

## 8. Compositional analyses of key components, evaluation of metabolites, food processing and nutritional modification

### Compositional analysis

Food composition analysis is concerned with both beneficial and harmful components in the human diet: nutrients, bioactive non-nutrients, antinutrients, toxicants, contaminants and other potentially useful and dangerous elements. The composition of any food varies, and the differences are caused by plant variety, growth and storage conditions, climate, processing and several other factors. As a result, compositional data are used mainly as an estimate or starting point to guide further analysis, if deviations from expectations are seen.

Possible changes in the composition of the recombinant-DNA plant are assessed using comparative analyses of the key nutrients, antinutrients, toxicants and other important components of the crop with the corresponding compounds in an appropriate comparator crop. Data on the composition of recombinant-DNA plants and their conventional counterparts are obtained from samples produced in controlled field trials and analysed using validated methods and appropriate statistical techniques. Samples are normally analysed in a random order using the same methods in order to prevent bias.

Based on the comparative approach, it is important to decide which nutrients the evaluation should be focused on. Generally, the food safety assessment considers the potential for any change in the concentration of key elements that have a significant impact on the diet, as well as the potential for any change in the bioavailability of key nutritional components.

Key compositional data that are statistically non-distinguishable collected from both the recombinant-DNA crop plant and the isogenic counterpart, grown under near identical conditions, are essential to establish substantial equivalence. Moreover, the compositional data should be shown to fall within the published range for conventional varieties that are considered to be safe for consumption based on a history of safe use.

Where significant changes are detected, analytical methods traditionally applied in the evaluation of food constituents, such as measurement of total protein, fat, ash, fibre and micronutrients, may need to be augmented with additional analyses to identify the nature of the changes observed, and to determine whether the observed differences could adversely affect health. Paragraphs 44 to 46 of the Codex Guideline outline the key considerations for key components and metabolites in recombinant-DNA plants.

There may be instances where reference values are not available for a particular food crop e.g. crops that are nutritionally modified and/or indigenous to a specific region. In such cases, the purpose of the assessment is to gather data to establish a compositional profile. It is important to note that all plant breeding methods, conventional and modern, have the potential to alter the compositional profile and nutritional value of plants or lead to unexpected or unintended changes in concentrations of various natural toxicants or antinutrients<sup>30</sup>.

Unintended changes in levels of nutrients can theoretically arise in several ways. Insertion of genetic material could disrupt or alter the expression of normally expressed plant genes. Expression of the introduced gene - through protein synthesis - might lead to enzymatic activity and substrate ranges beyond the intended target molecule, and a high transgene expression

<sup>30</sup> International Food Composition Tables Directory, see "additional resources" section.



**CODEX GUIDELINE PARAGRAPH 44.** Analyses of concentrations of key components<sup>31</sup> of the recombinant-DNA plant, and especially those typical of the food, should be compared with an equivalent analysis of a conventional counterpart grown and harvested under the same conditions. In some cases, a further comparison with the recombinant-DNA plant grown under its expected agronomic conditions may need to be considered (e.g. application of an herbicide). The statistical significance of any observed differences should be assessed in the context of the range of natural variations for that parameter to determine its biological significance. The comparator(s) used in this assessment should ideally be the near isogenic parental line.

In practice, this may not be feasible at all times, in which case a line as close as possible should be chosen. The purpose of this comparison, in conjunction with an exposure assessment as necessary, is to establish that substances that are nutritionally important or that can affect the safety of the food have not been altered in a manner that would have an adverse impact on human health.

**CODEX GUIDELINE PARAGRAPH 45.** The location of trial sites should be representative of the range of environmental conditions under which the plant varieties would be expected to be grown. The number of trial sites should be sufficient to allow accurate assessment of compositional characteristics over this range. Similarly, trials should be conducted over a sufficient number of generations to allow adequate exposure to the variety of conditions met in nature. To minimise environmental effects, and to reduce any effect from naturally occurring genotypic variation within a crop variety, each trial site should be replicated. An adequate number of plants should be sampled and the methods of analysis should be sufficiently sensitive and specific to detect variations in key components.

**CODEX GUIDELINES PARAGRAPH 46.** Some recombinant-DNA plants may have been modified in a manner that could result in new or altered levels of various metabolites in the food. Consideration should be given to the potential for the accumulation of metabolites in the food that would adversely affect human health. Safety assessment of such plants requires investigation of residue and metabolite levels in the food and assessment of any alterations in nutrient profile. Where altered residue or metabolite levels are identified in foods, consideration should be given to the potential impacts on human health using conventional procedures for establishing the safety of such metabolites (e.g. procedures for assessing the human safety of chemicals in foods).

<sup>31</sup> Key nutrients or key antinutrients are those components in a particular food that may have a substantial impact in the overall diet. They may be major constituents (fats, proteins, carbohydrates as nutrients or enzyme inhibitors as antinutrients) or minor compounds (minerals, vitamins). Key toxicants are those toxicologically significant compounds known to be inherently present in the plant, such as those compounds whose toxic potency and level may be significant to health (e.g. solanine in potatoes if the level is increased, selenium in wheat) and allergens.

<sup>32</sup> Changes in gene expression will also occur when conventional breeding methods are used. Unintended changes in plant composition have been argued to be less frequent in recombinant-DNA plants because only a limited number of genes are transferred during the genetic modification process.

level might reduce the availability of amino acids used for synthesis of other compounds. Finally, either the expressed protein or altered levels of other proteins or metabolites might have antinutritional effects<sup>32</sup>.

In general, to assess the effects (if any) of a novel protein expressed in a recombinant-DNA plant a number of key parameters are selected: (i) prior history of safe use of the protein in food; (ii) knowledge of the mode of action e.g. enzyme function; (iii) digestibility of the protein in *in vitro* models; (iv) absence of amino acid sequence similarity to sequences in available databases of known mammalian protein toxins and protein allergens or pharmacologically active proteins; (v) predictable levels of expression of the newly introduced protein.

For recombinant-DNA plants that were not developed to have intentionally altered nutritional value, the aim of the nutritional evaluation is to demonstrate that there has been no unintentional change in the levels of key nutrients, natural toxicants or antinutrients, or in the bioavailability of nutrients. In this case, food substitution using products from the recombinant-DNA plants should not adversely affect the health or nutritional status of the consumer. Implications for the population as a whole and for specific subgroups (e.g. children and the elderly) should be considered.

Nevertheless, information on the composition of many plant species is limited, especially with regard to the antinutrient and natural toxin profiles. Because of this, compositional analysis is often hampered when used as a screening method for unintended effects of genetic modification. It is necessary to develop alternative analytical methods that are more informative in these cases. More advanced methodologies, such as mRNA fingerprinting and metabolomic analysis, are being developed but remain to be validated as alternative means of detecting important differences in gene expression and establishing the toxicological significance of the alteration.

Metabolites are dependent on the nutrient profile of a food, which is assessed using the following steps: compositional analysis, morphological and physiological analysis in the form of *in vitro* tests, animal studies and clinical analysis through human studies. Because a broad

selection is made of nutritionally relevant compounds, and known antinutritional and toxic compounds, the targeted analytical approach, i.e. measuring the content of single substances, offers the assurance that unintentional alterations in plant metabolic pathways will be detected. Where changes in plant metabolites raise significant safety concerns, it may be possible to test their safety individually, or when they are present as a component of the food derived from the recombinant-DNA plant.

The basic information required for recombinant-DNA plants includes measurement of various carbohydrates, proteins, fats, energy and water (Greenfield and Southgate, 1996). Data on key vitamins and minerals are required where deficiencies are a cause of disease and for nutritionally modified foods.

The measurement of carbohydrates (McCleary *et al.*, 2006) can be conducted by various means: (i) analytical methods, which measure total starch, resistant starch and dietary fibre; (ii) chemical – the enzymatic degradation of polysaccharides or oligosaccharides to basic sugars; (iii) physical methods, which assess the food structure retained or conferred; (iv) an assessment of functional properties, such as whether the product is glycaemic, digestible, fermentable, etc.

Amino acid analyses are used to determine the protein content of novel foods. This can be achieved by using the Kjeldahl method (or similar) (Association of Official Analytical Chemistry, 2002), which in principle measures the nitrogen content in order to determine the protein content<sup>33</sup>. Alternatively, relying on their structure, proteins can be hydrolysed to their component amino acids, which can then be measured by ion-exchange, gas-liquid or high-performance liquid chromatography. The sum of the amino acids then represents the protein content (by weight) of the food.

Most of the fat in food is in the form of triglycerides. Fats are analysed either as fatty acids and the result expressed as triglycerides or are measured as the fraction of the food that is soluble in lipid solvents.

## Food processing

Processing methods can cause a significant variation in the nutrient content of a food compared with the nutrient profile of the crop as it was grown in a field (Morris *et al.*, 2004).

Modern separation techniques, such as milling, centrifugation, and pressing, change the nutritional content of food, preserving certain nutrients while removing others. Because of reduced nutritional value, processed foods are often “enriched” or “fortified” with some of the most critical nutrients (usually certain vitamins) that were lost during processing. Nonetheless, processed foods tend to have an inferior nutritional profile to whole, fresh foods, with respect to the content of sugar, starch, potassium/sodium, vitamins, fibre, and intact, unoxidized (essential) fatty acids. In addition, processed foods often contain potentially harmful substances such as oxidized fats and trans-fatty acids.

Heating techniques may reduce the content of many heat-labile nutrients such as certain vitamins and phytochemicals, and possibly other as yet undiscovered substances. For example, boiling a potato can cause a significant amount of the potato’s B and C vitamins to be lost through an osmotic reaction between the potato and the boiling water. Similar losses occur when food is roasted or fried in oil. The actual nutrient losses observed are affected by many factors such as food type, cooking time and temperature.

<sup>33</sup> This approach is based on two assumptions: that dietary carbohydrates and fats do not contain nitrogen and that nearly all of the nitrogen in the diet is present as amino acids in proteins.

**CODEX GUIDELINE PARAGRAPH 47.** The potential effects of food processing, including home preparation, on foods derived from recombinant-DNA plants should also be considered. For example, alterations could occur in the heat stability of an endogenous toxicant or the bioavailability of an important nutrient after processing. Information should therefore be provided describing the processing conditions used in the production of a food ingredient from the plant. For example, in the case of vegetable oil, information should be provided on the extraction process and any subsequent refining steps.



## Nutritional modification

For recombinant-DNA plants that were intentionally developed to have altered nutrients, the aim of the nutritional evaluation is to demonstrate that there are no additional unintentional changes in the levels of nutrients, including changes in the bioavailability of these nutrients.

The approach to the safety assessment of products with intentionally modified nutrient profiles is fundamentally the same as for the first generation of recombinant-DNA plants (OECD, 2001). However, the compositional differences between these products and their conventional counterparts are likely to be greater, thus increasing the potential for unintended effects. In essence, the utility of current methods for assessing the safety of recombinant-DNA plants may be found to be limited, due to the fact that the nutritionally modified crops will not be substantially equivalent to their conventional counterparts and will share fewer compositional values for comparison.

Nutritionally modified products may be produced to address a specific dietary or nutritional need. The safety assessment, however, must consider not only the target group but also groups in the population that may be at risk, thus recognizing the presence of population diversity. This requires validated data on food consumption patterns, nutrient intake and in some instances the nutritional status of a population or target group. The safety assessment of a nutritionally modified food must be considered in the context of a total diet.

Due to the potential for broad changes in nutrient levels and interactions with other nutrients, and unexpected effects, it may be necessary in certain instances to undertake feeding

**CODEX GUIDELINE PARAGRAPH 48.** The assessment of possible compositional changes to key nutrients, which should be conducted for all recombinant-DNA plants, has already been addressed under 'Compositional analyses of key components'. However, foods derived from recombinant-DNA plants that have undergone modification to intentionally alter nutritional quality or functionality should be subjected to additional nutritional assessment to assess the consequences of the changes and whether the nutrient intakes are likely to be altered by the introduction of such foods into the food supply.

**CODEX GUIDELINE PARAGRAPH 49.** Information about the known patterns of use and consumption of a food, and its derivatives should be used to estimate the likely intake of the food derived from the recombinant-DNA plant. The expected intake of the food should be used to assess the nutritional implications of the altered nutrient profile both at customary and maximal levels of consumption. Basing the estimate on the highest likely consumption provides assurance that the potential for any undesirable nutritional effects will be detected. Attention should be paid to the particular physiological characteristics and metabolic requirements of specific population groups such as infants, children, pregnant and lactating women, the elderly and those with chronic diseases or compromised immune systems. Based on the analysis of nutritional impacts and the dietary needs of specific population subgroups, additional nutritional assessments may be necessary. It is also important to ascertain to what extent the modified nutrient is bioavailable and remains stable with time, processing and storage.

**CODEX GUIDELINE PARAGRAPH 50.** The use of plant breeding, including *in vitro* nucleic acid techniques, to change nutrient levels in crops can result in broad changes to the nutrient profile in two ways. The intended modification in plant constituents could change the overall

nutrient profile of the plant product and this change could affect the nutritional status of individuals consuming the food. Unexpected alterations in nutrients could have the same effect. Although the recombinant-DNA plant components may be individually assessed as safe, the impact of the change on the overall nutrient profile should be determined.

**CODEX GUIDELINE PARAGRAPH 51.** When the modification results in a food product, such as vegetable oil, with a composition that is significantly different from its conventional counterpart, it may be appropriate to use additional conventional foods or food components (i.e. foods or food components whose nutritional composition is closer to that of the food derived from recombinant-DNA plant) as appropriate comparators to assess the nutritional impact of the food.

**CODEX GUIDELINE PARAGRAPH 52.** Because of geographical and cultural variation in food consumption patterns, nutritional changes to a specific food may have a greater impact in some geographical areas or in some cultural population than in others. Some food plants serve as the major source of a particular nutrient in some populations. The nutrient and the populations affected should be identified.

**CODEX GUIDELINE PARAGRAPH 53.** Some foods may require additional testing. For example, animal feeding studies may be warranted for foods derived from recombinant-DNA plants if changes in the bioavailability of nutrients are expected or if the composition is not comparable to conventional foods. Also, foods designed for health benefits may require specific nutritional, toxicological or other appropriate studies. If the characterization of the food indicates that the available data are insufficient for a thorough safety assessment, properly designed animal studies could be requested on the whole foods.

studies in other animals to determine the outcomes that result from changes in nutrient profiles and nutrient bioavailability.

## New analytical methods

Improved methodologies and more sensitive techniques allow detection of unintended alterations in the composition of foods in a way that was once not possible. The application of profiling methods such as DNA/RNA microarray technology, proteomics, gas chromatography coupled to mass spectrometry (GC-MS) and liquid chromatography coupled to nuclear magnetic resonance (HPLC-NMR) has the potential to provide indications of changes at the level of mRNA expression, protein production and/or at the level of metabolism without prior knowledge of specific changes in plant constituents.

The utility and applicability of these non-targeted techniques for risk assessment needs further exploration, in particular with respect to establishing and validating the relevance to food safety of the observed changes. One of the major difficulties is to distinguish between natural variations and variations that have resulted from genetic modification. It is essential that databases of plant constituent profiles under different conditions contain the full range of values of each measured parameter under a wide range of environmental, genetic, and development conditions. This information would need to be correlated with the presence or absence of associated food safety hazards.

Profiling methods are not yet suitable for routine risk assessment purposes, and further development and validation are needed. A more promising application of these methods may currently lie in a hypothesis-driven analysis of relevant categories of compounds that may be altered. Thus profiling methods are not aimed at replacing conventional single compound analyses, but may be useful, when validated, to confirm and supplement other data.

## References

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## Additional resources

International Life Sciences Institute (ILSI). Crop Composition Database.

A comprehensive online crop composition database that provides up-to-date information on the natural variability in the composition of conventional crops and provides a reference for comparing the composition of new crop varieties, including those developed through biotechnology. <http://www.cropcomposition.org/>

See also: ILSI. 2003. *Best practices for the conduct of animal studies to evaluate crops genetically modified for input traits*. Washington, DC, ILSI Press. <http://www.ilsil.org/AboutILSI/IFBIC/BESTPRACTICES.htm>





FAO INFOODS. The International Food Data Systems Project (INFOODS) is a comprehensive effort, begun within the UN University's Food and Nutrition Programme, to improve data on the nutrient composition of foods from all parts of the world, with the goal of ensuring that adequate and reliable data can be obtained and interpreted properly worldwide.

[http://www.fao.org/infoods/directory\\_en.stm](http://www.fao.org/infoods/directory_en.stm)

OECD. 1998. *Report of the OECD workshop on the toxicological and nutritional testing of novel foods*. Paris, Organization for Economic Co-operation and Development (OECD).

USDA National Nutrient Database for Standard Reference. The Nutrient Data Laboratory (NDL) has the responsibility to develop the USDA's National Nutrient Database for Standard Reference, the foundation of most food and nutrition databases in the United States, which is used in determining food policy, research and nutrition monitoring. <http://www.nal.usda.gov/fnic/foodcomp/search> ●