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The certification of major components and major elements in five food reference materials

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Summary. The development of five food reference materials (whole milk powder, pork muscle, rye and wheat flour, and haricot beans) is described. Homogeneity and stability of three categories of nutrients, major components, major elements and vitamins, proved to be adequate. Certification of Kjeldahl nitrogen, total fat, lactose, total dietary fibre (AOAC method), ash, Na, K, Mg, Ca, and Cl contents was successful. In contrast, only indicative values could be given for starch and sugars, nonstarch polysaccharides and P because of insufficient agreement between laboratories. The measurement of these components and elements need further study to obtain the improvement needed. Indicative values for retinol, β-carotene, α-tocopherol, vitamin B_1, vitamin C, and niacin in some of these materials could be given. Prospects for future certification of vitamins are favourable.

Introduction

Determination of the major components in food is required for the control of nutritional labelling (EC Council Directive 90/496/EEC), for nutritional surveys and for the compilation of food composition tables and databases. Although the methodology is generally considered to be well established, inter-laboratory studies have revealed a surprisingly poor level of reliability in many laboratories.

This paper describes the development of five reference materials and the procedures used to establish certified or indicative values for major components, major elements and vitamins. These CRMs are intended to provide a basis for method validation and laboratory quality control.

Materials, major components and major elements

Selection of reference materials, major components and major elements

The following materials were selected to cover the main categories of food:
- dairy products: whole milk powder (CRM 380)
- meats: freeze-dried pork muscle (CRM 384)
- cereals: rye flour (CRM 381) and wheat flour (CRM 382)
- vegetables: freeze-dried haricot beans (CRM 383)

The preparation of these reference materials as dry homogeneous powders packaged under dry nitrogen gas in double heat sealed plastic/aluminium laminated sachets, has been described previously [2].

The certification of the following major components (often called proximate constituents or major nutrients) was proposed: Kjeldahl nitrogen, total fat, lactose, available carbohydrates, starch, individual sugars, total dietary fibre, and ash.

For inorganic constituents, only certification of the major elements, Na, K, Mg, Ca, P and Cl, was undertaken because adequate CRMs for trace element analysis already exist. The feasibility of certification of the vitamin content in food CRMs was also studied in these materials. Results of a first intercomparison of methods showed that indicative values for retinol, α-tocopherol, vitamin B_1, vitamin C and niacin in some of these reference materials could be established.

Homogeneity

Major components proved to be homogeneously distributed in these materials as was shown by comparing the within-sachet and between-sachet variation with the method repeatability [2]. Retinol and α-tocopherol in milk powder (CRM 380) and Vitamin B_1 and B_2 in milk powder and pork muscle (CRM 384) were also shown to be sufficiently homogeneous.

Within-sachet and between-sachet homogeneity of elements were studied with inductively coupled plasma emission spectrometry on sample sizes of 100 to 500 mg. The within-sachet homogeneity was expressed as the coefficient of variation (CV) of 15 replicates from one sachet, the between-sachet homogeneity as the coefficient of variation of single analyses from 15 different sachet. The results obtained with the recommended minimum sample sizes, are summarised in Fig. 1 and demonstrate excellent homogeneity.

Stability

A 24 months stability study was carried out in one laboratory. All major components were monitored at regular intervals (3, 6, 12, 18 and 24 months) in sachets stored at −18°C, 20°C and 37°C. Vitamins were only monitored in sachets stored at −18°C and 4°C. Additionally, a limited study on the stability of α-tocopherol and vitamin C in haricot beans (CRM 383) was carried out.

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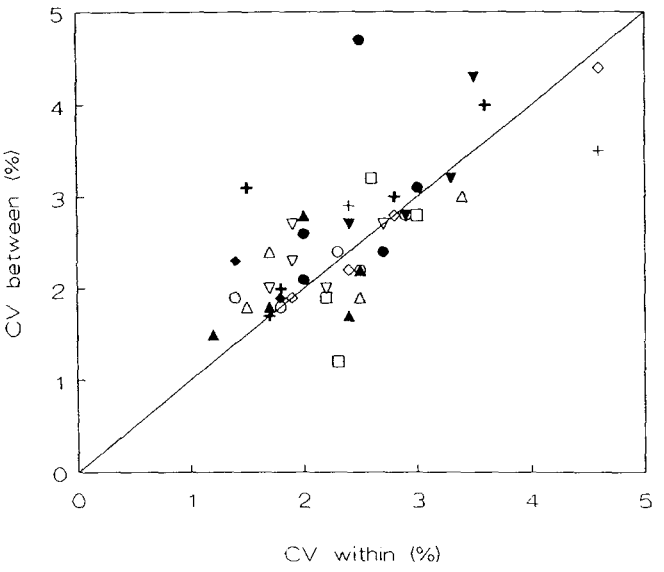

Fig. 1. Results of the homogeneity study for elements, comparing, for each material and element, the between-sachet variation (CV between) within-sachet variation (CV within). + Na; △K; ○ Mg; + Ca; ▲ P; ● Fe; ▽ Zn; ◇ Sr; □ Mn; ▼ Cu; ◆S

No evidence of change was found for major components with time or temperature for: total protein nitrogen, fructose, total dietary fibre and ash in any of the CRMs. In some of the CRMs, the following nutrients showed slight evidence of degradation but only at the highest storage temperature (37°C): total fat, available carbohydrates and lactose.

Vitamin B_1 and α-tocopherol proved to be stable in the CRMs at each temperature. No change in the content of vitamin B_2 in milk powder (CRM 380) was observed, however vitamin B_2 in pork muscle (CRM 384) showed a tendency to degrade with time at each temperature. The level of retinol in milk powder (CRM 380) fell during the first six months of storage, but stabilised afterwards. No difference in the vitamin C content of haricot beans (CRM 383) stored for 24 months at −40°C and 4°C, was observed. However, storage at 37°C resulted in almost complete destruction of vitamin C. It is concluded that the major components and vitamins tested are stable, provided the CRMs are stored at a temperature not higher than 4°C for long storage periods. They are sufficiently stable to allow normal postal shipments [11]. Vitamin B_2 in pork muscle (CRM 384) shows evidence of degradation at each temperature.

Intercomparison of methods for major components

A preliminary intercomparison of methods for major nutrients was organised in 1987 [2]. This intercomparison followed the design of the Eurofoods study of 1985 [1], but generally showed lower between-laboratory variation. In both studies, widely different values for fat, dietary fibre and carbohydrates were reported, caused largely by differences in methods.

Fat comprises a group of diverse components and those which are included in the fat value depend essentially on the type of method chosen, e.g. Folch-type [3], Weibull-Stoldt (WS)-type sometimes called Berntrop-type [4] or Schmid-Bondzynski-Ratzlaff (SBR)-type [5]. On both the 1985 and 1987 studies, it was found that with all samples, SBR-type methods gave systematically higher values than WS-type methods. Likewise results obtained for dietary fibre depend on the method used. It was concluded that separate certified or indicative values for fat and dietary fibre should be given for each type of method. Discussion of the results for carbohydrates with the participants indicated that inadequate solubilisation and incomplete hydrolysis were the major sources of error for starch determination. In the certification exercise general guidelines regarding solubilisation and hydrolysis of starch were given.

The coefficients of reproducibility (before deletion of outlying results) of the Eurofoods study (1985) and the certification study (1990) are compared in Fig. 2. Results for fat and fibre in the certification exercise are based on WS-type methods and AOAC method, respectively. A general improvement in results is noticeable.

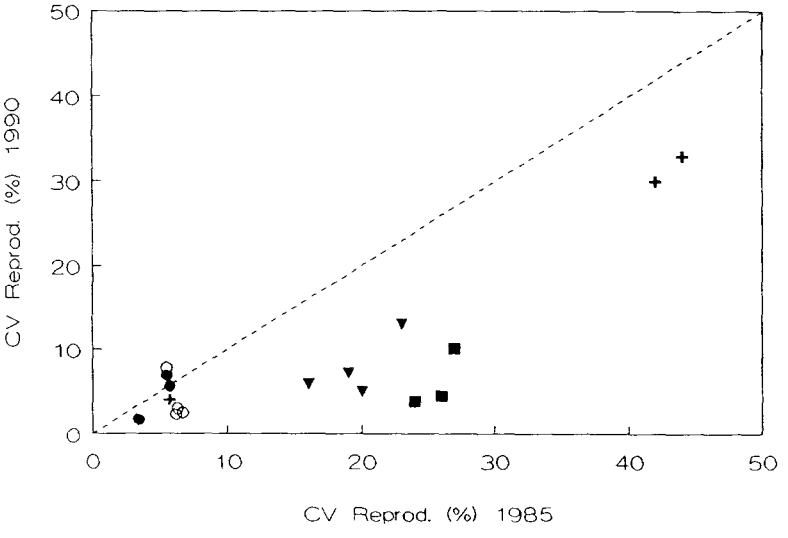

Fig. 2. Summary of the coefficients of variation of reproducibility (CV Reprod.) of the Euro-foods study 1985 and the Euro-foods study 1985 and the certification study 1990. Results for milk powder, wheat and rye flour and haricots beans are compared. The following nutrients were determined: ○ protein N; + total fat; ▼ available carbohydrates; ■ total dietary fibre; ● ash

Certification measurements

Participants made five separate determinations for each component on at least two different days. Each determination was made in a separately weighed subsample taken from at least two sachets. At the same time participants determined the moisture content of each sachet by a specified method involving either vacuum oven drying at 70 ± 2°C or drying at atmospheric pressure at 103 ± 2°C. No significant difference between the results of the two methods was evident. Each participant expressed his results on a dry mass basis.

Results were subjected to careful technical evaluation at a meeting of the participants.

Kjeldahl nitrogen

A wide range of catalysts (Cu, Se, HgO, TiO_2, Se/Cu, and TiO_2/Cu) and mineralisation procedures was used. The majority of the laboratories steam distilled the ammonia formed into boric acid, which was subsequently determined by titration using automated equipment. No difference in the results due to variations in the method was detected, and the certified values were based on the means of all accepted sets of results.

Total fat

For whole milk powder (CRM 380) certification is based on results by both Röse-Gottlied (5 sets) and Weibull-Stoldt (7 sets) methods (Table 1). Results were combined because no evidence of significant difference (T-test, $p < 0.05$) between these two types of methods was found (Table 2). Röse-Gottlieb methods applied were all nationally or internationally standardized methods. As can be expected results obtained by Röse-Gottlieb methods show better agreement between laboratories (Table 2).

Certification of total fat in pork muscle (CRM 384) is based on Weibull-Stoldt type methods only (Table 1). Four laboratories used (inter)nationally standardized methods. The certified values of cereals (CRM 381 and 382) are obtained with Weibull-Stoldt type methods only (Table 1).

Schmid-Bondzynski-Ratzlaff (SBR) type methods give erroneous results due to ether extractable compounds originating from acid hydrolysis of carbohydrates [2]. Table 2 illustrates that SBR-type methods give significantly (t-test, $p < 0.05$) higher values.

Lactose

The certified value for lactose in whole milk powder (CRM 380) includes enzymatic methods (7 sets) and HPLC methods (3 sets) (Table 1). Three laboratories used (inter)nationally standardized enzymatic methods. The expression of the results (anhydrous lactose or monohydrate) was found to be a common source of confusion. The certified value is expressed as anhydrous lactose.

Total dietary fibre

Total dietary fibre is largely defined by the type of method chosen. In the last few years, two different approaches for measuring total dietary fibre have evolved: the enzymatic-gravimetric procedures and procedures determining the sum of non-starch polysaccharides (Englyst-type).

Certified values for dietary fibre are based on the enzymatic-gravimetric procedure standardized by AOAC in 1985 [6] and on the "one-step AOAC procedure" of 1988 [8], which is almost identical to the 1985 procedure. A high level of agreement was achieved (Table 3). The sets of results obtained by Englyst-type procedures [9, 10] exhibited considerably larger scatter than those by the AOAC procedure (Table 3) and were used to calculate an indicative value.

Ash

The ash content is defined here as the residue remaining after ashing to constant mass at 550 ± 25°C (Table 1).

Table 1. Certified values for major components (g/100 g dry mass), between brackets the half-width of the 95% confidence interval of the certified value (g/100 g dry mass)

Major Component	Whole milk powder (CRM 380)		Pork muscle (CRM 384)		Rye flour (CRM 381		Wheat flour (CRM 382)		Haricots verts beans CRM 383)	
	Cert. value	p	Cert. value	p	Cert. value	p	Cert. value	p	Cert. value	p
Kjeldahl nitrogen	4.50 (0.04)	17	13.7 (0.2)	13	1.3 (0.1)	16	2.1 (0.1)	15	1.1 (0.1)	16
Total fat	26.9 (0.3)	12	10.8 (0.2)	9	1.1 (0.1)	8	1.3 (0.1)	7	-	-
Lactose (anhydrous)	36.3 (0.5)	10	-	-	-	-	-	-	-	-
Total dietary fibre (AOAC)	-	-	-	-	8.2 (0.2)	9	3.3 (0.1)	7	11.9 (0.2)	9
Ash at 550° ± 25°C	6.07 (0.5)	12	4.6 (0.2)	6	0.9 (0.1)	9	0.9 (0.1)	9	2.4 (0.1)	8

p = number of sets of results

Table 2. Summary of the results of the total fat determination (g/100 g dry mass) by Röse-Gottlieb (RG), Weibull-Stoldt (WS) and Schmid-Bondzynski-Ratzlaff (SBR) methods

	Whole milk powder CRM 380		Rye flour CRM 381		Wheat flour CRM 382	
	RG[a]	WS[a]	WS	SBR[b]	WS	SBR[b]
Means of means	26.92	26.84	1.06	1.71	1.26	1.70
Standard deviation	0.15	0.53	0.08	0.40	0.05	0.39
Number of labs	5	7	8	4	7	4
Difference significant?	no		yes		yes	

[a] not certified separately

[b] not certified

Table 3. Summary of the results of the total dietary fibre determination (g fibre/100 g dry mass) by AOAC and Englyst-type methods

	Rye flour CRM 381		Wheat flour CRM 382		Haricots verts beans CRM 383	
	AOAC	Englyst	AOAC	Englyst[a]	AOAC	Englyst[a]
Mean of means	8.2	7.7	3.3	3.1	11.9	10.9
Standard deviation	0.2	0.7	0.1	0.8	0.2	1.1
p	9	5	7	5	9	5

[a] not certified, indicative only

p = number of sets of results

Major elements: Na, K, Ca, Mg. Cl and P

The certification of major elements was attempted in rye flour (CRM 381), haricot beans (CRM 383) and pork muscle (CRM 384) using the techniques summarised in Table 4. Because of poor agreement of results no certified value for Ca in CRM 384 and Mg in CRM 383 can be given.

The poor agreement of the results for P in CRM 381, 383 & 384 and Cl in CRM 383 & 384 did not permit certification of these major elements. A summary of the certified values for major elements is given in Table 5.

Indicative values

Available carbohydrates (starch + sugars)

Despite preceding intercomparisons the level of agreement for starch and available carbohydrates was insufficient to allow certification of reference values in cereals and beans. Results for starch obtained with enzymatic-enzymatic (2 sets), enzymatic-HPLC (1 set) and polarimetric (2 sets) methods are summarised in Table 6. For haricot beans (CRM 383) no indicative value can be given because of major discrepancies between laboratories.

Indicative values given for available carbohydrates (starch + sugars) in Table 6 are obtained by enzymatic-HPLC (1 set) and enzymatic-reductiometric (4 sets) methods.

Table 4. Summary of techniques applied for the determination of major elements

Element	Technique	Number of labs
Na, K	Inductively couple plasma emission spectroscopy (ICP)	2
	Instrumental neutron activation analysis (INAA)	2
	Atomic Absorption spectrometry (AAS)	1
Ca	Inductively coupled plasma emission spectroscopy (ICP)	2
	Isotope dilution mass spectrometry (IDMS)	1
	Instrumental neutron activation analysis (INAA)[a]	1
	Atomic absorption spectrometry (AAS)	1
Mg	Inductively coupled plasma emission spectroscopy (ICP)	2
	Isotope dilution mass spectrometry (IDMS)	1
	Atomic absorption spectrometry (AAS)	1
Cl	Ion chromatography	1
	Instrumental neutron activation analysis (INAA)	1
	Titration	1

[a] not included in certified value

Table 5. Certified values for major elements (mg/g dry mass), between brackets the half-width of the 95% confidence interval of the certified value (mg/g dry mass)

Major element	Pork muscle CRM 384		Rye flour CRM 381		Haricots verts beans CRM 383	
	Cert. value	p	Cert. value	p	Cert. value	p
Na	2.8 (0.1)	4	0.02 (0.01)	5	0.08 (0.01)	5
K	15.5 (0.8)	5	2.9 (0.2)	5	7.8 (0.2)	5
Mg	1.00 (0.04)	4	0.4 (0.1)	4	-	-
Ca	-	-	0.2 (0.1)	4	2.9 (0.1)	4
Cl	-	-	0.5 (0.1)	3	-1	-

p = number of sets of results

Phosphorus and chlorine

Indicative values for Cl in pork muscle (CRM 384) are based on 3 methods given in Table 4. For CRM 383 no indicative value can be given because of large differences between laboratories. The indicative values for phosphorus were obtained with ICP (2 sets) and INAA (1 set).

Vitamins

An intercomparison of methods gave good agreement for retinol, α-tocopherol, vitamin B_1, vitamin C and niacin in some of these materials. Since short- and long-term stability of the most labile vitamins proved to be adequate (see above) indicative values for several vitamin/RM combination are proposed.

The methods used and the indicative values are summarised in Tables 7 and 8, respectively.

Table 6. Indicative values (g/100 g dry mass) for major components

Major component	Rye flour CRM 381			Wheat flour CRM 382			Haricot beans CRM 383		
	Mean	1SD	p	Mean	1SD	p	Mean	1SD	p
Starch[a]	75.9	2.6	5	79.6	1.8	5	-	-	-
Available[b] carbohydrates	90	5	5	89	4	5	78.9	1.3	5
Glucose	-	-	-	-	-	-	12.4	0.9	4
Fructose	-	-	-	-	-	-	4.6	0.3	5
Sucrose	-	-	-	-	-	-	1.0	0.4	4
Total dietary fibre (Englyst)	7.7	0.7	6	3.1	0.8	6	10.9	1.1	6

SD = standard deviation of means of laboratories
p = number of sets of results
[a] expressed as g polysaccharides/100 g dry mass
[b] expressed as g monosaccharides/100 g dry mass

Table 6a. Indicative values (mg/g dry mass) for major elements

Major element	Pork muscle CRM 384			Rye flour CRM 381			Haricot beans CRM 383		
	Mean	1SD	p	Mean	1SD	p	Mean	1SD	p
Ca	0.2	0.1	4	-	-	-	-	-	-
P	8.5	0.3	3	1.6	0.1	3	1.8	0.1	3
Cl	3.0	0.3	3	-	-	-	-	-	-
Mg	-	-	-	-	-	-	0.9	0.1	4

Table 7: Summary of methods as applied for the determination of vitamins

Vitamin	Method in key words	Number of labs
Retinol	Saponification, reversed phase HPLC, UV-detection	5
	Saponification, reversed phase HPLC, fluoresc. detection	2
	Saponification, normal phase HPLC, UV-detection	3
	Saponification, normal phase HPLC, fluoresc. detection	2
α-Tocopherol	Saponification, reversed phase HPLC, UV-detection	2
	Saponification, reversed phase HPLC, fluoresc. detection	2
	Saponification, normal phase HPLC, UV-detection	1
	Saponification, normal phase HPLC, fluoresc, detection	7
Vitamin B_1	Reversed phase HPLC, separation as thiamin, fluorescence detection	3
	Reversed phase HPLC, separation as thiochrome, fluorescence detection	3
	Fluorimetric thiochrome	2
	Microbiological assay, *lactobacillus viridescence*	1
Vitamin C	Reversed phase HPLC, separation as quinoxaline derivative, fluorescence detection	4
	Reversed phase HPLC, separation as L-absorbic acid, UV-detection	2
	Fluorimetric method	3
Niacin	Microbiological assay, *lactobacillus plantarum*	All

Table 8. Indicative values (mg/100 g dry mass) for vitamins

Vitamins	Whole milk powder CRM 380			Pork muscle CRM 384			Haricots verts beans CRM 383		
	Mean	1SD	p	Mean	1SD	p	Mean	1SD	p
Retinol	0.27	0.03	12	-	-	-	-	-	-
α-Tocopherol	0.6	0.1	11	-	-	-	0.34	0.06	10
Vitamin B_1	0.32	0.05	8	3.1	0.3	9	0.22	0.04	9
Vitamin C	-	-	-	-	-	-	15.1	1.4	9
Niacin	0.83	0.07	7	24	2.7	6	1.7	0.3	7

SD = standard deviation of means of laboratories
p = number of sets of results

Conclusions

Preliminary intercomparisons showed the need for urgent improvement in the quality of the analyses of major components and major elements in food laboratories, and identified many specific sources of errors. The resulting improvement in methodology allowed certified values for Kjeldahl nitrogen, total fat, lactose, total dietary fibre (AOAC method), ash, Na, K, Mg, Ca, and Cl in five stable and homogeneous food reference materials to be established.

However, methodology for carbohydrates, starch and sugars, and non-starch polysaccharides, still needs further attention. Surprisingly, results obtained by highly specialised laboratories for P and, for two out of three materials, Cl showed an unacceptable level of agreement.

Prospects for the future certification of vitamins are favourable both from an analytical point of view and stability. Indicative values for retinol, α-tocopherol, vitamin B_1, vitamin C, and niacin in some of these RMs are given.

These food reference materials will provide a basis for quality control programmes and the preparation of secondary RMs in food laboratories, which will lead to the improvement of the quality of nutritional analysis.

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