DEVELOPMENT BY COLLABORATIVE STUDY OF 6 STANDARDIZED METHODS FOR DETERMINATION OF PROTEIN, FAT, SUGARS, MASS LOSS AFTER DRYING, AND ASH

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INTRODUCTION

The **Regulation (EU) No 1169/2011** of the European Parliament and of the Council of October 25, 2011, defines the mandatory information for **nutrition labelling** and specifies the rules for the declaration of the components of food products on the packaging. The amounts of **fat, carbohydrates, sugars, and protein** must be indicated in the nutrition declaration, but no reference methods are specified, and laboratories apply the methods of their choice on food matrices not yet covered by standardization (such as dairy, cereal, or meat products), which may lead to differences in analytical results. To overcome this lack of reference methods, new standards for the determination of fat, loss of mass after drying, ash, sugars, and protein have been developed that can be applied to any type of food not yet covered by a standard. A **collaborative study** was conducted to determine the **repeatability (r) and reproducibility (R) limits** of each method. This study was performed by 30 European laboratories on 10 samples of different compositions. Participating laboratories committed to strictly applying the methods described and to analyzing each sample under repeatability conditions. The statistical treatment was carried out according to the ISO 5725-5 and 5725-6 standards. Using statistical evaluation, it was possible to build a model for the distribution of precision values as a function of the concentration levels for the entire application field of the methods. Six new French standards have recently been published, providing laboratories with applicable reference methods for the determination of fat, loss of mass after drying, ash, protein, and sugar content in foodstuffs for nutritional labeling purposes.

DESIGN & IMPLEMENTATION

Main steps: drafting of the analytical methods, selection of participating laboratories, choice of the products to analyze, preparation of the samples, analysis of the samples by the laboratories, data collection, and statistical treatment of the results to evaluate the precision standard deviation of each tested method under repeatability conditions (variation under the same conditions) and reproducibility conditions (variation under different conditions).

Analytical methods	Tested products	Analyses	Statistical treatment
- **Mass loss after drying** (at 70°C ± 3°C under vacuum and at 103°C ± 3°C) - **Protein** (Kjeldahl method) - **Fat** (gravimetric method) - **Ash** (weight after incineration at 525°C ± 25°C) - **Sugars** (by two different methods : high-pressure liquid chromatography with refractometric detection (HPLC-RI) and high-performance anion- exchange chromatography with pulsed amperometric detection (HPAEC-PAD).	- Nutritional liquid mix - Biscuits with chocolate - Ready-to-eat meal - Fish (frozen) - Mayonnaise - Dried fruits - Sweet spread - Industrial pastry - Pistachio dessert cream - Egg The matrices were chosen to cover a large concentration range for each analyte.	• 30 participating laboratories from Austria (1), France (18), Germany (9), Portugal (1), and Spain (1). • One sample of each matrix was sent to each laboratory. For each tested method, laboratories performed two replicates under repeatability conditions. • Participation-is-dependent on the analysis and matrix, with a maximum of 30 participating laboratories for proteins in frozen fish.	Collected data was first analysed to identify outlier results and then treated according to the ISO 5725-5 and ISO 5725-6 standards [2]. Analyses of variance (ANOVA) were performed to determine the intra-laboratory and interlaboratory robust variances: for each sample, mean (x_{pt}), repeatability and reproducibility standard deviations (s_r and s_R), repeatability and reproducibility limits at 95 % (r and R), and repeatability and reproducibility coefficients of variation (CV_r and CV_R) were estimated.

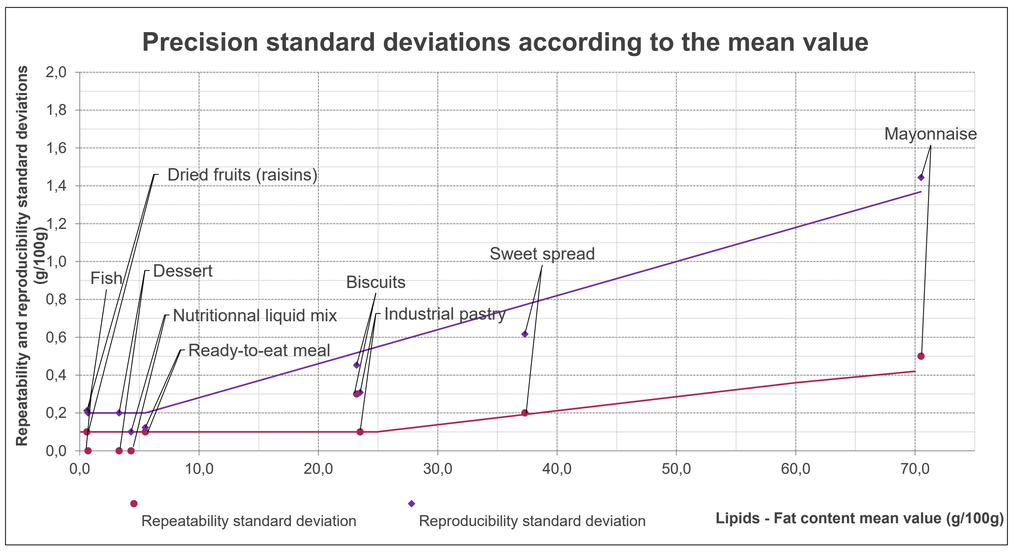
RESULTS & DISCUSSION

As an example of the statistical analysis, the results obtained with the proposed method for fat analysis are presented in Table 1 and Figure 1. Data analysis shows that the standard deviations tend to increase with higher mean values, which permitted the determination that, for this particular study:

- The difference between two independent single results, obtained using the same method on an identical sample in the same laboratory by the same operator using the same equipment within a short interval of time will, in no more than 5 % of the cases, exceed approximately 1.7% of the result, with a minimum absolute value of 0.3 g/100 g (repeatability limit, r).
- The difference between two independent single results, obtained using the same method on an identical sample in different laboratories by different operators using different equipment, will, in no more than 5 % of the cases, exceed approximately 5% of the result + 0.3, with a minimum absolute value of 0.6 g/100g (reproducibility limit, R).

FAT (g/100g)	Nutritional liquid mix	Biscuits	Ready-to-eat meal	Fish	Mayonnaise	Dried fruits	Sweet spread	Industrial pastry	Dessert
Total number of results, p	27	28	28	30	27	24	28	28	27
Number of results without outliers, p(x_{pt})	27	28	28	30	27	24	28	27	27
Robust mean, x_{pt}	4.3	23.2	5.5	0.7	70.5	0.6	37.3	23.5	3.3
Robust standard deviation, s*(x_{pt})	0.1	0.4	0.1	0.2	1.4	0.2	0.6	0.3	0.2
Repeatability standard deviation, s_r	0.0	0.3	0.1	0.0	0.5	0.1	0.2	0.1	0.0
Relative standard deviation, CV_r (s_r / x_{pt}) - %	0.0	1.3	1.8	0.0	0.7	16.7	0.5	0.4	0.0
Limit of repeatability, r	0.0	0.8	0.3	0.0	1.4	0.3	0.6	0.3	0.0
Reproducibility standard deviation, s_R	0.1	0.5	0.1	0.2	1.4	0.2	0.6	0.3	0.2
Relative standard deviation, CV_R (s_R / x_{pt}) - %	2.3	2.0	2.2	28.6	2.0	35.4	1.7	1.3	6.1
Limit of reproducibility, R	0.3	1.3	0.3	0.6	4.0	0.6	1.7	0.9	0.6

Table 1: Fat - Statistical results of interlaboratory tests



Function (Repeatability standard deviation s_r): Y = 0.6 % X - Minimum = 0.1 (g/100g)
Function (Reproducibility standard deviation s_R): Y = 1.8 % X + 0.2 - Minimum = 0.2 (g/100g)

Figure 1: Fat - Statistical results of interlaboratory tests

Content ranges validated:

- Mass loss after drying: 0.2 - 80.1 g/100 g
- Protein content: 1.1 - 12.7 g/100 g
- Fat content: 0.6 - 70.5 g/100 g
- Ash content: 0.50 - 2.01 g/100 g
- Sugars (total sum of glucose, fructose, sucrose, lactose, maltose and galactose): 0.40 - 71.16 g/100 g for HPLC-RI and 0.69 - 67.41 g/100g for HPAEC-PAD.

The repeatability and reproducibility limits estimated for all the tested methods are shown in Table 2. These limits were re-evaluated and compared with the first statistical estimations. as, Given the diversity of the matrices, the concentration ranges covered by the proposed methods and the performances of the laboratories, repeatability s_r and reproducibility s_R could be underestimated which would make the methods difficult to apply. for certain foods.

Analysis method	Repeatability limit (r)	Reproducibility limit (R)
Mass loss after drying at 70°C under vacuum	1.5%, with a minimum of 0.1 g/100g	8%, with a minimum of 0.4 g/100g and a maximum of 2.0 g/100g
Mass loss after drying at 103°C P_{atm}	1.5%, with a minimum of 0.2 g/100g	12%, with a minimum of 0.6 g/100g and a maximum at 2.0 g/100g
Protein	2%, with a minimum of 0.3 g/100g	9%
Fat	3%, with a minimum of 0.1 g/100g	6%, with a minimum of 0.2 g/100g
Ash	4.5%, with a minimum of 0.03 g/100g	10%
Sugars (HPLC-IR & HPAEC-PAD methods)	5%, with a minimum of 0.1g/100g	15%

Table 2: Repeatability limit (r) and reproducibility limit (R) defined for method application

CONCLUSION

Repeatability (r) and reproducibility (R) limits for 6 new analytical methods for mass loss after drying, ash, fat, protein and sugar content in food were estimated. Thanks to this study, the following French official standards could be published:

- ✓ **NF V03-801:2025** - Foodstuffs - Determination of **loss of mass on drying**
- ✓ **NF V03-032:2024** - Foodstuffs - Determination of the nitrogen content by the Kjeldahl method and calculation of the **protein content**
- ✓ **NF V03-426:2024** - Foodstuffs - Determination of total **fat content**
- ✓ **NF V03-802:2024** - Foodstuffs - Determination of **ash content**
- ✓ **NF V03-033-1:2025** - Foodstuffs - Determination of **sugars content** - Part 1: Method by liquid chromatography (high pressure) with refractometric detection
- ✓ **NF V03-033-2:2025** - Foodstuffs - Determination of **sugars content** - Part 2: Method by high-performance anion exchange chromatography coupled with detection by pulsed amperometric detection (HPAEC-PAD).

Next step → Implementation of these standards in the framework of the CEN (European Committee for Standardization).

REFERENCES

(1) Regulation - 1169/2011 - EN - Food Information to Consumers Regulation
(2) ISO 5725-5:1998 - Accuracy (trueness and precision) of measurement methods and results - Part 5: Alternative methods for the determination of the precision of a standard measurement method
(3) ISO 5725-6:1994 - Accuracy (trueness and precision) of measurement methods and results - Part 6: Use in practice of accuracy values