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Production and certification of "fresh" reference material for macronutrient analysis

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Summary. Reference materials for carrying out in-house quality assurance by food laboratories that analyse macronutrients have to date been inadequate. The freeze-dried, very specialized, materials that exist on the market are not always comparable with ordinary food products analysed at those laboratories.

A homogeneous, "fresh", canned meat material was produced by an ordinary cannery. The total amount of material (pork, nitrite salt and water) was 1700 kg. During production, the fat content was continuously analysed in the different sub-batches and combinations are made accordingly. The material was packed in tin cans containing 200 g, and tested for homogeneity. The shelf life is, by experience, at least five years. A large number of authorized public and industry laboratories participated in the certification procedure. For each constituent different types of standard analytical methods were used. The material is offered for sale together with a certificate, giving mean values for moisture, ash, fat, nitrogen, sodium, sodium chloride and hydroxyproline content. The uncertainty is given as standard deviations including the analytical error and the variations between laboratories, methods and units.

Introduction

Quality assurance of in-house accuracy in meat industry and public laboratories is best accomplished using reference materials. Other techniques, such as standard additions and the use of other analytical methods, are often unsuitable because of the routines. In those food laboratories, there has been, for quite a while, a demand for "fresh" reference material, since the samples analysed are nearly always fresh foods. A meat material would be preferable, since many food regulations are directed to meat products, and the sampling of such products dominates. Analytical errors in the production control can result in unlawful products or bad economy for the manufacturer, and wrong conclusions can be drawn during product control in public laboratories.

On the market, very few reference materials are available for macronutrient analysis. None of them are fresh materials. The purpose of this project was to produce a homogeneous, canned reference material including certificate, for analysis of macro-nutrients in meat and meat products, to encourage frequent checks of the in-house accuracy.

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Production of the material

The material was produced in February 1989, by the Swedish Meat Research Institute (MRI) and the Swedish meat processing plant, Scan Syd in Kalmar.

The ingredients were 88% pork trimmings containing about 23% fat, 2.5% nitrite salt of which 0.6% was sodium nitrite, and 9.5% water. The pork trimmings were ground through 10 mm plate openings, split into two batches and mixed with the other ingredients. Each batch was emptied into four containers. The fat content of the mixed material in each container was determined.

The homogenizing step was performed in a vacuum cutter. Each batch to be cut consisted of the material from two containers. The variation of the fat content between these different cutting batches was minimized by combining the material in the containers according to the fat content.

Finally, the material was packed into tin cans in quantities of about 200 g, and heat-treated according to the factory's normal routine for canned goods. The canned goods were labelled „REF MTRL. KÖTT 89. MEATRES-SLV" and transported to the MRI for distribution.

Homogeneity assessment

The material was tested for homogeneity by duplicate analyses of 30 randomly selected units (cans) at one laboratory, using the same analytical method for each constituent. The variations, expressed as standard deviations, are presented in Table 1. The material was considered homogeneous if the variation between units, (s_{bu}), including inhomogeneity and analytical error (repeatability), was not significantly larger than the analytical error (s_r). This was tested with an F-test. Repeatability and reproducibility standard deviations were calculated using the analysis of variance as described by Youden and Steiner [1] and ISO 5725 [2].

No inhomogeneity was observed for water, ash, nitrogen, protein, salt and hydroxyproline. The inhomogeneity for fat was of the same magnitude as the analytical error. The uncertainties given in the certificate [3] includes variation

Table 1. Results of homogeneity test. Variations between units (s_{bu}) and analytical error (s_r). Test parameter F, between units relative standard deviation (CV_{bu}) per cent

Constituent	s_{bu}	s_r	F-test s^2_{bu}/s^2_r	CV_{bu} %
	g per 100 g			
Moisture	0.18	0.16	N.S.($p>0.05$)	0.3
Fat	0.35	0.22	$p<0.05$	1.7
Protein	0.14	0.14	N.S.	1.0
Ash	0.043	0.040	N.S.	0.2
Sodium chloride	0.015	0.015	N.S.	0.6
Hydroxyproline	0.0044	0.0055	N.S.	2.5

Table 2. Anaytical methods

Moisture	Drying with and without sand at 100° – 105 °C
Ash	Incineration at 550 °C
Fat	Schmid-Bondzynski-Ratslaff (SBR) method 22 laboratories, nuclear magnetic resonance spectroscopy (NMR) 2, Soxhlet 3, Soxhlet with acid hydrolysis 3 and EC method 1 laboratory
Nitrogen	Modified Kjeldahl methods
Sodium	Atomic absorption spectroscopy 16 and flame photometry 4 laboratories
Chloride	Electrochemical titration, complexometry and ion-selective electrodes
Hydroxyproline	Colorimetry

due to inhomogeneity for fat. The variations between units for the different constituents in terms of relative standard deviations (CV_{bu}) were between 0.3 and 1.7%, except for hydroxyproline, where a higher value (2.5) was observed, due to a low concentration.

Certifying procedure

Analyses of moisture, fat, protein, ash and sodium were performed by laboratories authorized by the Swedish National Food Administration (NFA). This authorization programme started ten years ago and includes regular supervision visits and participation in proficiency tests (intercalibrations). Sodium chloride and hydroxyproline analyses were performed by meat industry laboratories supervised by the MRI, in a manner similar to the NFA authorization.

During the long period of time that these proficiency tests have been running, many errors have been eliminated, and the variations have become lower. This is indicated in an investigation concerning the present laboratories [4], reporting the results obtained when the same material was analysed on two different occasions, with a three-year time lag.

Two cans of the meat material were distributed by mail to each laboratory together with instructions for pretreatment before sampling. Analyses were performed as blind duplicates simultaneously, as in an ordinary proficiency test. When returned to the NFA, the results were examined, and the outlying results were eliminated, using the normal probability test of the Minitab Statistical Program ($p<0.05$) [5].

Table 3. Composition of reference material "Meat 1989". Number of laboratories, eliminated results in brackets. Certified values, reproducibility standard deviation (s_R), 95/95 tolerance limits

Constituent	No. of labs	Content g/100 g	s_R g/100 g	95/95 tolerance limits
Moisture	34 (2)	61.7	0.49	60.8 – 62.6
Ash	29 (6)	3.15	0.053	3.05 – 3.25
Fat	31 (3)	20.8	0.58	19.8 – 21.8
Nitrogen, N	32	2.28	0.049	2.19 – 2.37
Protein (Nx6,25)	32	14.3	0.31	13.7 – 14.9
Sodium, Na	20	0.99	0.080	0.83 – 1.15
Salt, NaCl[a]	20	2.52	0.204	2.12 – 2.92
Salt, NaCl[b]	14	2.5	0.07	2.3 – 2.7
Hydroxyproline	6	0.22	0.008	0.20 – 0.24

[a] Calculated from the sodium content
[b] Calculated from the chloride content

Table 4. Precision in different studies. Reproducibility relative standard deviations, CV_R per cent

	Eurofoods trial [7]	Mixed diet material [8]	Elkins [9]	Horwitz „achievable" [10]	Present study
Moisture				2.2	0.8
Ash	3.4 – 7.2	3.5		3.4	1.7
Fat	5.7 – 59.8	13.9	6 – 12	2.5	2.8
Nitrogen, N				3.5	2.2
Protein (Nx6,25)	3.0 – 7.1	2.1	2 – 4	2.7	2.2
Sodium, Na				4.0	8.1
Hydroxyproline				4.9	3.6

Repeatability and reproducibility standard deviations were calculated using the same technique as in the homogeneity test.

The analytical methods used in the test are seen in Table 2. The certificate is designed according to directions from ISO Guide 31 [6].

Results and discussion

The trimmed pork used to produce the reference material is in its natural state heterogeneous, with variations in muscle, fat and connective tissue from piece to piece. The homogenizing is for that reason hard to perform. The homogenizing of a fresh material containing fat is furthermore very delicate because of the risk of fat separation. If the homogenizing step is prolonged, in order to increase the homogeneity, it might instead lead to inhomogeneity. We confirm that this material is homogeneous concerning all certified constituents except for fat, where the variation depending on inhomogeneity amounts to 20% of the total variation (s^2_R). This inhomogeneity is judged to be of no practical significance.

The certified values of the different constituents are seen in Table 3. The uncertainties are given as reproducibility standard deviations (s_R), including analytical error and variations between laboratories, methods and units. The 95/95 tolerance limits for each nutrient, as well as the relative reproducibility standard deviations, (CV_R), are also given.

The 95/95 tolerance limits give a range within which 95% of future determinations will fall, with a probability of 95%. They are presented as a guide to laboratories who use the material.

The lack of accuracy is the most important cause of error in a determination. In the present test many laboratories and different analytical methods are involved. The methods are issued by official organizations and designed for food analysis. The laboratories are accustomed to the procedures. This implies accurate determinations of the concentrations of the different constituents.

The precision of the results in the present study are compared to earlier studies in Table 4. No elimination of outliers was performed in the Eurofoods Trial [7], concerning six different materials and twenty laboratories, and this of course leads to the larger variations observed. In a Mixed Diet Material [8], the reproducibility CVs for protein, fat and ash correspond better to the present test, fat, however, still being higher. Elkins [9] reports variabilities for protein and fat of about the same levels or higher for two comparable products, analysed in collaborative tests organized by the Committee of Canning Industry Chemists.

Horwitz [10] states that the analytical variability is independent of the nature of the analyte or of the analytical technique used, and only due to the analyte level measured. The variability can be calculated, in reproducibility CV per cent, using an empirical equation drawn from a great number of collaborative tests. The reproducibility CVs in the present study in Table 4 are very much in agreement with the very rough values for "achievable" CV, calculated according to this equation, also in Table 4.

According to available literature, the variations obtained in this test can be considered sufficiently small for the material to be used as a reference material. Horwitz also states that improved experience improves the between-laboratory results, to the limit of his equation. The laboratories involved in this test are all experienced in the process of intercalibration, and the results of the present intercalibration, used as a certification trial, could perhaps be taken as proof of this statement.

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