Functional Foods

A conceptual model for assessing their safety and effectiveness

Under the auspices of Innovation Network Rural Areas and Agricultural Systems, this study was carried out by:

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Preface

Even during the discussions which ultimately led to NRLO-report no. 2000/15E on the position and future perspectives of Functional Foods, already the question was raised whether potential consumer risks might result from an uncontrolled intake of physiologically active compounds added to a variety of food components or products. Simply consider the hypothetical situation that selenium, as an alleged cancer-preventing element, is added (albeit in low concentrations, given its acute toxicity) to many of the basic ingredients of your breakfast, making them functional foods. Combining several of those ingredients into a varied breakfast, then, might eventually lead to an undesired accumulation of selenium, thus undermining the potential benefit of a single functional food.

Several questions should be posed on how to deal with this issue, e.g.:

- What are the possibilities to predict the effects of the food chain on the ultimate level of physiologically effective compounds on the dining table at home?
- Is it possible to develop predictive models?
- Who will be accountable in case of unforeseen casualties: the consumer, the producer of the food ingredients or the keeper of the Intellectual Property Right of the claim?

Researchers from Wageningen University (with assistance from TNO Nutrition and Food Research) were invited by Innovation Network Rural Areas and Agricultural Systems to make an interdisciplinary effort to develop the study on Functional Foods mentioned above into an evaluation of the safety and effectiveness of Functional Foods. The project was managed by Prof.dr. W.M.F. Jongen.

As a result of this study a number of fundamental recommendations could be formulated, which have given rise to follow-up studies even now. Also, the Advisory Council on Health Research (RGO), which was consulted during preparations for this study, has shown an interest in the outcome.

It is not considered unlikely that the present study will be a kick-off for broader discussions dealing with various aspects of Functional Foods.

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Executive summary

Today, various food components are being studied for their potential role in promoting health and preventing – chronic – diseases beyond the conventional nutritional concepts. Newly found knowledge is commercialised by companies in the form of functional foods, i.e. products containing greater quantities of bioactive components than conventional products, which are marketed with a health claim. This new group of products raises the question as to how to integrate this development into the conventional recommendation of a well-balanced diet.
Firstly, companies do not sell diets, they sell products and supplements and optimisation at product level may result in dietary levels being too high. Besides, dietary recommendations do not exist for a large number of – potential – bioactive components. Accumulation of bioactive components may result in unwanted side-effects.
Secondly, the food production chain can have a great effect on variation in the composition of food products with regard to bioactive components used in functional foods. This variation is additional to the variation that is seen in consumption patterns of individual consumers.
A previous study identified pre-market modelling as a tool to obtain realistic information on intake data for bioactive compounds from different types of - functional and conventional - food products and supplements. The present study has made an attempt to integrate the information on intake and composition. Such an integration should result in a more realistic view of the component intake distribution in the entire population. The information required to implement this type of approach has been identified. In general terms it consists of:

- Quantitative information on the distribution of intake data (including functional foods and food supplements).
- Quantitative information on the composition (including variability and uncertainty) of – functional - food products.
- Quantitative information on the effects of the food production chain on that composition (including variability and uncertainty).

In conclusion, the report shows that the product-diet dilemma can be solved by developing a predictive model. The model integrates food intake data, dynamic consumption patterns and the production chain model and combines them with a risk-benefit approach. Based on the suggested approach the following recommendations are made regarding future research needed for implementation:

1. The processes of collecting and managing intake data, including functional foods and food supplements, should be developed in more detail to cope effectively with variability and uncertainty.
2. Developing production chain models in order to predict the effects of the chain on the level of bioactive compounds in consumed products (including variability and uncertainty).
3. Developing integrated probabilistic models to combine intake data and compositional data, including their predicted variability and uncertainty.
4. Incorporating food-matrix and food-processing effects on the bioavailability of bioactive compounds into predictive models.
5. Developing more detailed Risk Reduction Models (virtual cell models, target function distribution and epidemiological models).

To demonstrate the power of the proposed integrated approach, recommendations 1-3 are suggested for a case study on a few selected bioactive compounds. As a follow-up to the present study, the authors have made a research proposal.

1. Introduction

1.1. General

The recommendations made by the NRLO/RGO project on Functional Foods that generally received most support dealt with the importance of information on safe and effective levels in functional food products. Instruments such as pre-market modelling and knowledge of the overall effects of the food production chain on health aspects were seen as indispensable (Plaami et al., 2001). Pre-market modelling (see Appendix 1 for a general introduction to modelling) is a useful tool to obtain realistic information on intake data of bioactive compounds from different types of - functional and conventional - food products and supplements. Also, it makes it possible to observe any changes introduced to the total diet pattern by new types of products while the risk of overdosing as a result of cumulative consumption can be predicted and prevented.

The production chain of functional foods also introduces high variability in levels of bioactive compounds in end products. Research on the production of apple juice has shown that some 90% of the flavonoid content of apples is lost during their conventional processing into juice. By optimising the process it is possible to retain almost all flavonoids in the final juice (Van der Sluis et al., 2002 in press). Flavonoids are known to be much more potent antioxidants than the well-known dietary antioxidant of vitamin C. The average reported antioxidant activity of flavonoids is about 5 times higher compared to vitamin C. Recommended daily intake of vitamin C is set to 50 mg in most countries. The average reported intake of flavonoids is around 100 mg. The bioavailability of the well-known flavonoid of quercetin is high: absorption levels of around 50% (depending on type of glycoside) were reported. By extrapolation this would mean that the available antioxidant activity in the body originating from flavonoids is five times as high as the activity from recommended vitamin C intake.

If the production chains of many fruit and vegetable products are optimised, the flavonoid content of those products can be expected to increase, perhaps even as much as tenfold (the apple juice example). Thus, if consumers were to buy mostly those “healthier” foods, their antioxidant levels derived from flavonoid intake would increase to 50 times that of vitamin C. In other words, the antioxidant capacity of dietary flavonoids would be equivalent to a daily intake of 2500 mg vitamin C! Any additional intake of antioxidants through other food constituents or by consuming supplements, herbs, etc. might increase the figure further still.

Combining predictive modelling of effects in the food production chain with pre-market modelling of consumption data will result in a powerful tool for developing safe and effective functional food products.

1.2. Project purpose and approach

Developing a conceptual model to establish effective and safe levels of bioactive compounds in functional food products will require an integrated combination of information from the food production chain, consumption habits and biological effects of active compounds.

In this report we wish to address the following issues:

- A conceptual model to establish effective and safe levels of bioactive compounds in functional food products.
- Sources of information, variability, uncertainty and knowledge gaps for constructing predictive models for the safety and effectiveness of functional food products.
- Suggestions for an approach to translate the conceptual model into predictive models describing specific cases.

2. Conceptual framework

In order to predict the distribution of intake levels of bioactive compounds from - functional - foods in consumer groups it is necessary to integrate information on intake data with information on the composition of those products. To predict the effectiveness and safety of functional foods the outcome of the integrated model must be used as the input of a risk-benefit analysis. The underlying conceptual framework is depicted in Figure 1.

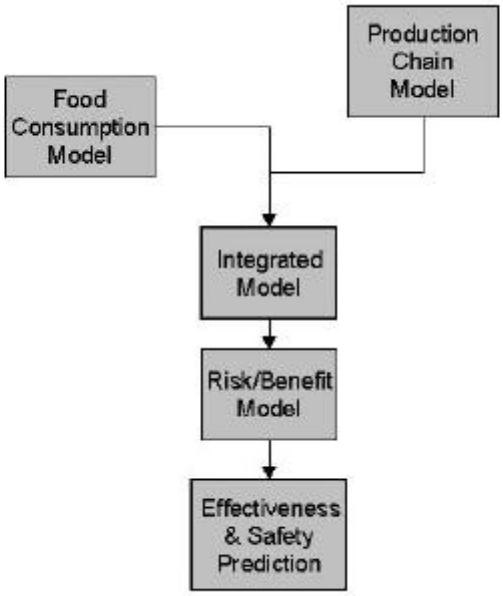

Figure 1. Conceptual framework for modelling the effectiveness and safety of functional foods.

The following chapters of the report will discuss the various aspects of this modelling approach, resulting in specific recommendations.

In order to predict the distribution of intake levels of bioactive compounds from – functional - foods it is necessary to integrate information on - the distribution of - intake data of food products and on - the distribution of – the composition of those products. In order to predict the effectiveness and safety of functional foods it is necessary to feed the data resulting from the integrated model into risk-benefit models.

3. Production chain

3.1. General

There are numerous sources of variation in levels of health-promoting compounds in foods throughout the food production chain (Figure 2).

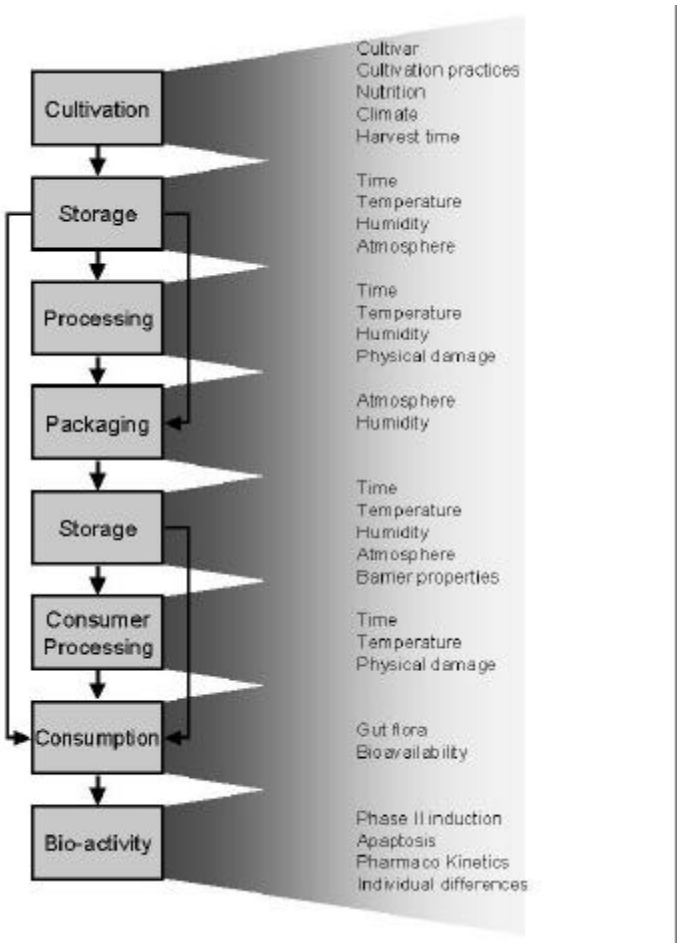

Figure 2. Diagram representing the food production chain, including potential factors affecting the level of health-promoting compounds (Dekker et al., 2000).

This variation is caused by the natural variation in cultivars and by the fact that improvements in production methods and processing conditions in the past were not aimed at retaining health-protecting compounds and their activities, but rather at achieving maximum production yield and improving aspects like storage qualities.

Both to understand the health effects of those compounds and to develop products containing them, an integration between nutritional/health sciences and food technology is essential. The need for this can be illustrated by the fact that variation in consumer intake levels of bioactive secondary plant metabolites can easily vary 10-100 times (Figure 3) as a result of the existing variation at different steps in the food production chain (Dekker et al., 2000).

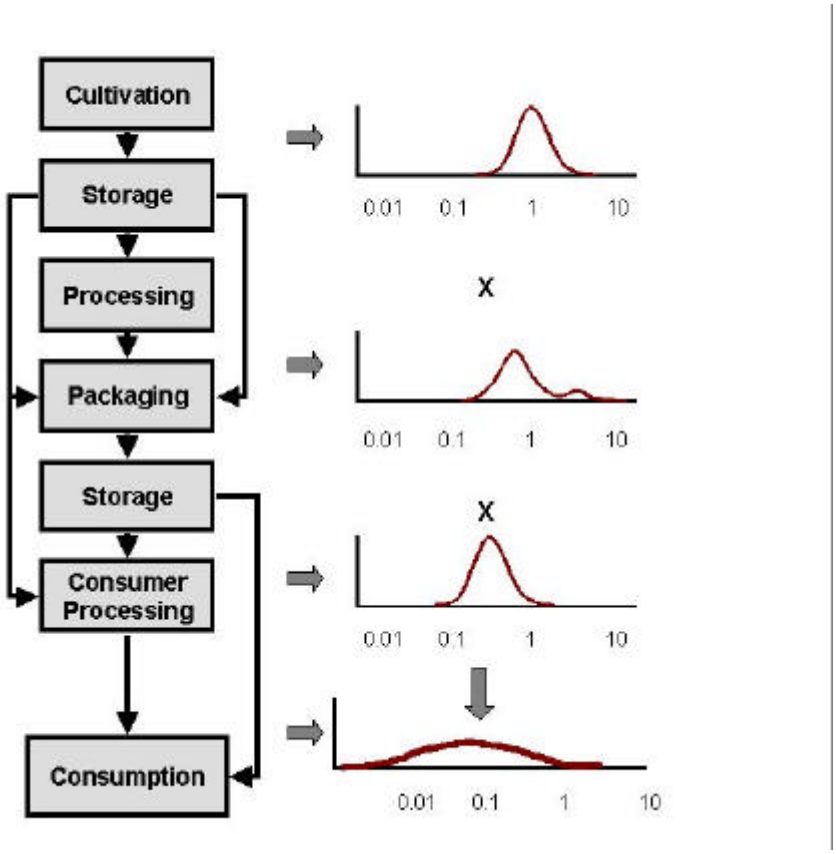

Figure 3. Diagram representing sources of variation in the level of bioactive components in the food production chain and the resulting variation in intake levels at consumption (Dekker et al., 2000).

In order to produce food products with enhanced, guaranteed levels of certain bioactive components it is essential to have more knowledge and control of the various steps within the food production chain. Since potential sources of variation in the food production chain are numerous and many interactions exist, an experimental approach to investigate potential effects is almost impossible. Therefore, research should focus on using limited experimental data and knowledge as a source to build predictive models of the steps in the food production chain (Dekker et al., 2000). For more technical details on how to build a predictive model see Appendices 2 and 3.

The sources of variation in the various steps of the food production chain that should be taken into consideration when building predictive models are discussed systematically and in more detail in this chapter and, in connection with traditional food composition databases, in chapter 4.
For a more detailed overview the reader is referred to www.iefs.org/montecarlo report of WP2 (due August 2001).

First, Figure 4 presents a diagram of the food production chain resulting in food composition data.

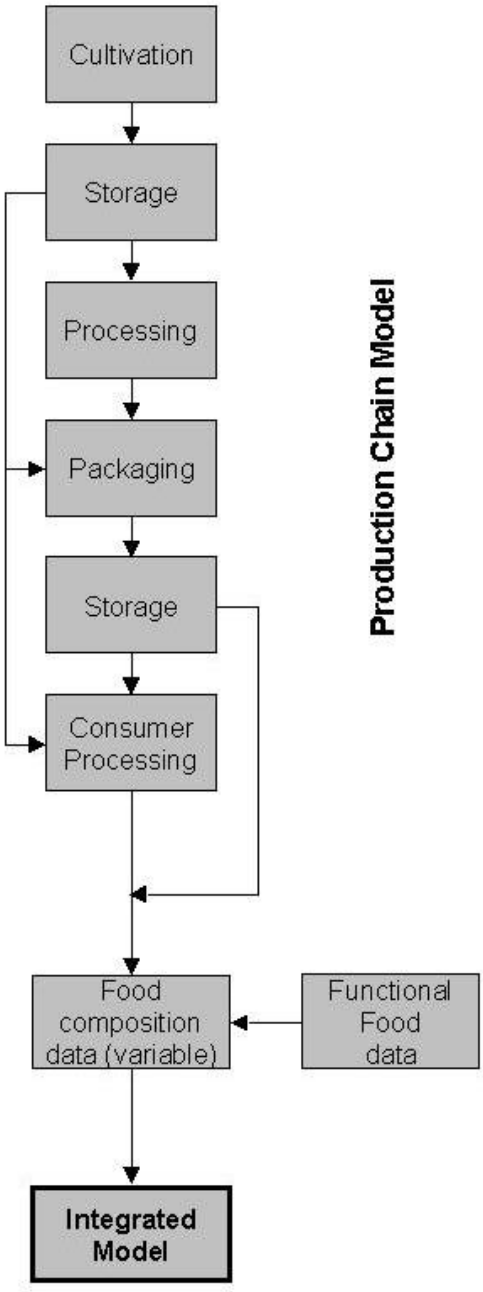

Figure 4. Food production chain model to produce food composition data.

In general, variation in the composition data of food products and intermediate products within the production chain arises from:

- applied methods of analysis
- quality of analyses
- food-related factors

3.2. Applied methods of analysis

Variation in food composition data caused by using different analytical methods must be seriously considered. Variability may result from:

- what exactly is measured by the method used
- the quality of sampling procedure and analysis

It is essential that methods used and compounds studied are specified. A major technical barrier is that analytical techniques are not available for many phytochemicals. In many cases, moreover, appropriate standards are not yet available. The problem is that although the number of plant-derived compound groups thought to have potential beneficial health effects today is impressive (see Appendix 4), they can never be studied in detail in view of the great number of single compounds and their different forms and complexes with other compounds. For example, 8,000 phenolic structures are currently known to exist in plants which, moreover, primarily occur in conjugated form, i.e. with one or more sugar residues linked to hydroxyl groups. Biological activity varies between different compounds and is dependent on the conjugated sugar unit.

Already, however, a set of 'model' crops and model compounds within different groups of compounds has been selected which may be used to begin a systematic research approach (Goldman, 1999; Plaami et al., 2000).

Sample procedures and analytical methods should be validated. Validation characterises methodological reliability and should be addressed as part of an evaluation of analytical procedures. A number of validation criteria has been set up. They include sensitivity, selectivity, recovery and reproducibility.

3.3. Food-related factors

Food-related sources of variability in food composition data arise from:

- the origins of food components
- processing effects

3.3.1. The origins of food components

Sources of variation due to the origins of food and food ingredients include:

- in plants: cultivar selection, cultivation practices, climate and harvest maturity
- in meat: species, race, feeding, handling, slaughter age
- in other animal-based ingredients (milk, eggs, etc.): race and feeding of the producing animal
- in other ingredients: chemical structure, microbial family, purity.

Traditionally, production yield, disease resistance and visible quality have been the main criteria to motivate decisions in plant breeding. Only recently, the idea of targeting to increase the concentration of nutrients in the edible parts of a plant, i.e. intrinsic quality, has become important.

Differences in contents of bioactive compounds due to differences in origin are especially important if plants are consumed fresh and, consequently, changes resulting from processing steps are limited. However, when mineral contents in food composition data are examined, the content in raw materials often is of greater importance than the processing steps (Watzke, 1998).

Recent advances in molecular biology such as gene isolation, manipulation and transfer between species make it possible to alter plant properties with aesthetic and commercial values without changing overall production. Accordingly, it has been possible to produce transgenic tomato lines containing 70-fold higher levels of quercetin in their fruit peel as compared to their parent plants and potato plants with up to 60-fold higher levels of kaempferol in their fruit, mainly in the flesh, as compared to parent plants (Vos et al., 2000).

The importance of cultivation practices for the composition of plant-based food products and food intake data is illustrated by selenium fertilisation. The intake of selenium (cofactor in antioxidants) derives from the selenium content of the soil. In Finland fertilisers have been supplemented with selenium from year 1984 on and the intake of selenium has increased from 30-40 g/day to almost 100 g/day. As for animal-based foods, an illustrating example is the production of -3-enriched eggs by feeding the hens.

Another example is the significance of harvest maturity, the primary factor affecting vitamin and phenolic contents. Most fruits and vegetables reach their maximum vitamin content when mature (Awad, 2001). However, in order to facilitate handling and transportation, harvest usually takes place at an earlier stage. In addition, food crops are generally harvested within a relatively narrow frame of maturity. Thus, in

practice, the largest differences in contents of bioactive compounds within fresh products resulting from differences in harvest maturity are seen between products used locally (harvested as ripe, no need for transportation) and those imported.

3.3.2. Processing effects

The steps that may lead to variability in food composition after the raw material has been brought into the production chain are:

- raw material storage
- processing
- packaging
- storage
- processing by consumer
-

Raw material storage:

Variability resulting from raw material storage depends on storage duration and conditions. In fresh vegetable products, loss of vitamins is greatest during the first 24 hours; after that, vitamin levels remain steady during storage while the visible quality also continues to be acceptable under appropriate circumstances. Transit of fresh products takes approximately 7 to 14 days and post-harvest handling before commercial freezing usually takes less than 12 hours. The faster the cooling, the smaller the loss (also if the product is chilled).

Processing:

Food processing has significant and complex effects on the compounds and food matrix, resulting in significant variation and uncertainty in food composition data. Variability may develop as a result of:

- applied processing techniques
- duration of processing
- order of techniques used in the processing chain

Uncertainty depends on:

- level of processing detail that can be included in the model
- stability and reproducibility of processing conditions

Industrial processing methods vary from minimal to extensive, involving procedures such as washing, cutting, blanching, adding processing chemicals, drying, fermenting, freezing, canning and sterilising. Composed foods are created by an interplay of ingredients and processes such as crystallisation, emulsification, gelation, phase separation, extrusion, aggregation, baking, agglomeration and granule formation. These texturisation processes are essential elements of industrial processes and also,

partly, constitute stabilisation processes used to secure food safety and physical food stability (preservation of sensory quality). Stabilisation processes are generally based on inactivation (heating), inhibition (fermentation, drying, cooling/freezing), removal (membranes) or prevention (packaging).

The impact of processing can be positive or negative with respect to the nutritive value of foods, i.e. the contents of nutrients and their bioavailability. For example, all processes where food components are separated change the distribution of mineral content of the single component and should therefore be considered as critical subprocesses in modelling the fate of minerals. Milling removes the majority of minerals from the flour and so this type of processing can have a positive or negative impact depending on which part is the stream used (i.e. the flour or the bran). Watzke (1998) gives other examples of processing effects on mineral losses.

Novel non-thermal food-processing technologies are becoming more important (Figure 5). These processes aim at gentle, minimum, sometimes even invisible processing in order to maintain as much of the quality of raw materials, their physical-chemical properties and functionality as possible while ensuring microbial safety and, ideally, reducing environmental problems.

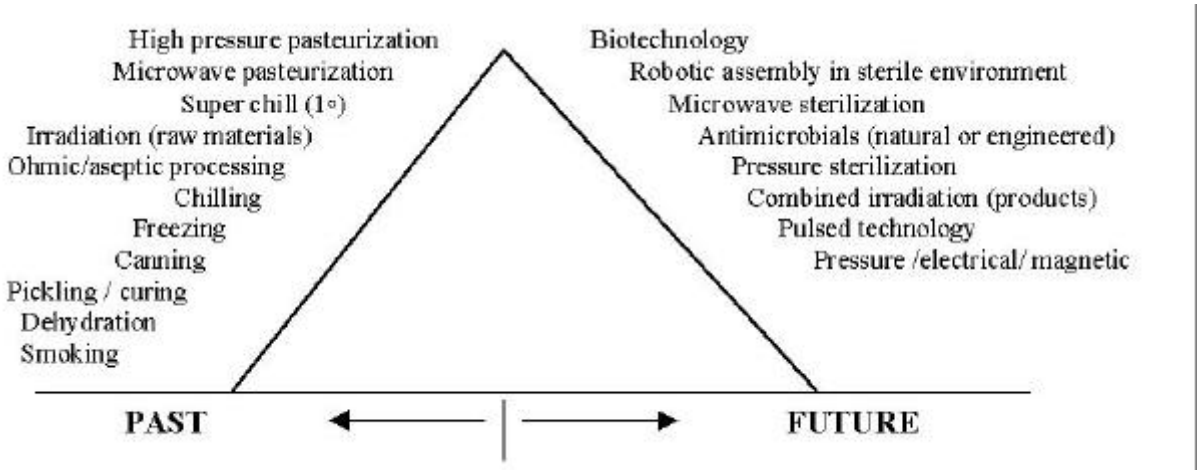

Figure 5. Traditional and future preservation technologies (Knorr, 1998).

In general, the main considerations in developing new stabilisation technologies have been given to effects on microbial populations and visible quality rather than on nutritional quality, as the latter are often thought to be automatically positive!

Functional foods (i.e. foods with health claims) are intended to produce health benefits via modifications in food ingredients or food structure when compared to traditional foods. Thus, they constitute a clear source of extra variability as compared to traditional food composition data.

Functional foods can be produced in two ways:

- By using technologies that
  - produce functional ingredients for the product during manufacturing (biotechnology, e.g. fermentation),
  - preserve, concentrate or increase the bioavailability of functional ingredients even in raw materials (e.g. processing effects on the bioavailability of lycopene, enzyme hydrolysis used to release the bioactive compound from the cell matrix),
  - modify the product to make it functional (e.g. fat technology)
- By adding a functional ingredient or by adding ingredients that enhance the bioavailability of existing functional ingredients (e.g. adding vitamin C to enhance the bioavailability of iron).

Plaami et al. (2001), Korhonen et al. (2000) and Knorr (2000) have reviewed processing effects on functional ingredients.

Packaging:

Variation in food composition data resulting from packaging depends on:

- technology used
- storage conditions

In recent years a number of new food processing and packaging technologies have been applied by the food industry. They include modified atmosphere (MA), controlled atmosphere (CA) and vacuum packaging, designed to extend shelf life. In modified atmosphere packaging, the package is filled with a gas of a specific composition, gasses used mostly being oxygen, nitrogen and carbon dioxide. Controlled atmosphere packaging is used mainly in bulk storage (also raw material storage) and transport while conditions are maintained as desired, for example by using ventilation. The package is called active if it does not only form a barrier to the environment but also actively affects internal conditions. For example, active package material may contain an oxygen scavenger and/or releaser to change the gas composition inside the package during storage.

Models have been developed to describe the O_2 and CO_2 levels in polymeric film packages as affected by film permeability, temperature and rate of gas exchange of the stored product in order to predict (and optimise) the keeping quality of varying products packed under MA (Hertog et al., 1999, Chen et al., 2000, Devlieghere et al., 1999). The spoilage rate of the product is used as an indication of quality in these models. However, the effect of minimal processing and packaging on the nutritional quality of products, including maintenance of vitamin levels, has not been clearly established.

Final product storage:

As with raw material storage, variability resulting from final product storage depends on:

- duration
- conditions

Processing by consumer:

Variability may result from:

- different processing techniques
- duration of different processing steps
- order of techniques used in the processing chain

Processing by consumer partly includes the same subprocesses that are used in industrial processing. Since processing by consumer is difficult to control, the resulting variability and, especially, uncertainty in food composition and intake often are more significant than those resulting from industrial processing, in which homogeneity of the end product is one of the objectives of quality control.

In a single process, for example peeling an apple, consumers can remove almost all anthocyanin and quercetin 3-glycosides, vitamins C and E and beta-carotene (Awad, 2001). On the other hand, processing by consumer can introduce complicated reaction cascades whose effects on the food components are very difficult to predict, e.g. by cutting or cooking plants from the Cruciferae family. Glucosinolates are an important group of phytochemicals that are widely distributed throughout the Cruciferae, a family that includes the Brassica vegetables such as cabbage, Brussels sprouts, broccoli and cauliflower. Glucosinolates coexist with, but are physically separated from, the hydrolytic enzyme of myrosin in the intact Brassica plant. On mechanical injury of the tissue, the enzyme and the substrate meet, resulting in hydrolysis. Thus, any process (industrial or domestic) disrupting cellular integrity may result in some glucosinolate hydrolysis. The breakdown products of glucosinolates have been found to exert anticarcinogenic activity in experimental animal models (Guo et al., 1992; Conaway et al., 1996). Also, processes taking place during cooking (partial inactivation of myrosin, heat degradation of glucosinolates, enzymatic breakdown of glucosinolates, loss of enzymatic cofactors ascorbic acid and iron, leaching of glucosinolates and breakdown products in cooking water) tend to considerably reduce the amounts of glucosinolates found in cooked vegetables.

Organosulphur compounds of garlic have been linked to be the bioactive compounds being responsible for the anticarcinogenic potential of garlic. Like glucosinolates,

processing in garlic also initiates a cascade of chemical conversions of the original limited number of organosulphur compounds, such as alliin and gamma-glutamyl-S-allylcysteine. Thus, processed garlic preparations contain different constituents with different types of effectiveness and safety than does raw garlic. For example, garlic that is aged exhibits antioxidative activities whereas other preparations may act as a pro-oxidant (Petesch and Sumiyoshi, 1999).

Since it is impossible to study all possible sources of variation in the production chain, research should focus on using limited experimental data and knowledge as a source to build predictive models of the steps taken in the food production chain.

4. Food consumption

4.1. Food consumption data

Figure 6 presents a diagram of a food consumption model resulting in food consumption data. Integrating the model's outcome data with the data from the food production chain model produces a realistic component intake distribution for the entire population.

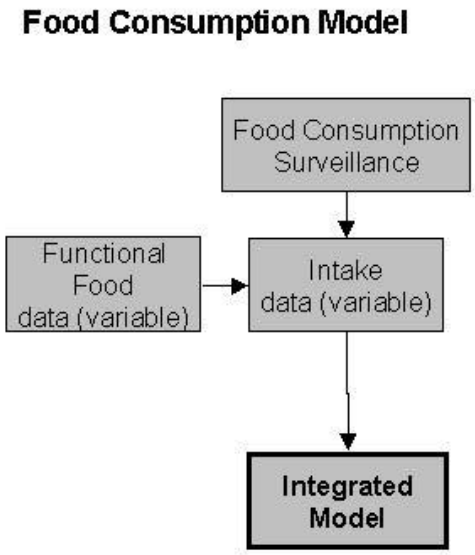

Figure 6. Food consumption model to produce food intake data.

In order to obtain information on food consumption four types of studies can be used, which lead to different types of data (FOSIE-report ITG E):

- Food supply data
- Household surveys
- Individual dietary surveys
- Total diet studies

Each type of data corresponds with a different stage in the food chain and is obtained by applying different methods.

Biomarkers of intake, i.e. components which can be measured in biological material that is indicative of the intake of specific foods or dietary components, constitute a

fifth type of exposure data. A sixth type of data is obtained from post-launch monitoring studies.

4.1.1. Food supply data

Food supply data refer to food availability, giving only a crude (overestimated) idea of potential average consumption. The data are gross annual estimates of the national availability of food commodities. Food supply data are calculated in food balance sheets that are accounts of annual food production, changes in stocks, imports and exports, agricultural use and industrial use on a national level. Food and nutrient losses prior to consumption and waste are not adequately accounted for.
The result is an estimate of the average value per head of the population rather than actual intake.

The FAO provides food balance sheets for most countries.

4.1.2. Household surveys

Food available at household level can be estimated by conducting budget surveys or consumption surveys. The first type of survey provides information on the purchase of food in terms of expenditure. In household consumption surveys, the amounts of foods and drinks brought into the household are recorded. Mostly, only expenditures on meals taken outdoors are noted. Data on food quantities and/or food expenditure may be collected by keeping a record, by having interviews or by using both methods.

In general, household surveys do not provide information on how food is handled within the household or on actual consumption by its members.

The DAFNE (Data Food Networking) project is a European initiative to assess household budgets spent on foods in five to nine European countries (Trichopolou and Lagiou, 1997). The Netherlands does not participate in the initiative.

4.1.3. Individual dietary surveys

To collect dietary intake data on an individual level, several methods can be used. Briefly, they can be divided into two categories: record and recall methods. Record methods collect information on current or actual intake over one or more days. Recall methods reflect past consumption, varying from intake over the previous day (24-hour recall) to habitual food intake (dietary history or food frequency questionnaire).

Moreover, repeated recalls or records can be performed, offering an opportunity to estimate habitual intake.

The EFCOSUM project provides an overview of European, nationwide food consumption surveys on an individual level from 1985 onwards. Methods used differ between studies and populations studied differ as well. In the Netherlands, three nationwide food consumption surveys using individual 2-day dietary records were conducted, i.e. in 1987/88, 1992 and 1997/98. The EPIC study is a European initiative to collect national food consumption data on an individual level in comparable ways in order to study nutrition in relation to the risk of developing cancer (Riboli and Kaaks, 1997).

4.1.4. Total diet studies

In total diet studies, representative samples of widely consumed foods are collected and analysed for their components of interest. The accuracy of population intake estimates based on these studies depends on the extent to which the analysed foods represent important dietary sources of the constituents studied.
Three approaches are distinguished in total diet studies:

- Market basket All food items that are part of the average diet are purchased, prepared according to standard household procedures and aggregated into a number of food groups. These food groups are analysed for their components of interest.
- Individual food items Based on national food consumption surveys for several age-sex categories, a list of foods representing the products most commonly consumed is composed. All selected food items are prepared according to methods most commonly used and then analysed.
- Duplicate portion A duplicate of the individual daily diet as consumed is analysed.

Total diet studies are used mostly to monitor the general situation. In the Netherlands, for example, food additives and food components were measured in the 'market baskets' of 16-year-old male adolescents (van Dokkum et al., 1982). In the same way, the minerals and trace elements found in the 'market baskets' of 18-year-old male Dutch adolescents were studied (van Dokkum et al., 1989, Brussaard, 2001).

4.1.5. Biomarkers

Biomarker studies involve two main stages. In the first stage, a human volunteer study is undertaken to examine whether a quantitative relationship can be established between the dietary intake of the component in question and the amount of the corresponding biomarker detected in biological material such as blood, urine and adipose tissue. Having established a relationship, the second stage can be performed, i.e. biomarkers are measured in individuals of the target population and dietary intake is deduced from those biomarkers.

Because dietary intake is measured with error and, therefore, the relation with biomarkers can never be a perfect one, a method has been developed to estimate 'true dietary intake', i.e. the triad method (Kaaks, 1997).

4.1.6. Post-launch monitoring

Post-launch monitoring implies that the amount and pattern of consumption is assessed and the nature and degree of expected and unexpected effects are identified after a new food product has been launched. Consumption of specific novel foods can be monitored by using EAN codes.

4.1.7. Discussion

Household and individual dietary surveys and total diet studies depend on the cooperation of respondents. Moreover, all questionnaire and interview methods are relying on the memory of those responding. This memory can be influenced by actual health status. For instance, it is known that obese people tend to underestimate their – energy - intake. A potential bias for household and individual dietary surveys is that participants may alter their dietary habits because of the assessment. This factor is absent from biomarker studies. However, most biomarker studies measure - nutrient - status rather than – nutrient - intake. Total diet studies differ from other methods in that their intake estimate does not depend on composition data from other data sources. The concentration of components is measured by making a chemical analysis of the duplicate diet.

The value of different study types for the conceptual model to evaluate the effectiveness and safety of functional foods depends on the situation. In principle, the best thing to have is individual dietary information that can be combined with composition data of individual food products prepared for consumption.

Important criteria for selecting an assessment method include:

- Method application
- Reference period of intake, i.e. short-term intake vs. long-term intake/usual intake
- Level of observation (food groups, foods, food components, selection of index numbers)
- Subjects studied, e.g. sex, age, educational level, smoking habits, level of physical activity
- Precision required
- Costs

The analogy between intake data from different surveys depends both on the method and on the criteria applied.

Food intake data may vary widely, showing some uncertainty. The sources of variability and uncertainty in food intake data are given below.

Sources of variability in food intake data are:

- Temporal variation and time frame of measurements: this refers to the year(s) of data collection (trends in consumption over time can be observed) as well as to the reference period used for dietary assessment
- Geographical variation: referring to differences between regions and countries
- Inter-individual, e.g. as a result of cultural, ethnic or lifestyle factors: differences in intake between individuals
- Intra-individual: differences in intake within individuals, i.e. day-to-day variation
- Portion sizes: measuring intake frequencies and using standard portion sizes to calculate the amount of intake in grams versus measuring actual portion sizes by making photographs and/or an assessment of real volume/weight measures
- Losses as a result of food preparation and/or storage
- Seasonal variation in quality parameters
- Product heterogeneity: concentration per consumer portion

Sources of uncertainty in food intake data are:

- Missing or incomplete data
- Measurement errors in assessing true intake, e.g. as a result of underreporting or overreporting by respondents
- Sample size: larger samples will produce more precise measurements and smaller uncertainties
- Sampling error: resulting in a non-representative sample of the target population
- Using surrogate data: extending available data to other populations or other food groups for want of data for the populations or food groups under study
- Codes and definitions of foods, recipes and ingredients: coding and definitions may differ between studies or populations. For example, potatoes are not included in the food group of ‘vegetables’ in the Netherlands. However, in other countries such as the US and Italy, potatoes are considered vegetables.

4.2. Food composition data

Food composition databases provide nutrient contents per food. They can be used to convert food intake into nutrient intake. Many European countries have compiled their own national food composition database. The following types of sources underlying those databases can be distinguished:

- Published data versus analysed data; data published by others versus data obtained by analysing foods
- Data deduced from other composition data; if no data are available for specific foods or food components, such data may be deduced from existing data

Food composition data may be variable and uncertain. Sources of variability in food composition data are:

- Date of publication or analysis: foods may differ over time
- Country of origin: foods differ between countries or regions
- Methods used for analysis: different methods may lead to different food composition data
- Brand: food components may differ between brands because ingredients or processes differ
- Losses due to food preparation: raw product versus food as consumed; e.g. peeling fruits before eating or cooking them
- Product heterogeneity: concentration per consumer portion or composite sample or multi-sample average
- Any – other - factors affecting variability in the production chain (see Chapter 3)

Sources of uncertainty in food composition data are:

- Availability/completeness of data: often, food composition data are not available for all foods or information on specific dietary components is absent
- Measurement errors
- Sampling procedure underlying food composition. Since food shows a wide variety, a balanced sampling procedure must be followed to result in a useful figure for the food composition table
- Sample size: see sampling procedure
- Level at which food composition data are known, i.e. total - raw - food, meal, food groups, composite dishes, foods as consumed, brand

In the Netherlands, the NEVO table is the most commonly used food composition table. The most recent table dates from 1996 (NEVO, 1996). This table includes information about the content of energy, protein (total, plant-based), fat (total, saturated, mono-unsaturated, polyunsaturated, linolenic acid, cholesterol), carbohydrates (total, mono/disaccharides, polysaccharides, dietary fibre), alcohol, water, polyols, Na, K, Ca, P, Mg, Fe, Cu, Se, Zn, retinol equivalents, vitamins D, E, B1 and B2, nicotinic acid, vitamin B6 and vitamin C for 1,476 foods (Hulshof et al., 1996; Westenbrink et al., 1996). A new edition of the table is due to be published in 2001. That version will also include information about folic acid (vitamin B11), vitamin B12 and carotenoids in fruits and vegetables.

For some dietary components not included in the NEVO table, fairly adequate and complete data are also available, for example for catechins in the Netherlands (Arts et al., 2000a; 2000b). However, for many components of potential relevance to health protection (such as flavonoids and other polyphenols, lignans, glucosinolates, peptides, lactic acid bacteria) only limited food composition data are available - or even none at all.

The value of different types of food consumption studies to evaluate the effectiveness and safety of functional foods depends on the situation. In principle, the best thing to have is individual dietary information that can be combined with the composition data of individual food products prepared for consumption.

5. Integration of consumption and consumption data

In order to calculate the intake of components, the amount of food eaten is multiplied by the content of the specific components of that food. The composition of food supplements can be treated in a similar way. In order to have a valid estimate of the intake of components it is necessary that all food products of interest have been assessed in the food consumption survey used.
The following calculations can be used:

- A point estimate of intake, preferably with a simple distribution, such as standard deviation and percentile points
- A probabilistic estimate, using statistical modelling techniques such as Monte Carlo Simulation Estimates of intake for populations, can be based either on the mean intake for the total population or on the users of specific relevant foods or food components only.

Monte Carlo simulation:
Monte Carlo simulation involves a random sampling of each probability distribution within the model to produce many scenarios. The analysis takes into account every possible value of a variable and, in addition, it weighs each possible scenario by the probability of its occurrence. The structure of the model is similar to a deterministic model, with all its multiplications, additions, etc. that link variables together. However, instead of a single value, each uncertain variable is represented by a probability distribution. Single-point or deterministic modelling uses a single 'best guess' estimate. Sensitivities of the assumptions can be performed on the model to show their impact on the outcome. These 'what if' scenarios are combinations of the input variables of the model. By taking into account actual variation, 'what if' scenarios in a Monte Carlo simulation are far more realistic than the deterministic models that are commonly used (Vose, 1996). Monte Carlo involves a random sampling of each probability distribution within the model to produce thousands of scenarios through iterations. Existing software of @RISK can be used here.

The ambition to obtain component intake distribution patterns that are characterised by high reliability requires an integration of the traditional 'composition table' approach with the 'production chain' concept.

6. Biological effects

6.1. Bioavailability

The term ‘bioavailability’ is used to describe those processes that occur after the consumption of nutrients, including their absorption at intestinal level and the subsequent distribution and metabolism or storage of nutrients in the body. A working definition of bioavailability that is often used originates from pharmacology, i.e. ‘the proportion of a nutrient ingested that becomes available for usage or storage in a target tissue’.

When De Pee and West (1996) described the factors that may interfere with the bioavailability of carotenoids and their provitamin A value they introduced the SLAMENGHI mnemonic. These factors include: Species of carotenoids, Linkages at molecular level, Amount of carotenoid, (food) Matrix, Effectors, Nutrient status, Genetics, Host-related factors and Interactions among these factors (de Pee and West, 1996).

Presumably, the same factors also play a role in the bioavailability of other dietary components.

Already, a great deal is known about the bioavailability of iron and this knowledge is brought together in algorithms. These algorithms facilitate the estimate of heme and non-heme iron absorption from diets, including the enhancing effects of ascorbic acid, meat, poultry or fish and the inhibiting effects of phytate, polyphenols, calcium, egg, soy protein or alcohol on non-heme iron absorption (Hallberg and Hulthén, 2000).

6.2. Risk-benefit models

Various risk-benefit models that can be used to substantiate effectiveness and safety predictions of functional foods are presented in Figure 7.

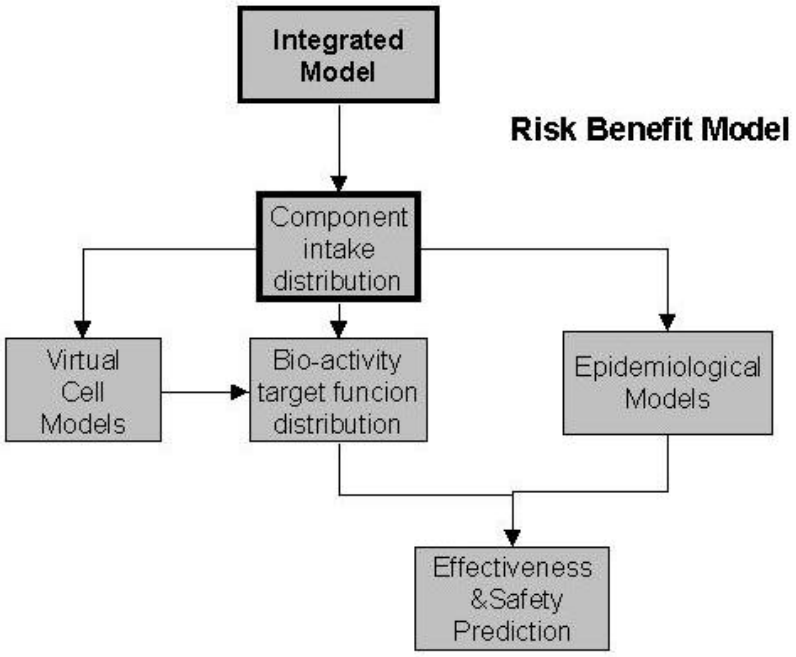

Figure 7. Risk-Benefit Models to be used when studying the biological effects of functional foods.

6.3. In vitro/in vivo studies

To examine the biological effects of functional foods and their functional ingredients, studies can be performed at three levels:

- In vitro studies
- Animal studies
- Human studies

In general, all the factors that may be of influence other than the factor of interest can be easily controlled in animal and in vitro studies. In studies with humans it is usually difficult to control the other factors. Potentially influential factors can be genetic or environmental, including other dietary factors. The main question is, however, whether results from studies on cells and animals can be generalised to humans. Since we are interested to know the biological effects in humans in the context of functional foods, epidemiological studies that are possible in humans are discussed in more detail in the next paragraph and in Appendix 5.

Developing 'virtual cell' models, for example when testing the effects of a compound in a dynamic environment at cell level by using computer modelling, will offer a valuable additional tool (Tomita, 2001). This type of approach may link the rapidly accumulating information from genomics and proteomics to biological activity, including the individual variation in man.

For biological effects, too, general sources of variability and uncertainty can be identified.

Sources of variability include:

- Amount of functional food/functional ingredient; associated with the nature of the exposure-response relation
- Form/source of functional ingredient; for example, synthetically created compounds may behave differently from naturally derived forms; also, isomers of compounds can have different characteristics
- Mixed exposures; effects may alter when compounds are combined with other compounds, e.g. due to competition in physiological processes
- Inter-individual differences due to genetic make-up, physiological factors, lifestyle factors, etc.; for example, effects can be different in men and women, smokers and non-smokers.
- For cell studies and animal studies: differences with effects in humans

Sources of uncertainty include:

- Availability of studies providing evidence of biological effects
- Lack of knowledge about underlying mechanisms
- Nature of exposure-response relation
- Doubts about possibility of generalising to humans as a whole
- Other interfering factors - and lack of knowledge thereof

6.4. Epidemiological studies

In epidemiological studies dietary intake is associated with health status. Dietary intake can be measured in terms of food groups, food items, nutrient intake or dietary patterns. Health status can be expressed in several ways, in terms of nutrient status, disease status, mortality and/or life expectancy.

The discipline of epidemiology studies the distribution and determinants of health-related states or events in specified populations. Associations between population characteristics and its disease risk are investigated to determine whether these factors directly alter the disease risk or else may act as markers for other underlying factors directly affecting the disease risk. Epidemiology is the only scientific discipline to address phenomena of disease occurrence in human populations directly, trying to explain them and advising public health bodies on preventive measures. Epidemiology

generates human data that can be used in the hazard identification and hazard characterisation steps of a risk assessment procedure.
Epidemiological studies use observational as well as experimental study designs. Basically, the following designs are used:

Experimental studies:

- Randomised controlled trials (clinical trials, intervention studies)

Observational studies:

- Ecological - correlation - studies
- Case-control studies
- Cohort studies

For observational studies, more detailed segmentation is based on the unit of observation: in ecological studies, populations or groups of individuals are studied, whereas individuals are the unit of interest in case-control studies and in cohort studies.

Experimental studies with human individuals provide the strongest evidence for causal risk relations while being least likely to suffer from any bias. However, they are the more expensive and usually less feasible studies. The - less expensive - cohort studies assess exposure and select study participants before the health outcome of interest occurs, thus providing relatively strong evidence. Although cheaper case-control studies generally assess exposure retrospectively in subjects with and without a specific health outcome, their evidence is more debatable. The lowest costs are associated with correlation studies, but they provide weak evidence and they are much more susceptible to bias (van den Brandt, 2000).

To enhance statistical power it is useful to combine the results of individual studies. Two methods can be used: a meta-analysis of published results or a combined analysis by pooling individual data.

A meta-analysis is a statistical procedure that integrates the results of several independent studies that are considered ‘combinable’ (Egger et al., 1997). Major problems in meta-analyses are publication bias, heterogeneity among studies and variations in the quality of studies, in exposure and response measures, in the presence of effect modifiers and in controlling interfering variables. Publication bias refers to the fact that studies with negative or null results are less likely to be published. Pooling, generally, is more costly and time-consuming than meta-analysis.

Going one step beyond pooling the results of independent studies is where multi-centre epidemiological studies are found.

To examine in vitro the biological effects of functional foods or their functional ingredients, animal and human studies can be performed. Virtual cell, bioactivity target function distribution and epidemiological models can be used to substantiate safety and effectiveness prediction.

7. Risk-benefit prediction

Risk reduction can be calculated in terms of a reduction in incidence (i.e. new cases in a specific time period), prevalence (i.e. total of cases prevalent at a time), mortality or life expectancy. Composite public-health measures have also been constructed, such as HLE (healthy life expectancy), DALY (disability-adjusted life years) and DALE (disability-adjusted life expectancy). These constructs combine public health measures, for example life expectancy and number of years lived in good health, because in addition to increasing the life expectancy an issue of growing interest has been to increase the number of years lived in good health.

Risk reduction at population level can be estimated by using several techniques. A simple method to estimate impact is to calculate a population attribute risk (PAR). In order to make the calculation it is necessary to have information about the relative risk, i.e. a quantitative estimate about the strength of the relation between dietary factor and health outcome under study and the prevalence of that health outcome.

Computer simulation models can provide more sophisticated methods to estimate the potential impact on disease incidence, disease mortality and/or - healthy - life expectancy. An example is the Chronic Diseases Model (CDM) developed at the National Institute of Public Health and the Environment. This model makes it possible to estimate the health effects of trends and interventions in lifestyle factors such as dietary intake. The model describes the relations between risk factors and diseases in a system-dynamic, multi-state model based on the life-table method. 'System-dynamic' refers to the fact that the population is not static, although births, deaths, migration and ageing are incorporated into the model. Population figures from Statistics Netherlands and national mortality figures have been included in the model. 'Multi-state' refers to the fact that the population under study is divided into different model categories or different risk-factor states, e.g. different intake levels, and different disease states, e.g. diseased or non-diseased. Thus, if the model is to be applied, the risk factor under study must be divided into classes. For example, the intake of a functional food can be divided into categories of zero intake, up to 50 grams daily and 50-100 grams daily. Moreover, the relation between intake in those categories and intended health outcome should be known. The model can then calculate the estimated figure for the health outcome of interest for the reference scenario; for example, before the functional food was introduced the whole population belonged to the lowest category of intake. Following this, the model can again calculate the health outcome of interest, although now using the intake figures after the product was launched. The difference between the two calculations is the estimate of expected

health gain. Estimates can be made for a one-year period as well as for longer periods of time.

All this makes it clear that all sorts of information are needed for calculating expected health gains in this way. When specific information is not available, it is possible to make assumptions. However, when several assumptions are used in a risk reduction model, the validity of the estimated health gain will not be unambiguous.

Other models are also available, such as those presented by Ponce et al. (2000). They used a quantitative method of risk-benefit analysis that allows for diverse health end points with differing impacts (i.e. duration and severity), applying dose-response modelling weighted by quality-adjusted life years saved. To demonstrate this method they evaluated the risks and benefits of fish consumption by using a single health-risk and health-benefit end point. Benefits were defined as a decrease in myocardial infarction mortality while risks were defined as an increase in neurodevelopment delay resulting from prenatal methyl mercury exposure.

All functional foods to be launched onto the market need to have a scientific record that demonstrates their potential to modulate target functions as well as their relevance to health and well-being, including any risk reduction for certain chronic diseases.

8. Summary and recommendations

The food production chain can have a great effect on variations in the composition of food products with regard to bioactive components used in functional foods. This variation is additional to the variation that is seen in the consumption patterns of individual consumers. Pre-market modelling is a tool to obtain realistic information on intake data for bioactive compounds from different types of - functional and conventional - food products and supplements. The present study developed a conceptual model for combining information on intake and composition. Such an integrated model can produce a realistic component intake distribution among the entire population. In this way, any risk of overdosing due to cumulative consumption (i.e. accumulation) can be predicted and prevented.

The information required to implement this type of approach was identified in this report. In general terms it consists of:

- Quantitative information on the distribution of intake data (including functional foods and food supplements).
- Quantitative information on the composition (including variability and uncertainty) of (functional) food products.
- Quantitative information on the effects of the food production chain on that composition (including variability and uncertainty).

Developing risk reduction models may further facilitate the evaluation process of functional foods.
Based on the suggested approach the following recommendations are made to initiate and stimulate future research:

1. The processes of collecting and managing intake data, including functional foods and food supplements, should be developed in more detail to cope effectively with variability and uncertainty.
2. Developing production chain models in order to predict the effects of the chain on the level (with variability and uncertainty) of bioactive compounds in consumed products.
3. Developing integrated probabilistic models to combine intake data and compositional data, including their predicted variability and uncertainty.
4. Incorporating food-matrix and food-processing effects on the bioavailability of bioactive compounds into predictive models.
5. Developing more detailed Risk Reduction Models (virtual cell models, target function distribution and epidemiological models).

To demonstrate the power of the proposed integrated approach recommendations 1-3 are suggested for a case study on a few selected bioactive compounds. As a follow-up to the present study, the authors have made a research proposal.

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Appendix 1: Modelling

A model can be seen as a hypothesis about how a system works or responds to changes in its input variables (Cullen and Frey, 1999). Models can be used for screening, research or decision-making purposes. A model is always a simplified version of reality.

The model should represent the system as accurately as is necessary for its intended application. A model used for screening purposes, for example, does not need to be highly accurate as long as it is conservative, that is, on the safe side. Models can be described in mathematical language, with equations and input variables, based on empirical or theoretical considerations.

For example, models can be used to screen the intake of additives (Löwik, 1996). These - mostly simple - models are used mainly for identifying areas relevant for legislation, data collection or research activities. If the estimated intake is far below maximum permitted levels, no further research is needed.

Models used for research purposes are focussed mainly on gaining a better understanding of system functions and structures, e.g. the biological availability of iron. These models tend to be more complicated than those used for screening purposes.

Two constructs are of major importance in modelling, i.e. variability and uncertainty. Variability refers to real differences, mainly due to differences in time, space or between individuals. Uncertainty stands for lack of knowledge, mainly as a result of measurement errors, small sample sizes or differences in interpretation.

Depending on its application, modelling can be performed in several ways. In deterministic models, point estimates are used while variability and uncertainty in input variables are not taken into account. Sensitivity analyses can be used to study the effect of variability in input variables on the outcome variables by changing one or more model items at a time. It often precedes probabilistic modelling. In probabilistic models, information on the distribution of input variables is used, resulting in a range of outcome variables and probabilities of those outcomes. In this way, both the uncertainty and variability of input variables are taken into account.

Thus far, probabilistic risk assessment modelling has been used more often in environmental sciences than in health sciences. For example, the United States

Environmental Protection Agency has widely used these techniques. See their web sites for more details:
http://www.epa.gov/superfund/programs/risk/rags3adt/index.htm
http://www.epa.gov/superfund/pubs.htm#r
http://www.epa.gov/scipoly/sap/index.htm

A model needs validation to ensure that it describes what it should describe, i.e. model outcomes must be compared with independent observations of the system that is being modelled. It should be realised that dietary intake, by its very nature, can never be measured without error. Part of this problem can be overcome by using biomarkers, i.e. concentrations of components in biological materials such as blood, adipose tissue and urine that are indicative of the intake of specific foods or dietary components. However, valid biomarkers are scarce. An alternative method of validation is to compare the outcomes of different models.

Appendix 2: Predictive modelling of the production chain

Modelling the production chain consists of a series of submodels describing the effects of different steps within the chain. A diagram of a submodel is given in Figure 1.
The submodel should be based on information and/or knowledge of the effects of various conditions on the compound(s) of interest. Submodels can be classified as one of two types:
A mechanistic model (e.g. a set of differential equations) based on a level of understanding of the essential processes involved.
An inductive model (e.g. a neural network) based on - large sets of - historic data describing the effects of conditions on that level.

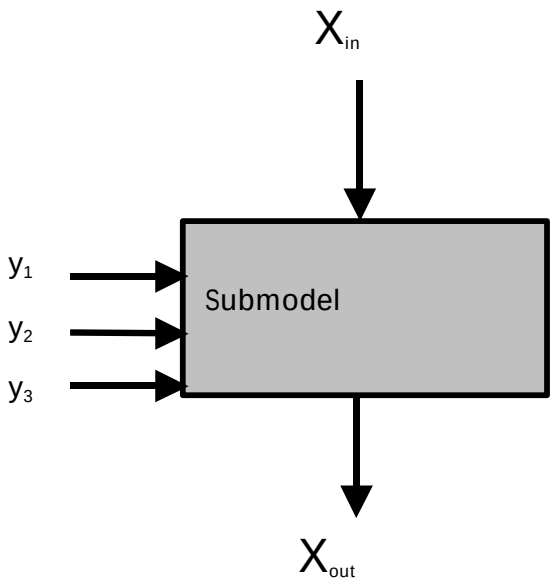

Figure 1. Diagram of a submodel describing the effect of a step in the production chain on the level of a bioactive compound ($X_{in/out}$) depending on the conditions during that step (y_i).

It is impossible to gather systematic information on all possible variations in all steps of the production chain. What should be used, therefore, is a predictive modelling approach to estimate the effects of variation in conditions and processes on food composition. To achieve this the following steps must be taken:

- essential parameters that determine the final content of the studied food compound must be identified

- critical subprocesses for each parameter occurring during the process under study must be defined
- subprocesses must be translated into mathematical equations which, in most cases, will be - partial - differential equations combined with mass balances, describing what happens during processing.

Thus, the model approach must be based on a sound mechanistic understanding of those conditions and processes within the entire production chain that have the greatest importance. A few examples are presented here:

A predictive model has been developed to study the stability of extra virgin olive oil during commercial activities up to public consumption (Pagliarini et al., 2000). Another example is the development of a model to predict the processing implications for microbial growth in food production, storage and distribution (Zwietering and Hasting, 1997a,b). In case studies, the model was applied to meat product and burger lines.

Dekker et al. (2000) applied predictive modelling to predict the fate of health-promoting compounds in the production chain. A case study investigated the effects of cooking on glucosinolates in cabbage. The first step in developing the model was to identify subprocesses that introduced variation to the composition of the final product in the production chain (Figure 1). Important parameters of health benefits were selected (content of glucosinolates, breakdown products, active myrosin) and critical subprocesses were identified (cell lysis, leaching, enzyme denaturation, enzyme activity). Independent analyses of selected subprocesses were performed and the processes were translated into mathematical equations. Using the temperature profile during cooking as well as the amounts of cabbage and cooking water as inputs to the model, the cooking process could be simulated and glucosinolate and breakdown product profiles during the cooking process could be calculated. In a validation procedure, the model was used to predict the effect of cooking water on the final level of glucosinolates in broccoli and this was compared with experimental data.

Data on the contents in raw materials and the effects of processing on those contents exist for macro- and micronutrients, although they are often incomplete. For bioactive compounds (plant-derived bioactive compounds, bioactive proteins and peptides or micro-organisms used as probiotics in functional food production), even fewer data tend to be available.

Appendix 3: Paper TIFS (pdf file)

Dekker, M, Verkerk, R and Jongen, W (2000) Predictive modelling of health aspects in the food production chain: a case study on glucosinolates in cabbage. Trends in Food Science and Technology 11: 174-181.

Appendix 4: Plant derived compound groups

Plant-derived compound groups that are thought to have potential beneficial health effects and their assumed working mechanisms (from Verkerk et al., 1998).

Bioactive components	Examples	Possible working mechanisms	Most important supplies
carotenoids	β-carotene, lutein, lycopene	antioxidant, inhibits cell-cell communication	yellow-green vegetables and fruits (spinach, carrots, oranges)
phenolic acids	chlorogenic acid, phloridzin, ferulic acid	antioxidant	vegetables, fruits
glucosinolates, indoles, isothiocyanates	indol-3-carbinol, phenylethyl-isothiocyanate	induction of detoxification enzymes	Cruciferae plants (sprouts, cabbage, broccoli)
organosulphur compounds	diallyl sulphide	induction of detoxification enzymes	garlic
catechins	epigallocatechin gallate	antioxidant	tea
flavonoids	quercetin, kaempferol	antioxidant	onions, apples, tea
selenium	selenomethionine	cofactor of antioxidants	eggs, milk and cereal products
terpenoids	D-limonene, geraniol, menthol	induction of detoxification enzymes	citrus fruits
phyto-oestrogens	genistein, daidzein, coumestrol	competitive receptors for sex hormones	soy, cereals, legumes
salicylates	acetyl salicylic acid		vegetables, fruits and spices
coumarins	dicoumarol, limettin	anticoagulators, induction of detoxification enzymes	vegetables (cassava), citrus fruits
phytate (inositol hexaphosphate)		regulation of cell division, antioxidant	cereals, nuts, seeds, legumes
(plant) sterols	β-sitosterol, campesterol	cholesterol absorption and metabolism	vegetables
saponins			soy
protease inhibitors	trypsin inhibitor	competition with protease inhibitors	vegetables, potatoes, cereals, legumes
peptides	glutenin	antioxidant, immune modulation	milk, wheat

Appendix 5: Epidemiological studies

1. Experimental studies

An experimental study generally is the most clear-cut way to study a dietary cause-effect relationship. This study design makes it possible to draw conclusions on whether a specific measure affects the disease rate directly or whether it should be considered as a marker of another, underlying cause affecting disease risk.
Experiments are said to be 'controlled' when an untreated group is involved, with the aim to control changes in disease incidence occurring independently of changes in exposure. In randomised experiments, human subjects are randomly allocated to treatment and control groups, thus minimising the likelihood of having a grossly unequal distribution of potentially confounding variables among the groups studied. Ideally, the experiment is carried out in a double-blind fashion, where both the subject and the observer of the study outcome are blinded to the exposure status, thereby avoiding subjective influences on the results of the study.
For ethical reasons, human experiments are generally carried out to study positive health effects. Therefore, these studies can be carried out only if sufficient observational data have shown reasonable probabilities of beneficial effects and/or if toxicological screening has produced reasonable proof of safety (Willett, 1998). Due to the enormous costs of large-scale intervention studies, exposure is usually limited to a few or even one exposure level. High costs are also a limiting factor when the aim is to study long-term health effects.

2. Observational studies

Ecological studies:
Ecological studies are also called correlation studies or aggregate data studies. Populations are studied to find geographical trends or time trends. The mean value for both exposure (e.g. population per capita consumption) and outcome (e.g. disease rate in the population) is obtained for each unit of observation. The design has the advantage that contrasts in dietary intake between populations can be very large. Also, average diets within a population are more stable than are the diets of individual persons. Finally, the health effect rates on which international studies are based are usually derived from relatively large populations; as a result, they are subject to only small random errors (Willett, 1998). Serious limitations, however, are that other factors associated with the population, such as genetic predisposition and other lifestyle factors, may confound results. Population per capita consumption may be only weakly related to the diet of those individuals that are at risk of disease. It is assumed that the

available food is eaten by humans, i.e. pets are not taken into account. Also, differences between populations in quality of measurement of both exposure and disease (diagnosing technology) may bias the results obtained.

Case-control studies:

Case-control studies start with a group of patients, or persons known to have the disease or condition. These cases are compared with a control group selected to represent the population from which the cases were drawn, except with respect to the exposures of interest. Exposure information is collected for a defined time period prior to diagnosing the disease. A case-control study usually compares the odds of past exposure to a suspected risk factor between cases and controls in order to obtain an odds ratio, which is an estimate of relative risk. One advantage is that no long follow-up periods are needed to get results. Another advantage is that, for rare diseases, a much smaller sample size may suffice. A major disadvantage of case-control studies, however, is that the disease may influence exposure information provided by the subjects, i.e. recall bias, or that recent exposure really is different from what it used to be, because of the disease. Also, selection bias may occur when the relation between exposure and disease in study participants is different in those subjects actually involved in the study from those who theoretically were eligible to participate.

Cohort studies:

Cohort studies start with a population which, initially, is free of the health outcome under study. Exposure is assessed at the start of the study (and is sometimes assessed several times during follow-up); besides questionnaire/interview data, this may also include the collection of biological material. During follow-up, some subjects will develop the disease (or health outcome) while others will not. The risk of developing the disease is related to the exposure and is generally expressed in terms of relative risks or relative rate, comparing risks in exposed and unexposed individuals. A main advantage of the cohort design is that, at entry, subjects are free of the disease in question. Therefore, the disease will not influence exposure of the subjects or their exposure information.

Both in cohort studies and in case-control studies, confounders may not be equally distributed between exposure groups.

A general limitation of cohort studies is that large populations are required, especially if rare health outcomes are studied. Substantial follow-up time is required for diseases that have long latency periods. Large sample sizes and long follow-up periods both make cohort studies relatively expensive.

Human populations are heterogeneous in behaviour and genetic susceptibility. Observational studies take this real-life situation into account when drawing conclusions about disease risks. In observational studies, individuals are not randomly

assigned to exposed versus unexposed groups. Consequently, these studies may be affected by bias distorting the factor-disease association. However, in their design and in statistical analyses epidemiologists try to minimise most sources of bias. In experimental studies, random allocation of subjects to different treatments will minimise the effect of variation.
Within epidemiological studies three concepts are of importance when estimating the value and the usefulness of a study or study design, i.e. bias, confounding and effect modification.

3. Bias

Bias is the result of systematic error in either the design or the execution of a study. Several types of bias exist:
Selection bias: error made during the selection of study participants because individuals have different probabilities of being included in the study, depending on exposure and health outcome;
Information bias: error made when measuring exposure or effect. This bias results from a systematic tendency for individuals selected for the study to be erroneously placed in different exposure/outcome categories, thus leading to misclassification. Information bias can be due to the fact that the ability to recall past exposure is dependent on the disease status, i.e. recall bias. Objective markers of exposure, including biomarkers of exposure, are less prone to bias than direct responses from study subjects. Another form of information bias is interviewer bias. This bias occurs when data collection in a case-control study is not masked with regard to the disease status of study participants. Observer bias occurs when outcome assessment is not independent of knowledge of the exposure status. Finally, respondent bias occurs when outcome information (in a cohort study) is obtained by participant response. Whenever possible, it should be confirmed by more objective means, such as a hospital chart view.

In general, due to bias, the observed result will tend to be different from the true result. In many cases, bias can be avoided by using an appropriate study design and valid and reliable methods of data collection.
Both types of bias may affect case-control studies more than cohort studies.

4. Confounding

Generally, a confounder has a causal association with the outcome and a causal or non-causal association with exposure, although it is not an intermediate variable in the causal pathway between exposure and outcome. Confounding refers to a situation

in which a variable is – partly - responsible for the statistical association between exposure and outcome. The result may be the appearance or strengthening of an association not due to any direct causal effect or the apparent absence or weakening of a true association. Identification of potential confounders is usually based on a priori knowledge of the dual association of the possible confounder with exposure and outcome. Presence of confounding can be verified by checking if associations with exposure and outcome exist, by studying the exposure/outcome relationship in different strata of the confounder or by checking changes in associations once adjustments have been made. The basic idea underlying adjustment is to use some statistical model to estimate the association between exposure and outcome, given a constant value or level of suspected confounding variables.
Residual confounding arises when a confounding factor cannot be measured with sufficient precision.

5. Effect modification

Effect modification is present if the magnitude of the association between exposure and disease varies across the level of another variable. In the presence of effect modification, any extrapolation to another population must take into account the distribution of the effect-modifying factor.