

Food nutrition evaluation

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1 Introduction

The variation in nutrient content is important because of the effects it can have in meeting nutritional requirements. It is therefore important to analyse the composition of the genetically modified food and to establish the extent to which the product is equivalent to its non-genetically modified counterpart. Statistically significant differences in composition may warrant closer examination during the safety assessment process of the food product with respect to the genetic modification. Furthermore, an estimate of the expected intake is necessary for the safety evaluation of the genetically modified food and for the evaluation of its nutritional significance.

2 Compositional analysis

2.1 Compositional data and methods

This section has to present the proximate analysis of the matter, to describe the sampling procedure, to refer to the analysis methods and to precise the statistical distribution of the results.

2.1.1 Major and minor constituents

A non-exhaustive checklist as presented hereafter provides information on critical parameters of food safety and nutrition. Depending of the crop and/or derived food product to be considered, several components may be not relevant.

Checklist for proximate composition analysis

- Moisture % of wet weight
- Protein % of dry weight
- Total fat % of dry weight
- Ash % of dry weight
- Total carbohydrates % of dry weight

- Amino acids % of dry weight and % of total amino acids
 - Essential and semi-essential amino acids: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine
 - Non-essential amino acids: alanine, asparagine, aspartic acid, cysteine, cystine, glutamic acid, glutamine, glycine, proline, serine, tyrosine

- Fatty acids % of dry weight and % of total fat
 - See Table 1 of Chapter V for the classification of fatty acids.

- Carbohydrates % of dry weight
 - Reducing sugars
 - Mono and disaccharides
 - Starch
 - Other polysaccharides

- Dietary fibre % of dry weight
 - soluble fibre, with further specification if relevant
 - insoluble fibre, with further specification if relevant

- Mineral composition and trace-elements
 - Na, K, Ca, P, Mg, Fe, Mn, Cu, Zn, S, I (mg / 100g)
 - Se, Cl, Pb, Co, Cr, Cd, Hg (mg / kg)

- Vitamins
 - Fat soluble vitamins: Vitamin A (retinol) ($\mu\text{g}/100\text{g}$), Vitamin D₃ (cholecalciferol) ($\mu\text{g}/100\text{g}$), Vitamin D₂ (ergocalciferol) ($\mu\text{g}/100\text{g}$), Vitamin E (-tocopherol) ($\text{mg}/100\text{g}$), Vitamin K (phylloquinone) (mg/kg), -carotene (mg/kg)
 - Water soluble vitamins: Vitamin B₁ (thiamine) (mg/kg), Vitamin B₂ (riboflavin) (mg/kg), Vitamin B₆ (pyridoxine) (mg/kg), Niacin (mg/kg), Pantothenic acid (mg/kg), Folic acid (mg/kg), Biotin (mg/kg), Vitamin B₁₂ (cobalamin) (mg/kg), Vitamin C (ascorbic acid) (mg/kg)

2.1.2 Analytical methods

Reference methods must be used and mentioned. European standardized validated methods will be preferred but other official methods will be considered.

Following considerations may be useful for the choice of these methods. There is not a unique model for chemical and nutritional analysis of a food product. The nature

and finality of the product are to be taken into account. Nevertheless the choice of the analytical method is crucial for the validation and the signification of the result.

Proteins

The "conventional" test for protein measurement is based on the N content (Kjeldhall method). AOAC has proposed the modalities for nitrogen analysis in grains, animal products and milk products. In some cases (e.g. milk products) it is recommended to precipitate proteins (with trichloroacetic acid) in order to estimate the real protein nitrogen quantity and not the total nitrogen quantity. Nutritional values are not the same for proteins and non proteic nitrogen compounds. In the USA the Dumas method is an official method for protein measurement in cereals. A strong correlation exists between the two methods (Kjeldhall and Dumas).

Amino acids

Total amino acids composition is obtained after acid hydrolysis of peptidic links and separation by ion exchange chromatography. Conventional methods can be applied to all amino acids with the exception of tryptophan (totally destroyed) and sulfur amino acid that are oxidized. Asparagine and glutamine are transformed in aspartic acid and glutamic acid. Alkaline hydrolysis is the obligatory pathway for tryptophan analysis. Sulfur amino acids can be estimated after oxidation.

Fatty acids

Lipids are mainly composed of hydrophobic units. Their solubility characteristics, rather than a common structural feature, are unique for this class of compounds. The majority of lipids are derivatives of fatty acids.

Some lipids act as building blocks in the formation of biological membranes. They occur in food but usually at less than 2%. Nevertheless, even as minor food constituents they must receive particular attention, since their reactivity may strongly influence the organoleptic quality.

Triacylglycerols (also called triglycerides) are deposited in several animal tissues and organs of some plants. Lipid content in such storage tissues are a commercial source of lipids that can rise up to 20%.

The class of lipids (see Table 1: classification of lipids according to "acyl residue" characteristics) also includes some important food aroma substances or precursors, as well as amphiphilic substances, pigments, vitamins, colorants....

Table 1 : Classification of lipids according to "acyl residue" characteristics

| Class of lipids | Constituents |
|---|--|
| Simple lipids (not saponifiable) | Free fatty acids, isoprenoid lipids (steroids, carotenoids, monoterpenes), tocopherols |
| Acyl lipids (saponifiable) | |
| Mono-, di-, triacyl-glycerols | Fatty acids, glycerol |
| Phospholipids (phosphatides) | Fatty acids, glycerol or sphingosine, phosphoric acid, organic base |
| Glycolipids | Fatty acids, glycerol or sphingosine, mono-, di- or oligosaccharide |
| Diols lipids | Fatty acids, ethane, propane, or butane diol |
| Waxes | Fatty acids, fatty alcohols |
| Sterol and stanol esters | Fatty acids, sterols, stanols |

Total fat can be determined after extraction and gravimetric estimation. Some methods (e.g. Folch) allow to identify the different categories of extracted lipids afterwards. Hydrolysis methods (e.g. acid hydrolysis) have been standardised but these methods do not allow to characterise the individual lipid classes, only the total fatty acids.. A lot of specific methods have been standardized with regard to the kind of raw materials (grains, oleaginous crops...).

Lipids in fats are a very heterogeneous category of components. The extraction procedure is very important in order to obtain all the fractions and to prevent the extraction of other materials (hydrophobic proteins...). Fatty acids can be determined by gas chromatography after saponification and esterification.

It will be very useful to distinguish the 6 and the 3 families for nutritional aspects.

Carbohydrates

Carbohydrates are commonly divided into monosaccharides, oligosaccharides and polysaccharides.

Total reducing power after chemical hydrolysis gives an approximation of the digestible glucids (sugars + starch). Chromatographic methods (CPG and HPLC) allow to obtain individual sugars.

Starch can be measured after gelatinization, liquefaction and hydrolysis into glucose.

Dietary fibres

Dietary fibres may be composed of soluble and insoluble constituents. Because of the large diversity of undigestible materials, analysis is difficult. Enzymatical methods are preferable to the Van Soest method even if this last technique has been standardized in some countries, especially for cereals.

The Van Soest method gives values similar to those obtained in vivo from digestibility studies with animals. This technique allows to determine the concentration of cellulose, lignin and hemicelluloses. Nevertheless, the Van Soest method does not correspond to the actual notion of dietary fibres including a lot of other constituents as soluble and insoluble fibres are not distinguished.

With enzymatical methods the digestible constituents (1-4 β -glucans, proteins) in the defatted sample are enzymatically hydrolysed (heat stable α -amylase, gluco-amylase, protease). Water soluble fibres are isolated by precipitation with ethanol. The proteins and mineral matter still remaining with the soluble and insoluble dietary fibres are deducted.

Minerals and Trace-elements

Minerals are the constituents remaining as ash after calcination. They may be divided into two categories: main elements (Ca, P, K, Cl, Na, Mg) and trace elements (Fe, Zn, Cu, Mn, I, Mo...).

The main elements and number of trace elements are essential because they have a biological role. In the same food raw material, the content can vary greatly according to genetic and climatic factors, agricultural procedures...

A lot of food constituents (protein, organic acid, polysaccharides...) bind minerals and influence their bioavailability.

Several trace elements may be toxic depending of the food intake.

Vitamins

Vitamins are minor but essential constituents of food. They are usually divided into fat-soluble and water soluble vitamins.

Several methods can be used to measure their content. Chromatographic methods are often possible. Attention must be paid to extraction procedures before analysis.

2.1.3 Statistical and sampling aspects

The sampling method must be explained and must take into account the requirements linked to the statistical analysis as well as the distribution of the components in the raw material.

A very important point to consider is the variability of the raw material for example by taking into account the impact of the geographical origin, the climate, the agronomical practices, the annual variations...

Enough samples are to be analysed with the help of a sampling plan and the results are to be evaluated on a statistical basis.

Plants used to obtain samples for compositional analysis should be grown under conditions that represent normal practice for the crop plant. For example, studies on herbicide tolerant crops should be done on herbicide treated crops (with a waiting period afterwards). As the transgenic plant inactivates the herbicide, (metabolised) degradation products might be present in the plant.

2.2 Nutritional aspects

Whenever changes are made to the way in which a food is produced or processed or uses non-conventional ingredients, the implications on the nutritional value require consideration. Information will be needed on any issue relating to this aspect. Foods are usually complex mixtures of macro- and micronutrients which provide energy and nutrients and contribute to human well-being.

2.2.1 Identification of key nutrients

If a genetically modified crop is expected to have an important role in the diet then appropriate information on nutritional composition is needed. Both macro- and micronutrients of nutritional value are already given in section 2.1.1. It is clear that not all these nutrients are relevant for every specific genetically modified crop. For every such crop, the place (value) within the human diet should be determined. It is well known that different food groups contribute in different ways to human nutrition. Depending on the composition and the (estimated) consumption of the genetically modified food, it appears justified to limit the testing to the most relevant nutrients, which are specified in Table 2. This table should be considered as an example and not as an exhaustive list.

Table 2: Identification of relevant nutrients for different food groups

| Food group | Key nutrients |
|--|--|
| Cereals | Carbohydrates (simple and complex), dietary fibre, B-vitamins, minerals and trace elements, proteins and amino acids (if present) |
| Fruits and vegetables | Water-soluble vitamins, dietary fibre, carbohydrates, minerals and trace elements |
| Milk and milk-products | Total protein content and specific amino acid composition, fatty acid composition, fat-soluble vitamins, calcium, other relevant minerals and trace elements |
| Meat, Poultry, Fish and Meat-replacers | Total fat and fatty acid composition, total protein (for meat replacers also specific amino acids), fat-soluble vitamins, vitamin B12, trans fatty acids |
| Fats and oils | Fatty acid composition, fat-soluble vitamins, total fat, trans fatty acids |
| Extras | Macro-nutrient composition |

2.2.2 Intake

The consumption pattern may show a major change when a genetically modified food is included in the diet and thus affects human nutritional status. As it may not be possible to predict such events, a surveillance programme should accompany the marketing of a genetically modified food. Such a programme should encompass information on changes in the conditions for processing and preparation as well as effects of possible replacement of other foods or food component of dietary importance. If surveillance reveals changes in those factors which raise concerns regarding wholesomeness, a reappraisal of the acceptability of the genetically modified crop would be required.

2.3 Toxicants and anti-nutrients

Naturally occurring toxins that are inherently present in the plant should be determined. Data on the sensitivity of the crop towards the formation of mycotoxins, pathogenic microorganisms, biogenic amines and other toxic substances or organisms formed in the product have to be given, if relevant.

Information is requested with respect to the presence of anti-nutrients. This applies particularly to the key anti-nutrients for the product. The examples given below are to be considered as examples and not as an exhaustive list.

2.3.1 Examples

- **Protease inhibitors** inhibit the activity of trypsin, chymotrypsin and other proteases. They are found in legumes such as beans and peas, but also in cereals, potatoes and other products. Their presence results in impaired growth and poor food utilization.
- **Amylase inhibitors** have a similar activity against amylases.
- **Lectins or hemagglutinins** are glycoproteins mainly found in legumes: beans, peas, lentils. They bind to intestinal epithelial cells. They cause agglutination of

erythrocytes in vitro. Their presence results in poor food utilization and impaired growth.

- **Cyanogens** are cyanogenic glucosides found in cassava, linseed, peas, beans and other products. They may cause HCN poisoning.
- **Glucosinolates** are thioglycosides found in cabbage and related species. Effects upon the thyroid function have been demonstrated.
- **Saponins** are also glycosides found in soybeans, peanuts, sugar beets and others. Haemolytic effects in vitro have been shown.
- **Gossypol** is particularly important in cottonseed. Several toxic effects have been demonstrated.
- **Phytic acid** occurs in several vegetable products. This compound has a strong chelating activity. Its presence may affect bioavailability of minerals.
- **Mycotoxins** are very diverse in chemical structure and in the characteristics of the mycotoxicoses they produce. These toxins include the aflatoxins, the tricothecenes, the fumonisins, zearalenone, moniliformin and fusaric acid.
- **Phytotoxins** (solanine, etc.).

2.3.2 *Potential effects of cultivation conditions and processes*

If relevant for the particular food, information is requested about the presence of the anti-nutrient and particularly about the quantity. Moreover this information should include data about the potential effect of different cultivation conditions. In addition it is very well established that processing may have profound effect upon the level of anti-nutrients present. Two approaches at least have to be followed:

- the effect of inactivation processes
- the effect of separation processes.

In order to have a real picture of these effects, the commonly applied processing steps have to be followed. The use of flow sheets is highly recommended.

Inactivation studies under conditions equivalent to normal processing may give information about the stability of the anti-nutrient. Inactivation may be due to heat treatments, enzymatic activity, leaching or others.

Separation processes like dry milling, wet milling, extraction, centrifugation or equivalent may affect the level of anti-nutrients. In this case it is easily understood that information on the localisation of the anti-nutrient is of great help, such as the distribution in endosperm, aleurone layer, bran or germ for cereals. Data about the presence of the particular anti-nutrient in fractions for food and for feed use are necessary.

2.4 **Secondary Plant Metabolites**

Secondary plant metabolites are neither nutrients nor anti-nutrients. They are part of the characteristic composition of a plant. They are important for the compositional analysis and for the comparative approach. Even more than in the previous paragraphs, an exhaustive list cannot be given.

Some of these substances may have undesirable effects. Others may have beneficial effects for human health or in terms of resistance to mould growth.

2.4.1 Examples

- **Phenolic compounds** are considered to be of great importance. Detailed information about the qualitative and quantitative composition of the phenolic fractions is necessary.
- **Key enzymes** may affect the utilization of the plant material. Information about the relevant enzymes is necessary.
- **Organic acids** are another group. This includes:
 - aliphatic plant acids like citric, malic and others,
 - aromatic acids like benzoic acid and analogues,
 - phenolic acids like caffeic, coumaric, ferulic acids and others.
- **Carbohydrates** are mainly covered in section 2.1.1 (major and minor constituents). In addition to simple sugars and polysaccharides, complex sugars such as raffinose, stachyose and verbascose have to be covered in this section.
- Additional information on **N substances**, if not covered in a previous section, is requested. This includes low molecular N substances, unusual amino acids and others.
- With respect to **lipids**, information not covered in Table 1 is requested. This includes complex lipids and others.

2.5 Derived Products

Processing may have a pronounced effect upon the content and distribution of nutrients and anti-nutrients.

This aspect is, by preference, covered by means of flow sheats indicating the major steps in the processing scheme. As the global and detailed composition is already dealt with in the previous sections, further information on particular nutrients and anti-nutrients is necessary if this information is essential for the assessment of the product.

As an example it is felt that when discussing soya-beans some aspects related to inactivation of anti-trypsin factors during toasting have to be included

3 Implications of genetically modified crops to human nutrition

If a genetically modified food is expected to have an important role in the diet then appropriate human nutritional assessment data are needed. Attention should be paid to the particular physiologic characteristics and metabolic requirements of specific groups of the population (infants, pregnant and lactating women, elderly) and to persons with chronic diseases (like diabetes). Information will be needed on long term as well as on short term effects of the consumption of the genetically modified food.

4 Conclusion

The assessment of possible compositional changes as a result of the genetic modification has to be carried out for genetically modified crops and derived products as presented in this chapter. This includes the analysis of the major and minor constituents, the anti-nutrients, the secondary plant metabolites and the possible

occurrence of toxicants. Investigation to what extent cultivation conditions and processes can lead to the concentration or to the elimination of the constituents in the final product should also be carried out. For every genetically modified crop, the place (value) within the human diet should be determined. As the genetic modification could change the overall nutrient profile of the crop and consequently affect the nutritional status of individuals consuming the food, a surveillance programme should accompany the marketing of such crops.