Reference material for improving the quality of nutritional composition data for foods

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Summary. Analytical values for the nutritional composition of foods are required for a range of purposes, in particular, for the construction of nutritional data bases. These provide the basis for estimating nutrient intakes in population groups, and for the nutritional labelling of foods. Both of these uses requires analytical values that accurately represent the composition of the foods. The requisite quality can only be achieved by the institution of a sound quality assurance programme in which reference materials have a vital role.

The special features of nutritional analyses and their control with reference materials are reviewed and the criteria for the production of these reference materials discussed.

Introduction

The growing evidence that the incidence of many non-infective diseases is related to dietary factors has lead to an increased interest in the nutritional composition of foods [17]. Many governments have, on the basis of expert examination of the evidence, introduced proposals for desirable dietary changes within their populations, and consider that the nutritional labelling of foods would assist consumers in their choice of an appropriate and, by inference, more healthy diet [5]. Those involved in the production and retailing of foods have also seen nutritional labelling as being useful and desired by their customers. These two factors are leading to substantial growth in the nutritional analysis of foods and the consequent need for the regulation and control of the analysis. At the same time nutritional research in the epidemiological studies is frequently dependent on international comparisons [13] and is therefore dependent on having access to a body of sound, comparable nutritional data for the composition of foods. The quality of this analytical data is crucial to ensuring compatability between data from different sources [16].

The world quality implies fitness for the purpose for which the analytical data are produced. This paper gives special attention to the major, and growing, areas where nutritional data are used. These are the construction of nutrient data bases and nutritional labelling.

While these two areas have some distinctive features they have a unifying requirement for the analytical values to accurately represent the composition of the foods being analysed. Achieving the required accuracy on a consistent basis requires the laboratories involved to operate a quality assurance programme [18]. In this respect nutritional analysis is not different from any other analytical activity.

Analyses for the production of nutritional data bases because of the widespread subsequent use of the data in nutritional research, therapeutic clinical nutrition, and in the development of food and nutrition policies by governments and international agencies has special requirements for representative analytical data [13]. Analogously nutritional labelling may form part of the statutory requirements for food labelling and food compositional standards and thus the analytical values are required for the regulatory process and therefore must stand the rigours of legal examination [6].

The major quality requirement is therefore, accurate compositional data for samples that are representative of the foods in question. Sampling is crucial to the production of reliable analytical values and it is vital that sampling protocols are designed and executed with the direct involvement of the analysts [8].

Quality assurance programmes

These have been the subject of detailed discussion [18] and the main features are summarised briefly in Table 1. One important feature of all programmes relates to staff training, management and motivation — analytical quality is determined to a great extent by the worker at the bench and a programme will not succeed unless all of those involved appreciate the need to maintain standards and feel that they can contribute. Thus as far as is possible, the analytical workers should understand the reasons for the analyses and the uses to which the data will be put. The analysts also appreciate the limitations of the data they generate and they therefore, also have a role to play in advising, and ideally controlling the subsequent use of the data they have generated [8].

Reference materials provide a means of controlling analytical procedures and are also valuable in the choice of procedure and in training of laboratory staff in new procedures [19].

Special features of nutrient analysis

Although many nutrients are not present in low concentrations when compared with many contaminants and natural toxicants, there are some special features of nutrient analysis that define the types of reference materials required and the criteria to be used in their selection and production.

First, the complexity and variety of food matrices in both natural foodstuffs and the complex mixtures found in many

Table 1. Main features of a quality assurance programme

Stage	Objective
Training, supervision and motivation of staff	Acceptance of need for maintenance of standards and means of ensuring standards
Organisation of record keeping	To follow progress of sample through the various analytical operations
Design and execution of sampling protocols	Samples are representative of the foods at the point of sampling
Storage and preparation of samples	Composition is preserved and contamination excluded
Choice of analytical methods	Analysis provides accurate values for the composition of the representative samples
Evaluation of analytical values	

Based on [8]

Table 2. Major factors in the selection of reference materials

Variety of food matrices	A series of matrices to cover range of types of matrix to be met in practice
Form and distribution of nutrients in foods	Materials must cover adequately the nutrients to be measured and the range of forms of the nutrients present
Species of nutrients	Materials must present the range of separation operations likely to be met in practice
Protection of labile nutrients	Materials must contain labile nutrients. Preservation and storage conditions must be devised to stabilise composition

Fig. 1. Matrix effects and sources of error in inorganic analyses

processed foods. This means that a variety of matrix effects are likely and reference materials must reflect this variety.

Second, the fact that many nutrients are either very closely associated with, or covalently linked to other components of the matrix. Many nutrients must therefore be extracted from the matrix, often after chemical or enzymatic treatment to release them from the matrix. These stages can only be controlled analytically by suitable reference materials.

Third, many nutrients, especially the vitamins, are members of classes of compounds where the biological nutritional activity differs between the various members, so that analysis of the nutritionally active components requires some separation of the extracted components during the analysis — these operations can best be controlled from the initial stage, the analytical sample [3]. Added to this is the need to protect labile nutrients, in the forms that they are present in foods, during extraction and separation.

These features are not confined to nutrients but they do define the major factors that must be considered in selecting reference materials, which are summarised in Table 2. The
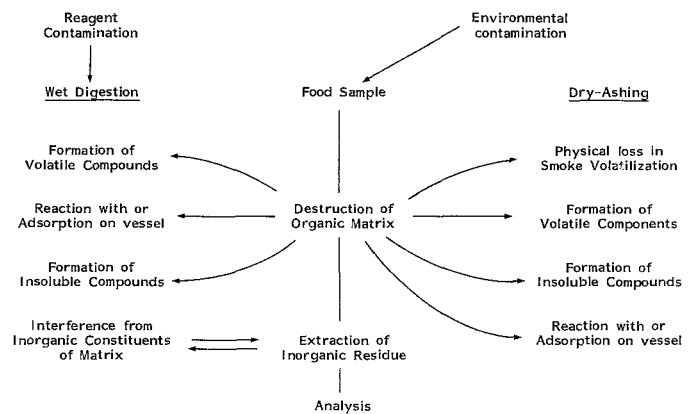
lability of some nutrients presents some very difficult issues regarding the stability of these nutrients in the reference materials. At the present time it is not possible to define the appropriate conditions for some nutrients until the results of studies on their stability in a reference matrix are available.

I would like to discuss the types of material that are required and to do this against the background of the stages of nutrient analyses.

Types of reference material

For the purposes of discussion it is convenient to group the nutrients into three major categories:

a) *Major components* — often called 'proximate constituents', water, protein, fat and the carbohydrates.

b) *Inorganic constituents* — it is customary to consider these in two subgroups — trace constituents with concentrations of the order of µg/kg such as Cu, Mn, Cr, I, F, Co and major inorganic constituents with concentrations of the order of mg/kg such as Na, K, Ca, Mg, P, Cl, although these divisions are arbitrary and two important inorganic nutrients, Fe and Zn lie at the boundary.

c) *Vitamins* — these are usually considered in two subgroups, water-soluble such as vitamins of the B-complex, vitamin C, folates, and lipid-soluble such as vitamins A, D and E. The distribution and stability of these two groups are distinctive and the division is convenient in the present discussion [10].

Reference materials for inorganic nutrients

There is already a wide experience of the use of reference materials in the control of inorganic nutrient analysis [20]. The analytical procedures used in inorganic analysis and the stages that can be controlled by reference materials are similar for both the trace and major inorganic nutrients. Contamination is a major concern in the analysis of trace constituents particularly those that are common in the environment.

The stages in a typical inorganic analytical protocol are set out in Fig. 1, illustrating that two different types of matrix effect are possible.

The first arises during the destruction of the organic matrix which typically is the first stage in the analysis. Two major procedures are used with several variants.

Wet-digestion — wet ashing. In this, the organic matrix is oxidised with inorganic acid with or without an oxidising agent. The major cause of contamination is the reagents used — and the matrix effects producing underestimation are broadly of three types — loss of volatile components, formation of insoluble constituents and adsorption on (or combination with) the reaction vessel [4]. In principle these latter two matrix effects may be controlled by the use of added standards in a recovery type study but they may depend on the actual form of the nutrient in the food. Losses of volatile components during oxidation are, entirely, matrix dependent and require standard reference materials.

Incineration — dry ashing. This procedure is subject to a similar range of matrix effects which include mechanical losses in smoke, volatilisation due to locally (or general) excessive temperatures, formation of volatile components, together with adsorption/combination with the vessel and the formation of insoluble materials. The temperatures involved are generally much higher and the complexity of the decomposition reactions of the matrix during pyrolysis [2] makes prediction of matrix effects very difficult. As with wet ashing the use of added (recovery) standards may control some stages likely to produce error.

Analysis of inorganic residue. The residual acidic solution usually requires dilution before analysis and some precipitation may occur with elements forming insoluble salts, e.g. sulphates. The solubilisation of the inorganic residue presents more problems such as adsorption to residual carbon, and the formation of insoluble complexes such as silicates and sulphates [4]. These stages can be controlled by standard additions.

Matrix interference in the actual analytical operation are of major concern since the analyte is accompanied by a range of other constituents in proportions that are typical of the food matrix. While it is possible to simulate these matrix effects by the preparation of appropriate complex standard mixtures — reference materials providing the inorganic constituents in the same proportions as those in the food are advantageous.

Thus, although many matrix effects can be controlled by procedures other than the use of reference materials — some cannot and in many other respects reference materials are more satisfactory since they provide an independent validation of the entire analytical operation. The practice of using deviant analyses of the reference material to derive 'correction' factors is totally unsound in a quality assurance programme since this assumes systematic and non-matrix related error. The proper approach is to seek out the source of the error by the use of other, recovery type, studies at the various stages.

It is appropriate at this point to consider some general points in relation to analytical operations and systematic errors. Strictly speaking systematic errors can only be identified by the use of a range of methods ideally based on different principles. It is, however, useful to consider the conditions that lead to the standardisation of systematic errors because variation of these conditions can identify where an analytical procedure is not robust. Table 3 lists the

Table 3. Operational factors that standardise systematic errors

Sample-size	Identical or closely similar sized analytical samples
Reagents	Reagents from the same batch
Standard solutions	Prepared from same stock, or same series of dilutions used
Replicate analyses	Analysed in the same batch or the same time
Operator	Only one analytical operator
Choice of procedure	One procedure alone

Work with replicates of different size. Analyse replicate analyses in different batches on different days and using different standard dilutions and different operators

operational factors that standardise systematic error and which should be abandoned as part of a quality assurance programme.

Separate reference materials are not required for trace and major inorganic constituents since the only real factors that are different in trace analysis are avoidance of contamination and the greater significance of absorption onto the surfaces of reaction vessels from dilute solutions.

Major components

It is incorrect to assume that because the proximate constituents of food have been measured by analysts for over 100 years that there is no requirement for reference materials to ensure accuracy and compatibility of results from different laboratories [1]. In collaborative studies carried out in Europe [9] the agreement between laboratories for protein — as measured from total-N values, fat and carbohydrates was often poorer than within laboratory replication. While some discrepancies were due to the use of different, although, standard procedures there was evidence of poor replication. Fat values were extremely discordant and carbohydrate values even more so.

Total N. Matrix decomposition in the Kjeldahl digestion is very complex and complete oxidation of all amino acid nitrogen cannot be assumed.

Fat. Matrix effects are particularly important in the measurement of fat and cannot be controlled by recovery studies because food lipids are often present complexed with protein or in emulsions — and chemical treatment is required prior to extraction.

Carbohydrates. Most foods contain a range of carbohydrates species [15], free sugars (mono-, di-, and oligo saccharides) which may be in solution within the matrix or which may form part of the solid food matrix; polysaccharides such as starch, which may be within a plant structure — and be contained within the intact native starch granule or in various stages of disrupted granule. In many foods, starch together with the polysaccharides of the plant cell wall structures and related polysaccharides, form the major part of the solid food matrix. The polysaccharides of the cell

Table 4. Provisional list of reference materials for nutrient analysis

Wheat flour, white, bread making 72% extraction
Rye flour, wholemeal
Oats, rolled
Potato, powder
Carrots, freeze-dried powder
Cabbage, freeze-dried powder
Milk, whole dried powder
Egg, powder
Beef, minced steak, freeze-dried
Liver, pig, freeze-dried
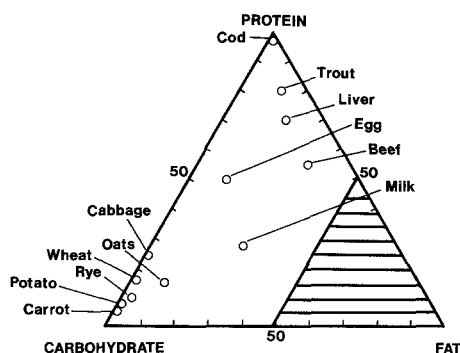Trout, freeze-dried
Cod, freeze-dried flesh

Fig. 2. Distribution of suggested reference materials within the protein, fat and carbohydrate matrix. (The *crosshatched area* includes foods with more that 50% fat)
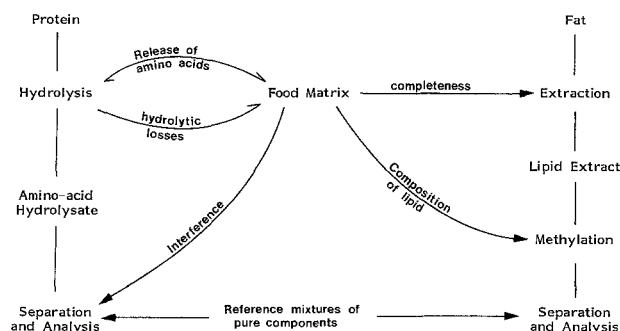

Fig. 3. Matrix effects in amino-acid and fatty acid compositional analyses

wall structure are combined in the complex supramolecular architecture of the cell wall.

Measurement of the available carbohydrates requires extraction and separation of the free sugars and extraction and hydrolysis of the starch [15]. The non-starch polysaccharides also require extraction before analysis and complete analysis requires separation and hydrolysis. Matrix effects on both composition and extraction are critical [4]. The principal matrix effects relate to completeness of extraction and the composition of the analyte. While the latter can be controlled with reference standard mixtures, complete control requires reference materials.

Criteria for reference materials

There are two major requirements. First, the matrices should be typical foods and the range of matrices cover the range

of foods likely to be met by the laboratory. Second, the range of concentrations of the analytes within the matrix should reflect the ranges likely to be met in practice.

Other technical criteria are that the matrix should be stable, preferably readily available and capable of being presented as a homogeneous powder to the analyst.

Table 4 lists a number of food materials that might be considered as providing a suitable range of matrices.

Figure 2 shows the distribution of matrix within the range of all possible mixtures, of protein, fat and carbohydrate of the food materials listed in Table 4.

This identifies regions of the food matrix that are not adequately reflected. Thus foods with more than 50% fat are not represented.

These materials would also provide reference materials for qualitative compositional standards for the amino acid composition of proteins and the fatty acid composition of fats. While the end analyses of these two attributes are routinely controlled with standard reference mixtures these do not control the critical stages of these two analytical operations (Fig. 3).

Certification of materials

Ideally a standard reference method should be used for certification — while this presents only minor difficulties for total nitrogen, and fat — the carbohydrates especially the non-starch polysaccharides are more problematical [14] and it will probably be necessary to proceed by a sequential certification process using closely defined methods [7,12]. In an ideal world one would like to see a number of procedures giving closely similar values.

Vitamins

A similar range of matrices is desirable because the most important matrix effects are on completeness of extraction and the composition of the analyte. The major problems are stability of the vitamins in the stored materials [10].

Water-soluble vitamins. A number of studies on cereals, milk powders and a few other foods provide information that suggests that the appropriate stability may be achieved for thiamin, riboflavin and niacin and possibly B_6 — but folates and vitamin C may present very nearly insuperable storage problems [11].

Lipid-soluble vitamins. These present greater problems as even within a lipid solvent system stability is difficult to maintain [3]. It may well be that, on the basis of present knowledge, a limited number of materials may be suitable for a few vitamins. I believe that we need to examine actual stability in some bulk reference materials (such as those prepared for the inorganic or major components) combined with a deliberate experimental search for suitable storage conditions. This is a complex task but the improvements in the quality of analyses that would result outweigh the difficulties.

Conclusion

It is possible to select a range of food matrices which provide a means for controlling nutritional analysis. These represent

the range of matrix composition and analyte composition likely to be met in practice and a programme to implement the preparation and certification of these materials should be set in hand.

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