The complete text of the method is attached as Appendix IV.

2. COMMITTEE ON NUTRITION AND FOODS FOR SPECIAL DIETARY PURPOSES

COMMODITY	PROVISION	METHOD	PRINCIPLE	Note
Guidelines for Nutrition Labelling	Saturated fat	AOAC 996.06	Gas liquid chromatography	AOAC is requested to clarify whether the method is applicable to the determination of polyunsaturated fat.

3. Codex Committee on Processed Fruits and Vegetables

Pickles	Benzoic acid	ISO 5518:1978	Spectrophotometry	The Commodity Committee is requested to review more modern methods such as the liquid chromatographic method IFU 63 (1995) or the gas chromatographic method NMKL 103 (1984)/AOAC 983.16 which has been endorsed as a Type II Codex general method.	IV	TE
Pickles	Lead	ISO 6633:1984	Flameless atomic absorption spectrophotometry		IV	TE
Pickles	Sorbates	ISO 5519:1978	Spectrophotometry	The Commodity Committee is requested to review more modern methods such as the liquid chromatographic method IFU 63 (1995) or the gas chromatographic method NMKL 103 (1984)/AOAC 983.16 which has been endorsed as a Type II Codex general method.	IV	TE
Pickles	Sulphur dioxide	ISO 5522:1981	Titrimetry followed by: Gravimetry (high levels) Nephelometry (low levels)	The Commodity Committee is requested to review the Optimized Monier-Williams method (AOAC 990.28), which has been endorsed as a Type II Codex general method.		NE
Pickles	Sulphur dioxide	ISO 5523:1981	Colorimetry	See above		NE
Pickles	Tin	ISO 2447:1974		The Commodity Committee is asked to consider whether it is necessary to express the provision using four significant figures.	IV	TE

The CCPFFV did not discuss the methods section in the standard for pickles or provide further information concerning the questions raised by CCMAS on the above methods. As the Draft Standard for Pickles was returned to Step 6, it is expected that the CCPFFV will have the possibility to finalize the section on methods at its next session.

ANNEX I

CODEX COMMITTEE ON SUGARS
DRAFT REVISED STANDARD FOR HONEY[9]

2.3. Literature references[10]

Bogdanov S, Honigdiastase, Gegenüberstellung verschiedener Bestimmungsmethoden, *Mitt. Gebiete Lebensmitt. Hyg.* **75**, 214-220 (1984)

Bogdanov S and Lischer P, Interlaboratory trial of the European Honey Commission: Phadebas and Schade Diastase determination methods, Humidity by refractometry and Invertase activity: Report for the participants 1993.

Chataway HD (1932) *Canad J Res* 6, 540; (1933) *Canad J Res* 8, 435; (1935) *Canad Bee J* 43, (8) 215.

DIN-NORM 10750 (July 1990): Bestimmung der Diastase-Aktivität.

DIN. Norm, Entwurf: Bestimmung des Gehaltes an Hydroxymethylfurfural: Photometrisches Verfahren nach Winkler (1990)

Determination of Diastase with Phadebas, *Swiss Food Manual*, Chapter 23A, Honey, Bern, 1995.

Figueiredo V, HMF Interlaboratory Trial, Report for the participants, Basel canton chemist laboratory, (1991)

Jeurings J and Kuppers F, High Performance Liquid Chromatography of Furfural and Hydroxymethylfurfural in Spirits and Honey. *J. AOAC*, 1215 (1980).

Determination of Hydroxymethylfurfural by HPLC, *Swiss Food Manual*, Kapitel Honig, Eidg. Druck und Materialzentrale 1995

International Honey Commission Collaborative Trial (in press).

Hadorn H (1961) *Mitt Gebiete Lebens u Hyg*, 52, 67.

Kiermeier F, Koberlein W (1954) *Z Unters Lebensmitt*, 98, 329.

Lane JH and Eynon L (1923) *J Soc Chem Ind* 42, 32T, 143T, 463T.

Schade J. E., Marsh G. L. and Eckert J. E.: Diastase activity and hydroxymethylfurfural in honey and their usefulness in detecting heat adulteration. *Food Research* 23, 446-463 (1958).

Siegenthaler U, Eine einfache und rasche Methode zur Bestimmung der α-Glucosidase (Saccharase) im *Honig. Mitt. Geb. Lebensmittelunters. Hyg*. 68, 251-258 (1977).

Turner JH, Rebers PA, Barrick PL and Cotton RH (1954) *Anal Chem*, 26, 898.

Walker HS (1917) *J Ind Eng Chem*, 2, 490.

Wedmore EB (1955), *Bee World*, 36, 197.

White JW Kushnir I and Subors MH (1964) *Food Technol*, 18, 555.

FW (1959) *JAOAC*, 42, 344.

White J, Spectrophotometric Method for Hydroxymethylfurfural in Honey. *J. AOAC*, 509 (1979).

Winkler O: Beitrag zum Nachweis und zur Bestimmung von Oxymethylfurfural in Honig und Kunsthonig. *Z. Lebensm. Forsch.* **102**, 160-167 (1955)

Harmonised methods of the European Honey Commission, Apidologie - special issue, **28**, 1997

[9] ALINORM 01/25, Appendix II

[10] CCS asked CCMAS to consider retaining only those essential references.

CODEX STANDARD FOR SALTED FISH AND DRIED SALTED FISH OF THE *GADIDAE* FAMILY

7.4 Determination of salt content[11]

1. Principle

The salt is extracted by water from the preweighed sample. After the precipitation of the proteins, the chloride concentration is determined by titration of an aliquot of the solution with a standardized silver nitrate solution (Mohr method) and calculated as sodium chloride.

2. Equipment and chemicals

- Brush
- Sharp knife or saw
- Balance, accurate to ±0.01 g
- Calibrated volumetric flasks, 250 ml
- Erlenmeyer flasks
- Electric homogenizer
- Magnetic stirrer
- Folded paper filter, quick running
- Pipettes
- Funnel
- Burette

- Potassium hexacyano ferrate (II), $K_4Fe(CN)_6 \cdot 3H_2O$, 15% w/v (aq)
- Zinc sulphate, $ZnSO_4 \cdot 6H_2O$, 30% w/v (aq)
- Sodium hydroxide, NaOH, 0.1 N, 0.41% w/v (aq)
- Silver nitrate, $AgNO_3$, 0.1 N, 1.6987% w/v (aq), standardized
- Potassium chromate, K_2CrO_4 5% w/v (aq)
- Phenolphthalein, 1% in ethanol
- distilled or deionized water

3. Preparation of sample

Before preparing a subsample adhering salt crystals should be removed by brushing from the surface of the sample without using water.

The entire sample should be subjected to a systematic cutting and randomization process to assure a subsample representative of the composition of the whole fish or fishery product.

At least 100 g of subsample should be thoroughly homogenized by using an electric homogenizer.

Determination should be performed at least in duplicate.

4. Procedure

(i) Five gram of homogenised subsample is weighted into a 250 ml volumetric flask and vigorously shaken with approximately 100 ml water.

(ii) Five millilitre of potassium hexacyano ferrate solution and 5 ml of zinc sulphate solution are added, the flask is shaken.

(iii) Water is added to the graduation mark.

(iv) After shaking again and allowing to stand for precipitation, the flask content is filtered through a folded paper filter.

(v) An aliquot of the clear filtrate is transferred into an Erlenmeyer flask and two drops of phenolphthalein are added. Sodium hydroxide is added dropwise until the aliquot takes on a faint red colour. The aliquot then diluted with water to approximately 100 ml.

(vi) After addition of approximately 1 ml potassium chromate solution, the diluted aliquot is titrated under constant stirring, with silver nitrate solution. Endpoint is indicated by a faint, but distinct, change in colour. This faint reddish-brown colour should persist after brisk shaking.

[11] Elaborated by a Norwegian/German Working Group.

To recognize the colour change, it is advisable to carry out the titration against a white background.

(vii) Blank titration of reagents used should be done.

(viii) Endpoint determination can also be made by using instruments like potentiometer or colorimeter.

5. Calculation of results

In the equation of the calculation of results the following symbols are used:

A= volume of aliquot (ml)
C= concentration of silver nitrate solution in N
V= volume of silver nitrate solution in ml used to reach endpoint and corrected for blank value
W= sample weight (g)

The salt content in the sample is calculated by using the equation:

Salt concentration (%) = (V x C x 58.45 x 250 x 100) / (A x W x 1000)

Results should be reported with one figure after the decimal point.

6. Reference method

As reference method a method should be used which includes the complete ashing of the sample in a muffle furnace at 550°C before chloride determination according to the method described above (leaving out steps (ii) and (iv)).

7. Comments

By using the given equation all chloride determined is calculated as sodium chloride. However it is impossible to estimate sodium by this methodology, because other chlorides of the alkali and earth alkali elements are present which form the counterparts of chlorides.

The presence of natural halogens other than chloride in fish and salt is negligible.

A step, in which proteins are precipitated (ii), is essential to avoid misleading results.

ANNEX III

DRAFT STANDARD FOR SOUPS AND BROTHS - DETERMINATION OF SODIUM CHLORIDE

Method 2/4 of the AIIBP Official Collection of Methods of Analysis, Revision 1998

Analytical Procedure: Status of Method:

Potentiometric Titration Final

PURPOSE OF DETERMINATION

Determination of salt concentration in savoury products by means of the chemical determination of chloride and consecutive stoichiometric calculations.

SCOPE

Applicable to bouillons, soups and seasonings with salt concentrations >1 %.

PRINCIPLE

The salt concentration of a product is calculated from the chloride concentration in a solution as determined by potentiometric titration with silver nitrate.

REFERENCE METHOD

- AOAC Official Methods of Analysis, 15th Ed.(1990) Methods 32.034 - 32.039

VALIDATION

The method has been validated in a collaborative study with 12 laboratories participating. The method has been applied to 12 samples from commercial production with salt concentrations between 70 g/kg to 500 g/kg. The 12 samples represent 6 different products For each product, two different production batches were analysed.
The statistical preevaluation of the submitted results suggested a clustering into three different data sets for the validation calculations:

Data Set 1: 4 different samples (one production batch of each product) with salt concentrations between 70g / kg and 440g / kg.

Data Set 2: 5 different samples (the second production batch of each product) with salt concentrations between 70g / kg and 440g / kg.

Data Set 3: 2 samples (two batches of one product) with salt concentrations of around 500g / kg and high fat content.

Results:	**Repeatability**	**Reproducibility**
Data Set 1	0.20	0.30
Data Set 2	0.27	0.32
Data Set 3	0.88	1.25

The complete description of samples and the data evaluation is given in Appendix I.

QUALITY ASSURANCE

REFERENCE MATERIALS - **SEE SECTION „CHEMICALS“**

TESTS DURING ANALYSIS

The determination is verified by the following tests during the analytical procedure.

GENERAL:

Per sample two determinations are performed.

QUALITY CONTROL

Molarity of silver nitrate solution **Interval: weekly**

The molarity of the 0.1M titration solution should be determined periodically. The molarity must not be determined if it is the first use of a freshly prepared titration solution. The test is performed with sodium chloride.

DOCUMENTATION

Calibration Data: Molarity of silver nitrate solution
(see, MEASUREMENT OF THE SILVER NITRATE MOLARITY; for formula see, CALCULATION OF SILVER NITRATE MOLARITY)

Weight of sodium chloride	Volume of $AgNO_3$ Solution	Molarity of $AgNO_3$ Solution	Date of Calibration	Remarks
$\mathbf{W_{NaCl}}$ [g]	$\mathbf{V_{st}}$ [ml]	**M** [mol / l]		

APPARATUS and CHEMICALS

INSTRUMENTATION AND AUXILIARY EQUIPMENT

- Laboratory blender
- High Speed Blender with optimal dispersion tool (e.g. Polytron with min. tool diameter of 25 mm)
- Analytical balance (accuracy: 0.001g)
- Titration unit for potentiometric titration consisting of :
  Silver reference electrode, pH glass electrode or Silver combination electrode, Automatic or manual dosing unit, Magnetic stirrer, Potentiometric recorder (alternatively an automatic or semiautomatic titration unit may be used)

GLASSWARE

- Erlenmeyer flasks: 250 ml / 300 ml
- Measuring cylinder: 500 ml
- Volumetric flasks: 250 ml, 100 ml
- Beakers: 250 ml
- Pipettes: 10 ml and 25 ml
- Funnel

EXPENDABLES

- Filter paper for quantitative filtration *or*
- Clean glasswool

CHEMICALS

- Deionised Water
- Nitric acid, 65%
- Silver Nitrate p.a. (Merck Titrisol 0.1 M)
- Sodium chloride p.a. Merck 6404

SOLUTIONS

- Nitric Acid 2M:
  Dilute approximately 120ml nitric acid to 1L (2M)
- Silver Nitrate 0.1 M: prepare from stock solution according to instructions

SAMPLE PREPARATION

HOMOGENISATION

Homogeneous Samples:
- Use without any further pre-treatment

Inhomogeneous Samples / Whole Consumer Packages:
- Homogenise a sufficiently large sample of a product with coarse pieces (e.g. Minestrone) or the whole content of the consumer package with a clean and dry blender.

SAMPLE SIZE

Select approximately 5 g, determined accurately to 0.01g, of this homogenised sample in order to obtain reproducible results.

PROCEDURE

- Weigh the sample into a 250 ml Erlenmeyer flask (SW)
- Add approximately 150 ml deionised water
- Homogenise the sample for 1 - 1½ minutes with the High Speed Blender at medium to high speed
- Quantitatively transfer the solution into a 250 ml volumetric flask.
- If necessary, filter the sample trough a filter paper or glass wool that an efficient filtration is reached. Use a vacuum sucking device if gravity filtration is not effective.

Remark:
Products with a high fat content (e.g. bouillons) do not require filtration after homogenisation. Fat accumulates in lumps during the preparation of the solution. They must be discarded. Alternatively, 2 to 4 ml of hexane may be added to dissolve all the fat. This solution can then be pipetted off and discarded.

For a quantitative transfer of the sample, thoroughly wash the homogenising tool and filters. Add the washings to the volumetric flask.

- Fill to the mark with cold water.
- Clean the High Speed Blender carefully after each use to avoid errors through cross contamination.

ANALYTICAL PROCEDURE

INSTRUMENT CONTROL:

Check all parts of the analytical instrumentation (dosing unit, electrodes, recording unit) according to the manufacturers guidelines. If necessary clean silver electrodes carefully with scouring powder, hot water and wipe clean with paper tissue.

MEASUREMENT OF THE SILVER NITRATE MOLARITY

- Weigh approximately 0.060g sodium chloride to an accuracy of 1mg (W_{NaCl}) into a 250 ml beaker,
- Add 10 ml nitric acid solution and fill to ~200ml with deionised water,
- Position the electrodes in the solution
- Titrate with 0.1 M silver nitrate solution to obtain a complete curve on the Potentiometric recorder. (Alternatively operate your instrument according to the manufacturers guidelines).

  Remark:
  Make sure to stir at constant rate producing vigorous agitation without splashing throughout the recording of the curve.

- Determine the inflection point of the curve and record the volume (V_{st}) of silver nitrate used. Calculate the molarity as given in the section „CALCULATIONS; CALCULATION OF SILVER NITRATE MOLARITY“.

MEASUREMENT OF THE SAMPLE

- Recommended volume for titration (5g sample in 250 ml) :

Salt concentration in sample:	Amount of salt in 250 ml	Volume to use for titration:	Dilution factor (D_f)	Amount of salt in aliquot:	
g / 100 g (%)	g	ml		mg	mmol
10 - 30	0.5 - 1.5	25	10	50 - 150	0.8 - 2.5
30 - 60	1.5 - 3.0	10	25	60 - 120	1 - 2

- Transfer the appropriate volume of the prepared sample solution to a 250 ml beaker,
- Add 10 ml nitric acid solution and dilute with approx. 200 ml deionised water.

Position the electrodes in the solution and titrate with the silver nitrate solution.

Remark:
Make sure to stir at constant rate producing vigorous agitation without splashing throughout the recording of the curve.

- Titrate with ~0.1 M silver nitrate solution to obtain a complete curve on the potentiometric recorder.
- Determine the inflection point of the curve and record the volume (V) of silver nitrate used.
- Calculate the salt concentration in the sample according to the formula given in the section CALCULATIONS, CALCULATION OF THE CONCENTRATION IN SAMPLES
- If an automatic or semiautomatic titration unit is used, follow the instructions of the manufacturer for the calculation of the chloride or salt concentration

CALCULATIONS

CALCULATION OF SILVER NITRATE MOLARITY

$$\text{Molarity of silver nitrate, } M = \frac{W_{NaCl} * 1000}{MW * V_{ST}} \left[\frac{g}{g / Mol * ml} \right]$$

W_{NaCl}: Weight of sodium chloride (g)
MW : Molecular weight of sodium chloride (58.443)
V_{st} : Volume of silver nitrate used for titration of standard solution (ml)

CALCULATION OF THE CONCENTRATION IN SAMPLES

$$\text{Salt concentration (in \%)} = \frac{V * M * MW * D_f}{SW * 10} \left[\frac{ml * mol / l * g / mol}{g} \right]$$

V: Volume of silver nitrate used for titration (ml)
M: Calculated molarity of silver nitrate solution (recommended: 0.1M)
MW Molecular Weight of sodium chloride (58.443)
D_f Dilution factor
SW Sample weight (g)
10 Correction factor to give percentage value

RESULTS

The result should be expressed in % with one digit after the decimal point.

LITERATURE

- CPC Europe, Quality Assurance
  Report "Determination of salt content"
  (Sample Preparation/Comparison of Chloride Meter and Potentiograph)
- AOAC Official Methods of Analysis, 15th Ed.(1990) Methods 32.034 - 32.039

VALIDATION PROCEDURE

Validation of the method has been performed by the collaborative study. New validation is only required if significant changes in sample preparation are introduced. The decision for additional validation studies must be based on reliable comparisons to the original procedure.

COLLABORATIVE STUDY: Data Evaluation

A collaborative study with 15 laboratories located in 9 countries of Europe has been performed in June 1995.

These laboratories received 12 samples from regular production runs. Of six commercial products, two different batches were supplied for analysis. The theoretical concentrations for salt, sodium and fat was calculated from the recipe. The real salt concentration as obtained during manufacturing, was unknown.

Sample	Theoretical Salt Concentration: [g / 100 g]	Theoretical Sodium Concentration: [g / 100 g]	Fat Concentration: [g / 100 g]	Batch Identification
Beef Bouillon Paste (High Fat)	48.6	21.4	23.1	9911/9503
Beef Bouillon Granulate (Low Fat)	43.5	19.3	2.0	2631/4957
Standard Cream Soup (Asparagus)	12.2	5.5	8.1	7454/5971
Standard Gravy (Demi Glace)	11.4	5.6	13.8	7471/1532
Cream Sauce (Béarnaise)	14.2	6.7	9.6	3556/4312
Instant Cream Soup (Tomato)	7.3	3.4	8.3	736/7780

The initial statistical data analysis could be performed on the results supplied by 12 laboratories.
The statistical preevaluation of the submitted results allowed for the acceptance of data from 12 laboratories and suggested a clustering into three different data sets for the validation calculations:

Data Set 1: 4 different samples (one production batch of each product) with salt concentrations between 70g / kg and 440g / kg. Sample identification: 2631, 7454, 1532, 3556

Data Set 2: 5 different samples (the second production batch of each product) with salt concentrations between 70g / kg and 440g / kg. Sample identification: 4957, 5971, 7471, 4312, 7780

Data Set 3: 2 samples (two batches of one product) with salt concentrations of around 500g / kg and high fat content. Sample identification: 9503, 9911

STATISTICAL ANALYSIS

Results:	**Repeatability**	**Reproducibility**
Data Set 1	0.20	0.30
Data Set 2	0.27	0.32
Data Set 3	0.88	1.25

	Molarity of AgNO3 Solution	Weight of sodium chloride (g)	Volume ofAgNO3 Solution (ml)	Date of Calibration / Analysis:
Use for Samples:	**M:**	W(NaCl)	V st:	
Example	**0.102**	0.58	97	
	0.000	0	1	
	0.000	0	1	
	0.000	0	1	
	0.000	0	1	
	0.000	0	1	

Molarity of silver nitrate, $M = \frac{W_{NaCl} * 1000}{MW * V_{ST}} \left[\frac{g}{g / Mol * ml}\right.$

W_{NaCl}: Weight of sodium chloride (g)

MW : Molecular weight of sodium chloride (58.443)

V_{st} : Volume of silver nitrate used for titration of standard sol

The table contains formulas for the calculations

Results of Analyses

Correction Factor (for percent output) **10** (should remain unchanged)

Molecular Weight of Sodium Chloride (MW) **58.443**

			Subsample 1						Subsample 2		
					Titration 1	Titration 2	Average				Titration 1
Sample ID	**Fill Weight (g)**	**Salt Concen-tration:**	Sample Weight 1 (g)	Dilution Factor	Volume of AgNO3 Solution	Volume of AgNO3 Solution	(of 2 Titrations)	**Salt Concen-tration:**	Sample Weight 2 (g)	Dilution Factor	Volume of AgNO3 Solution
			SW	Df	V:	V:	V:		SW	Df	V:
Example		**6.58**	5	10	5	6	5.5	**0.15**	22	29	19
		#DIV/0!					#DIV/0!	**#DIV/0!**			
		#DIV/0!					#DIV/0!	**#DIV/0!**			
		#DIV/0!					#DIV/0!	**#DIV/0!**			
		#DIV/0!					#DIV/0!	**#DIV/0!**			
		#DIV/0!					#DIV/0!	**#DIV/0!**			
		#DIV/0!					#DIV/0!	**#DIV/0!**			
		#DIV/0!					#DIV/0!	**#DIV/0!**			
		#DIV/0!					#DIV/0!	**#DIV/0!**			
		#DIV/0!					#DIV/0!	**#DIV/0!**			
		#DIV/0!					#DIV/0!	**#DIV/0!**			
		#DIV/0!					#DIV/0!	**#DIV/0!**			
		#DIV/0!					#DIV/0!	**#DIV/0!**			

ANNEX IV

Draft Standard for Soups and - Determination of Creatinine

AIIBP Method 2/5 (Revision 2000, HPLC)

DETERMINATION OF TOTAL CREATININE BY HPLC

Draft to be reformulated into the Standard Format of AIIBP Method Book

1 SCOPE AND FIELD OF APPLICATION

Description of a method for the determination of (total) creatinine in meat extract, bouillon's, soups and gravies.

2 DEFINITION

Total creatinine is the amount of creatinine present and that obtained from creatine and creatine phosphate and determined under the conditions outlined hereafter.

3 PRINCIPLE

Conversion of creatine and creatine phosphate into creatinine and extraction of creatinine by diluted hydrochloric acid, followed by neutralisation and addition of an internal standard. Except for meat extract, clean up over alumina and C18-cartridges. Separation using HPLC followed by UV detection.

4 REAGENTS AND MATERIALS

4.1 Material

- Beakers, low form, with spout, 250 ml

- Pasteur pipettes, 150 mm

- Volumetric flasks, class A, RN stopper PE, 100 ml.

- Idem , 500 ml

- Idem, 20 ml

- PH-meter, table type, digital

- Device for membrane filtration of solvents with fitting hydrophilic membrane filters for aqueous solvents. 0.45 µm pore diameter.

- Adjustable pipette Socorex, mod. 831, 5 ml: 0.5 – 5ml

- PP tips, white

- Disposable syringe, 10 ml

- Sep-Pak plus cartridges Alumina B, Waters no 20505

- Sep-Pak plus cartridges C18, Waters no 20515

- Membrane filter Millipore for degassing of HPLC liquids, 0.45 µm pore diameter.

4.2 Reagents

Before using chemicals refer to the Sigma/Aldrich Guide to Chemical Safety and/or other adequate manuals or safety data sheets approved by your local authorities. Methanol should be handled with care. It is a toxic and inflammable material.

- Creatinine, for biochemistry
- Cytosine, for biochemistry
- Hydrochloric acid 32 % GR
- o-Phosphoric acid, 85 % GR
- Dipotassium hydrogenphosphate (K2HPO4), GR
- Petroleum ether (boiling range 50-75 o C)
- Methanol, gradient grade
- Sodium hydroxide, pellets GR

5 PREPARATION OF REAGENTS

5.1 Hydrochloric acid, about 21%

Into a suitable polyethylene bottle pour 100 ml water and add slowly 200 ml of hydrochloric acid 32%. Mix and allow to cool to room temperature. This solution keeps for 6 months at room temperature.

5.2 Sodium hydroxide solution (about 25% w/w)

Into a suitable polyethelene bottle weigh 50 g of sodium hydroxide pallets and add 150 ml water. Dissolve and cool to room temperature. This solution keeps for one year at room temperature.

5.3 Creatinine - stock solution (1 mg/ml)

Into a 100 ml volumetric flask weigh 100.0 mg of creatinine. Add 2 ml of hydrochloric acid solution (5.1). Dissolve, make up to the mark with water and mix well. This solution keeps for one week at 4 °C.

5.4 Cytosine – stock solution (0.45 mg/ml)

Into a 100 ml volumetric flask weigh 45.0 mg of cytosine. Add 2 ml of hydrochloric acid solution (5.1). Dissolve, make up to the mark with water and mix well. This solution keeps for one week at 4 °C .

5.5 Standard solutions

5.5.1 Concentrated standard solution

Into a 100 ml volumetric flask pipette 2 ml of creatinine solution (5.3). Add 10 ml of hydrochloric acid solution (5.1), 4 ml of sodium hydroxide solution (5.2) and 5 ml of cytosine solution (5.4). Fill up to the mark with water and mix. This solution contains 20 µg creatinine / ml and 22.5 µg cytosine / ml.

5.5.2 Working standard solution

Into a 20 ml volumetric flask pipette 2 ml of solution 5.5.1. Fill up to the mark with water and mix. This solution contains 2 µg creatinine / ml and 2.25 µg cytosine / ml.

5.6 Mobile phases for HPLC

5.6.1 Mobile phase A (0.1 mol phosphate buffer, pH 7.0)

Into a 1000 ml beaker, weigh 17.4 g dipotassium hydrogen phosphate (K2HPO4). Dissolve in about 800 ml water. Adjust the pH to 7.0 with phosphoric acid (pH meter!). Transfer quantitatively into a 1000 ml volumetric flask, make up to the mark with water and mix. Filter over a membrane (0.45 µm pore width). The buffer keeps for one week at 4 o C.

5.6.2 Mobile phase B (rinsing solution)

Mix 500 ml of methanol with 500 ml of water. This solution keeps for 3 months at 4 o C. The mobile phases should have the ambient temperatures before they can be used. Degas the solutions as appropriate over Millipore filter.

6 SAMPLE PREPARATION

6.1 Meat extract

Pasty meat extracts tend to form a sediment of crystallised creatinine and mineral salts. To obtain a homogeneous test portion, heat the sample in a water bath to 45 - 50 o C and mix well. Cool to room temperature.

6.2 Food (bouillons, soups, gravies)

Homogenise a representative sample.

7 PROCEDURE

Perform the analyses in duplicate

7.1 Meat extract

In a 250 ml beaker weigh to the nearest mg about 2.00 g of meat extract . Determine the weight to the nearest mg. Dissolve under stirring in about 100 ml of water previously heated to 60 o C. Transfer the solution with more water quantitatively into a 250 ml volumetric flask and cool to room temperature. Make up to the mark with water and mix well. Into a 100 ml volumetric flask, pipette 5 ml of this solution and add 10 ml of hydrochloric acid solution (5.1). Place the flask in a boiling water bath for one hour. Cool to room temperature, add 4 ml of sodium hydroxide solution (5.2) and 5 ml of cytosine solution (5.4). Make up to the mark with

water and mix well. Into a 20 ml volumetric flask pipette 2 ml of this solution, make up to the mark with water and mix well. Filter the extract over a membrane filter suitable for aqueous liquids. Use the solution after filtration for the HPLC procedure.

7.2 (bouillon's, soups, gravies) Defatting and hydrolysis

7.2.1 Defatting and hydrolyses

In a 250 ml beaker weigh to the nearest mg about 10.00 g of sample. Dissolve under strong stirring in about 100 ml of water previously heated to 60 o C. Transfer the solution quantitatively into a 250 ml volumetric flask by adding about 100 ml of cold water and mix well. Add 3 ml of petroleum ether and fill up to the mark with water (the organic layer must be above the mark). Shake in order to extract the fat into the organic phase. Allow phases to separate then remove the organic layer with a Pasteur pipette. Remark: When analysing products containing less than 7% fat, the addition of petroleum ether can be skipped. Pipette 20 ml of the aqueous extract into a 100 ml volumetric flask. Add 10 ml of hydrochloric acid solution (5.1). Place the flask in a boiling water bath for one hour. Cool to room temperature, add 4 ml of sodium hydroxide solution (5.2) and 5 ml of cytosine solution (5.4). Make up to the mark with water and mix well.

7 PROCEDURE

Perform the analyses in duplicate

7.1 Meat extract

In a 250 ml beaker weigh to the nearest mg about 2.00 g of meat extract . Determine the weight to the nearest mg. Dissolve under stirring in about 100 ml of water previously heated to 60 o C. Transfer the solution with more water quantitatively into a 250 ml volumetric flask and cool to room temperature. Make up to the mark with water and mix well. Into a 100 ml volumetric flask, pipette 5 ml of this solution and add 10 ml of hydrochloric acid solution (5.1). Place the flask in a boiling water bath for one hour. Cool to room temperature, add 4 ml of sodium hydroxide solution (5.2) and 5 ml of cytosine solution (5.4). Make up to the mark with water and mix well. Into a 20 ml volumetric flask pipette 2 ml of this solution, make up to the mark with water and mix well. Filter the extract over a membrane filter suitable for aqueous liquids. Use the solution after filtration for the HPLC procedure.

7.2 (bouillon's, soups, gravies)Defatting and hydrolysis

7.2.1 Defatting and hydrolyses

In a 250 ml beaker weigh to the nearest mg about 10.00 g of sample. Dissolve under strong stirring in about 100 ml of water previously heated to 60 o C. Transfer the solution quantitatively into a 250 ml volumetric flask by adding about 100 ml of cold water and mix well. Add 3 ml of petroleum ether and fill up to the mark with water (the organic layer must be above the mark). Shake in order to extract the fat into the organic phase. Allow phases to separate then remove the organic layer with a Pasteur pipette. Remark: When analysing products containing less than 7% fat, the addition of petroleum ether can be skipped. Pipette 20 ml of the aqueous extract into a 100 ml volumetric flask. Add 10 ml of hydrochloric acid solution (5.1). Place the flask in a boiling water bath for one hour. Cool to room temperature, add 4 ml of sodium hydroxide solution (5.2) and 5 ml of cytosine solution (5.4). Make up to the mark with water and mix well. Into a 20 ml volumetric flask pipette 2 ml of this solution, make up to the mark with water and mix well.

7.2.2 Clean-up

Attach the alumina and C18 Sep-Pak cartridges and the membrane filter for aqueous liquids to a 10 ml disposable syringe (see drawing below). Fill the syringe with the diluted solution obtained under 7.2.1. Elute with a flow of approximately 1 drop per second. Collect 3.5 ml in a 5 ml measuring cylinder and discard. Then collect at least 2 ml of eluate and use this for the HPLC analysis.

Note: See the analytical flow sheet for a graphical representation of the procedure. The defatting step in the second text box is not necessary for meat extract. The complete clean up with Sep Pack columns is necessary for food and not for meat extract. This is mentioned in the 6 th text box. After completion of the ring test, the method description will contain two separate flow sheets (one for food and one for meat extract). This note will then be removed from the text.

8 HPLC

Allow the chromatographic system to equilibrate. Inject the same volume of standard (5.2.2) and sample solution and perform HPLC under the following conditions.

Separation column : Spherisorb ODS 2, particle size 5 µm, 250*4.6 mm.

Mobile phase : Phosphate buffer (see 5.6.1)

UV detector : ë = 234 nm

Injection volume : 10 µl

Flow rate : 0.8 ml / min

Identify creatinine and cytosine in the sample solution by comparison with retention times and areas or heights of the corresponding peaks obtained with standard solution. Under the above mentioned conditions cytosine and creatinine must be base-line separated.

Approximate retention times are:

Cytosine : 5 min

Creatinine : 6 min

A few minutes of flushing with buffer is recommended after each sample injection. The standard solution under 5.2.2 is to be injected 3 times at the beginning of the day, once after each fourth sample and twice at the end of a test series. The result (in peak area or height) should remain stable. Use under these conditions the average response for the calculation of the results.

Column handling and maintenance

Overnight:

- Run with 0.05-0.1 ml/min mobile phase 5.6.1. (buffer solution).

During extended period of non-use (e.g. weekend)

._Rinse the columns with pure water for about one hour (flow 0.8 ml/min) to wash out all buffer salts.

._Run the chromatographic system with mobile phase 5.6.2. (methanol / water 1:1) at 0.05-0.1 ml/min.

Restart for use:

._Rinse the columns with pure water for about 30 minutes (flow 0.8 ml/min).

._Condition the column with mobile phase 5.6.1. at 0.8 ml/min for about 30 minutes. The baseline must be stable and standard solution must be injected to evaluate system performance.

9 CHROMATOGRAPHIC RESOLUTION

Chromatographic resolution shall be determined at least by visual inspection of the calibration chromatograms.

The internal standard cytosine has to be properly separated from creatinine. Some tailing of the internal standard and creatinine peak is tolerable and will not significantly degrade the required performance of the analytical system. It is recommended to determine the resolution and peak skewness. The cytosine and creatinine peaks must be base-line separated.

10 CALCULATION, EXPRESSION AND INTERPRETATION OF RESULTS

10.1 Calculation

The total creatinine content, expressed in g / 100 g product, is equal to:

Qsple * Cstd V1 100 20 * 100 *

---------- * ---- * ---- * * ----------

Qstd V2 M 1 * 2 * 1000000

Or, simplified Qsple * Cstd V1 1 1

---------- * ---- * ---- * -----

Qstd V2 M 10

Where

Qsple ; Quotient of peak area or height of creatinine/cytosine sample solution

Qstd : Quotient of peak area or height of creatinine/cytosine in standard solution.

M : Mass of the test portion in g

Cstd : Concentration of creatinine standard solution (5.5.2), in µg/ml

V1 : Volume of initial solution of the test portion (250 ml)

V2 : Volume of V1 used for hydrolysis (5ml for meat extract, 20 ml for food)

10.2 Expression of results

Express the results with 3 significant digits

10.3 Repeatability and reproducibility of the method

The following data have been obtained in a collaborative test on a sample of meat extract, with a creatinine concentration of about 7.2 g/100 g and a sample of meat glace paste with a creatinine concentration of about 0.6 g/100 g.

10.3.1 Repeatability (r)

Meat extract : r = 0.30 g / 100 g. sr = 0.11 g / 100 g

Meat glace paste : r = 0.067 g/ 100 g. sr = 0.024 g / 100 g

10.3.2 Reproducibility (R)

Meat extract : R = 0.42 g / 100 g. sR = 0.15 g / 100 g

Meat glace paste : R = 0.082 g / 100 g. sR = 0.029 g / 100 g

11 AUTOCONTROL PROCEDURES

11.1 Reference samples

For routine use, include the analysis of a reference sample with a known concentration of creatinine. Stored at -20 o C, this sample can be kept for at least 18 month. At regular intervals it is recommended to verify the system by establishing a calibration curve (chapter 12) and control procedures by adding standard creatinine solution to creatinine free sample material (see chapter 13).

12 CALIBRATION CURVE

Use the concentrated stock solutions for creatinine (5.3) and cytosine (5.4). Prepare eight 100 ml volumetric flasks and label them CP1 to CP8. Transfer exactly the required volumes of creatinine stock solution (5.3) into the corresponding volumetric flasks CP1 to CP8. For each solution, perform the following steps. Add 10 ml hydrochloric acid (5.1), 4 ml sodium hydroxide (5.2) and 5 ml cytosine cytosine solution (5.4). Fill to the mark with water. Prepare eight 20 ml volumetric flasks and label them CP1.1 to CP 8.1. Transfer 2 ml of each calibration solution CP1 to CP8 into the corresponding flasks CP1.1 to CP8.1 and fill to the mark with water.

Label Concentration in µg / ml

Creatinine

Standard (5.3) in ml

Cytosine Standard (5.4) in ml

Label Concentration xi in µg / ml

Aliquot CP1-CP8 in ml

CP1 5 0.5 5 CP1.1 0.5 2
CP2 10 1.0 5 CP2.1 1.0 2
CP3 15 1.5 5 CP3.1 1.5 2
CP4 20 2.0 5 CP4.1 2.0 2
CP5 25 2.5 5 CP5.1 2.5 2
CP6 30 3.0 5 CP6.1 3.0 2
CP7 40 4.0 5 CP7.1 4.0 2
CP8 50 5.0 5 CP8.1 5.0 2

Inject 10 µl of each calibration solution CP1.1 to CP8.1 twice. Use peak height or peak area for evaluation as for sample solutions.

Calculations- Calibration curve

Definition:xi denominates the theoretical concentration of the calibration solution as calculated for CP1.1 to CP8.1. yi denominates the ratio Q(sample). Take the ration Q of each creatinine solution (CP1.1 to CP8.1) and the internal standard as calculated from your integrating system. Use peak height or area. Average the data from two injections of the same calibration solution. Perform the linear regression analysis (least square fit) for the pairs concentration peak height or area over the complete range of the calibration curve and calculate the regression coefficient r.

13 CREATININE ADDITION TO FOOD

SAMPLES NOT CONTAINING CREATININE

Perform the complete analysis procedure with a bouillon sample not containing creatine or creatinine, which is fortified at different levels.

Weigh 250 mg creatinine in a 100 ml volumetric flask. Dissolve and fill to the mark with water. One ml of this solution contains 2.5 mg creatinine. Follow the procedure, described under 7.2. Weigh in each of three 250 ml beakers 10.00 g of creatine/creatinine free sample. Dissolve under strong stirring in about 100 ml of water heated previously to 60 o C. Transfer the solution quantitatively into a 250 ml volumetric flask by adding about 100 ml cold water and mix well. Mark the volumetric flasks A, B and C. Pipette exactly 5.0, 10.0 and 15.0 ml of the creatinine solution into volumetric flasks A, B and C respectively. Fill to the mark with water and continue the procedure under 7.2. The theoretical creatinine content in sample A is 125 mg/100 g, in sample B 250 mg/100 g and in sample C 375 mg/100 g. Calculate the recovery.

14 REMARKS

Comparison Tests between a series of laboratories showed, that slightly more comparable results are obtained using peak heights versus peak areas. If the creatinine content of a sample is outside the calibration curve, repeat the analysis with adjusted sample weight. The clean-up step with Sep-Pak cartridges is necessary to remove matrix components which otherwise elute before and after the components of interest. If the same samples are analysed regularly it may be worthwhile to investigate if this step can be omitted (cost reasons).

._Evaluation of extracts from various samples showed no interfering components in the region of the internal standard cytosine. Thus the internal standard technique may be applied.

._Sample preparation is based on Waters Sep-Pak material. Other suppliers may provide similar or identical material. However, the performance of other materials must be checked carefully and procedures may need adjustment.

._Note that for complex samples, although sample clean-up (with Sep-Pak columns) is used in the method, "late" peaks may occur in the chromatograms. In such cases sufficient time must elapse before a next injection. Recommended time for "suspected" samples is 35 minutes.

Alternative column

Alternative columns to the one, mentioned in chapter 8, may be used provided that these have the same characteristics. An example is e.g.: TSK - Gel ODS 80 TM, 5 µm, 150 mm * 4.5 mm-ID TOSO HAAS 08148. The retention times for cytosine on this column is about 4.6 minutes and for creatinine about 5.7 minutes.

Pre Columns

The use of a pre column may be required in routine analyses. Select the pre-column, which corresponds to the analytical column. Use e.g.: TSK-Gel Guard Cartridge ODS80 TM TOSO HAAS 14123 with TSK Gel cartridge holder

TOSO HAAS 14100 or Cartridge with Spherisorb ODS 2.5 µm, 20*4 mm with appropriate cartridge holder.

ANNEX V

DETERMINATION OF LEAD, CADMIUM, ZINC, COPPER, AND IRON (NMKL METHOD)

DETERMINATION BY ATOMIC ABSORPTION SPECTRO-PHOTOMETRY AFTER WET DIGESTION IN A MICROWAVE OVEN

1. SCOPE AND FIELD OF APPLICATION

This method describes quantitative determination of the metals: lead, cadmium, zinc, copper and iron in various types of foods, with the exception of oils, fats and extremely fatty products. The method employs atomic absorption spectrophotometry (AAS) after microwave oven digestion under pressure. The method has been tested on dry materials only, but may under certain conditions be used for samples containing water.

2. PRINCIPLE

The sample is wet digested with nitric acid and hydrogen peroxide in a sealed container heated by microwaves. The sample solution is diluted with water and the concentrations of the metals are determined by flame or graphite furnace AAS.

3. REAGENTS

Reagents should be of at least analytical grade, preferably of suprapur quality, or equivalent.

3.1 Water, redistilled or deionised (Millipore or equivalent quality).

3.2 Nitric acid, concentrated. (65% w/w).

3.2.1 Nitric acid, 0.1 mol/l: Dilute 7 ml of conc. nitric acid with water to 1000 ml.

3.2.2 Nitric acid, 3 mol/l: Dilute 200 ml of conc. nitric acid with water to 1000 ml.

3.3 Hydrogen peroxide, 30% w/w.

3.4 Lead standard solution, 1000 mg/l: Dissolve 1.000 g Pb in 7 ml nitric acid in a 1000 ml volumetric flask. Dilute to volume with water.

3.5 Cadmium standard solution, 1000 mg/l:

Dissolve 1.000 g Cd in 14 ml of water and 7 ml of nitric acid in a 1000 ml volumetric flask. Dilute to volume with water.

3.6 Zinc standard solution, 1000 mg/l: Dissolve

1.000 g Zn in 14 ml of water and 7 ml of nitric acid in a 1000 ml volumetric flask. Dilute to volume with water.

3.7 Copper standard solution, 1000 mg/l: Dissolve 1.000 g Cu in 7 ml of nitric acid in a 1000 ml

volumetric flask. Dilute to volume with water.

3.8 Iron standard solution, 1000 mg/l: Dissolve 1.000 g Fe in 14 ml of water and 7 ml of nitric acid in a 1000 ml volumetric flask. Dilute to volume with water.

3.9 Working standard solutions

3.9.1 For graphite furnace analyses: Dilute standard solutions 3.4-3.8 with 0.1 mol/l nitric acid (3.2.1) to a range of standards covering the linear range of the element to be determined.

3.9.2 For flame analysis: Dilute standard solutions 3.4-3.8 with 0.1 mol/l nitric acid (3.2.1) to a range of standards covering the linear range of the element to be determined.

Note: Alternatively to preparing standard solutions from metals or metal salts, use commercially available standard solutions for AAS on the condition that they comply with the requirements for traceability.

4. APPARATUS

All glassware and plastic ware should be carefully cleaned and rinsed e.g. with nitric or hydrochloric acid, diluted 1:10, to prevent metal contamination.

4.1 Atomic absorption spectrophotometer with background correction and graphite furnace, alternatively air-acetylene burner or nitrous-acetylene burner.

4.2 Hollow cathode lamps, or Electrodeless

Discharge Lamps (EDL lamps) for Pb, Cd, Zn, Cu and Fe.

4.3 Graphite tubes, pyrolytically coated and with platforms for Pb and Cd.

4.4 Microwave oven, designed for laboratory use, e.g. CEM MDS-2000.

4.5 Digestion vessels, about 100 ml and with- standing a pressure of at least 1.4 Mpa (200 psi).

5. PROCEDURE

5.1 Pre-treatment of samples

If necessary, dry the sample to constant weight at 105 o C, alternatively use freeze drying. Freeze drying is usually preferable, since that renders the sample less compact and easier to homogenize. Homogenize the sample with apparatus suitable for each type of material. Check for leaching metals if the equipment contains metal parts.

5.2 Wet digestion

Weigh 0.2-0.5 g of the dry sample into the digestion vessel (4.5). If the sample material is not dry, the amount of sample is restricted to 2 g and the dry matter must never exceed 0.5 g. The maximum test portion from a sample having a water content of e.g. 50% is thus 1 g (= 0.5 g dry matter); for a material containing 95% water the test portion may be 2 g (<0.5 g dry matter). Include one blank sample in every round.

Add to the digestion vessel 5 ml conc. nitric acid (3.2) and 2 ml hydrogen peroxide (3.3). Seal the vessel and

place it in its holder in the microwave oven (4.4) and close the door. Set the oven program according to table 1 and start the program.

The program is valid on the condition that 12 samples are being digested simultaneously. If fewer samples are being digested the remaining vessels must be filled as described above and not with water.

5.3 Dilution

Remove the digestion vessels from the microwave oven and allow to cool thoroughly before attempting to open them. Open the vessel and rinse down the lid and the walls into the container. Transfer the solution to a 25 ml volumetric flask and dilute to the mark with water. Then transfer the solution to a plastic container. Treat the blank in the same way.

5.4 Atomic absorption spectrophotometry

The concentration of the metal in question determines the technique to be used - flame or graphite furnace technique. Use flame technique as far as possible since this technique is less sensitive to interference than the graphite furnace technique. The most appropriate wavelength, gas mixture/temperature program and other instrumental parameters for each metal are found in manuals provided with the instrument. Always use background correction unless it is proven to be unnecessary. It is important that the measurements are made in the linear range when the method of standard addition is used. A standard addition curve should consist of at least three points of which at least two are standards. The concentration of the highest standard should be 3-5 times the concentration in the sample solution. The lower standard should have a concentration between that of the highest standard and the sample solution.

5.4.1 Flame technique

The high acid concentration of the sample solution after digestion has detrimental effects on both results and the environment. It is therefore important that the solution is diluted as much as possible and that standard and sample solution have the same acid concentration. When this is done Zn and Cu can as a rule be determined against a traditional standard curve. Dilute the sample solution 1:2 with 0.1 mol/l nitric acid

(3.2.1). Dilute standard solutions 1:2 with 3 mol/l nitric

5.4.2 Graphite furnace technique

This method is generally required for determination of Pb and Cd. Use pyrolytically coated tubes with platforms. Since the method results in fairly large dilution of the sample the technique may often be useful in the determination of e.g. Cu. Always use the method of standard addition unless it is proven to be unnecessary. Program the autosampler to deliver a volume to the graphite furnace which gives an absorbance as large as possible within the linear range and producing a background absorbance of not more than about 0.5 absorbance units. To enhance the absorbance at very low concentrations, multiple injections may be employed. Table 3 shows examples of instrumental parameters applicable to a Perkin Elmer/HGA 600 instrument.

6. CALCULATIONS AND EVALUATIONS

6.1 Calculation of the results

Measure the peak area, not the height. Construct a standard curve and read the concentration of the metal from the curve. Calculate the concentration of the metal, C (mg/kg) from the formula:

$$C = \frac{(a - b) \times V}{m}$$

where

a = concentration in the sample solution (mg/l)

b = mean concentration in the blank solutions (mg/l)

V = volume of the sample solution (ml)

m = weight of the sample (g)

If the value (a-b) is lower than the detection limit (see section 6.2), substitute (a-b) with the detection limit for calculation of the limit of detection in the sample. If the sample has been diluted, take into account the dilution factor.

NOTE: Do not forget to recalculate the result to fresh weight if it is based on the dry matter content of the sample. Give the result with an applicable number of significant figures, two are normally enough.

6.2 Estimation of the detection limit

For each of the metals, estimate the detection limit as three times the standard deviation of the mean of at least 20 blank determinations. Thus a large number of blanks must be analyzed before the detection limit can be calculated. As the detection limit is not static, it must be reevaluated from time to time depending on the changes in the levels observed.

7. NOTES

7.1 When digesting unknown samples, observe caution since a too large amount of sample may rup-ture the safety membrane of the digestion vessels.

7.2 When using a microwave oven other than the one given as an example, it may be necessary to modify the given time/effect program case by case.

7.3 Regularly check the microwave oven used for delivered power. If the measured effect disagrees with the specification, adjust the program accordingly. Carry out a simple calibration e.g. as follows: Fill a plastic beaker with 1.000 kg of water at room temperature. Measure the temperature (Tb). Place the beaker in the oven and heat at full effect for 120 s. Remove the beaker, stir the water and measure its temperature (Ta). Delivered power in watts is P = 35 x (Ta - Tb).

7.4 The high concentration of acid in the sample solution will dampen the signal as compared to the signal from solutions of lesser acidity. It is also corrosive on the instrument and the laboratory environment. Therefore, sample solutions should be diluted before AAS measurement as described under 5.4.1.

7.5 A simplified version of the method of standard addition is to use a matrix-matched standard curve, which is applicable to samples with the same matrix and sample weight: The sample and standard solutions are mixed and used to make a standard addition curve. This curve is then parallel transferred to origin and is used as the standard curve for the following samples diluted in the same proportions. The matrix matched standard curve and the sample solutions will thus have the same matrix concentration. This function is often available in the software of modern AAS instruments.

7.6 Evaluate new matrices by means of ash/atomization curves in order to optimize parameters of the graphite furnace technique.

8. RELIABILITY OF THE METHOD

The reliability of this method has been tested in a method performance study with 14 participating laboratories. How many participants that analyzed each metal is listed in table 4, together with the means, the absolute and relative standard deviation for repeatability (Sr and RSD r, respectively) and reproducibility (SR and RSDR, respectively). The method precision shall, according to IUPAC-1987 be given as repeatability value, r and reproducibility value, R. The results of the method performance study show that the method yields satisfactory results within the tested intervals. The full report on the collaborative trial of this method will be published in Journal of AOAC International.

9. REFEREES OF THE METHOD

This method was elaborated by Lars Jorhem and Joakim Engman (Swedish National Food Administration, Uppsala), who also arranged the method -performance study and wrote the study report.

Adresse: c/o Veterinærinstituttet
Avdeling for fôr og næringsmiddelhygiene
Postboks 8156 Dep.
N-0033 OSLO
NORGE

Tabell 4 Metodens pålitlighet, avprövningens statistiska resultat. Resultaten anges i mg/kg.
Table 4. Method performance, the statistical result of the performance study.The results are given in mg/kg.

Bl=Blankar. MP=Mjölkpulver. L=Lever. W=Vetekli. D=Diet-CRM. BM=Nötmuskel F=Fisk. M=svamp-CRM.

Bl=Blanks. MP=Milk powder. L=Liver. W=Wheat bran. D=Diet CRM. BM=Bovine muscle. F=Fish. M=Mushroom CRM.

Metall *Metal*	**Prov** *Sample*	n	**Medelvärde** *Mean*	S_r	S_R	RSD_r%	RSD_R%	r (=2.8x S_r)	R (=2.8x S_R)
Pb	Bl	13	0.00048	0.00037	0.00056	77	117	0.0010	0.0016
	MP	10	0.005		0.019		381		0.052
	L	11	0.130	0.049	0.055	37	42	0.14	0.15
	W	12	0.155	0.088	0.091	57	59	0.25	0.26
	D	12	0.394	0.063	0.098	16	25	0.18	0.27
	BM	10	0.398		0.086		22		0.24
	F	12	0.48		0.13		27		0.36
	M	12	1.62		0.26		16		0.73
Cd	MP	11	-0.0000		0.0032		-8392		0.0090
	Bl	11	0.000025	0.000015	0.000033	58	131	0.000042	0.000094
	BM	12	0.0124		0.0034		28		0.0097
	L	13	0.164	0.025	0.034	15	20	0.070	0.094
	W	11	0.171	0.0078	0.022	4.6	13	0.022	0.063
	F	12	0.211		0.035		17		0.099
	M	11	0.482		0.053		11		0.149
	D	12	0.764	0.050	0.105	6.5	14	0.14	0.294
Zn	Bl	13	0.0016	0.0020	0.0022	123	142	0.0055	0.0063
	F	13	4.45		0.43		9.7		1.2
	MP	14	35.3		3.3		9.3		9.1
	D	13	47.8	1.9	2.5	4.0	5.3	5.4	7.1
	M	14	56.9		3.0		5.3		8.4
	W	13	73.5	2.5	3.5	3.4	4.8	7.1	9.9
	BM	11	147.3		2.5		1.7		7.0
	L	12	181.9	2.8	8.8	1.6	4.8	7.9	25
Cu	Bl	11	0.00159	0.00060	0.00196	38	123	0,00168	0.00548
	F	13	0.254		0.071		28		0.199
	MP	11	0.58		0.16		27		0.444
	BM	14	2.89		0.42		14		1.15
	W	14	10.34	0.75	1.06	7.2	10	2.1	2.92
	M	14	37.7		2.2		5.7		6.0
	D	12	63.42	0.95	1.9	1.5	3.0	2.7	5.3
	L	14	107.5	3.3	4.1	3.1	3.8	9.3	12
Fe	Bl	13	0.0137	0.0039	0,0177	28	129	0.0108	0.0495
	MP	12	3.3		1.6		47		4.4
	F	12	7.6		1.4		18		3.9
	BM	12	73.0		5.0		6.9		14.1
	M	13	103.8		8.5		8.2		23.7
	W	13	124.2	5.3	10.6	4.2	8.6	14.8	29.8
	D	11	303	12	17	4.0	5.7	34	48
	L	13	484	27	32	5.6	6.7	75	90